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Reproduction in Domestic Animals

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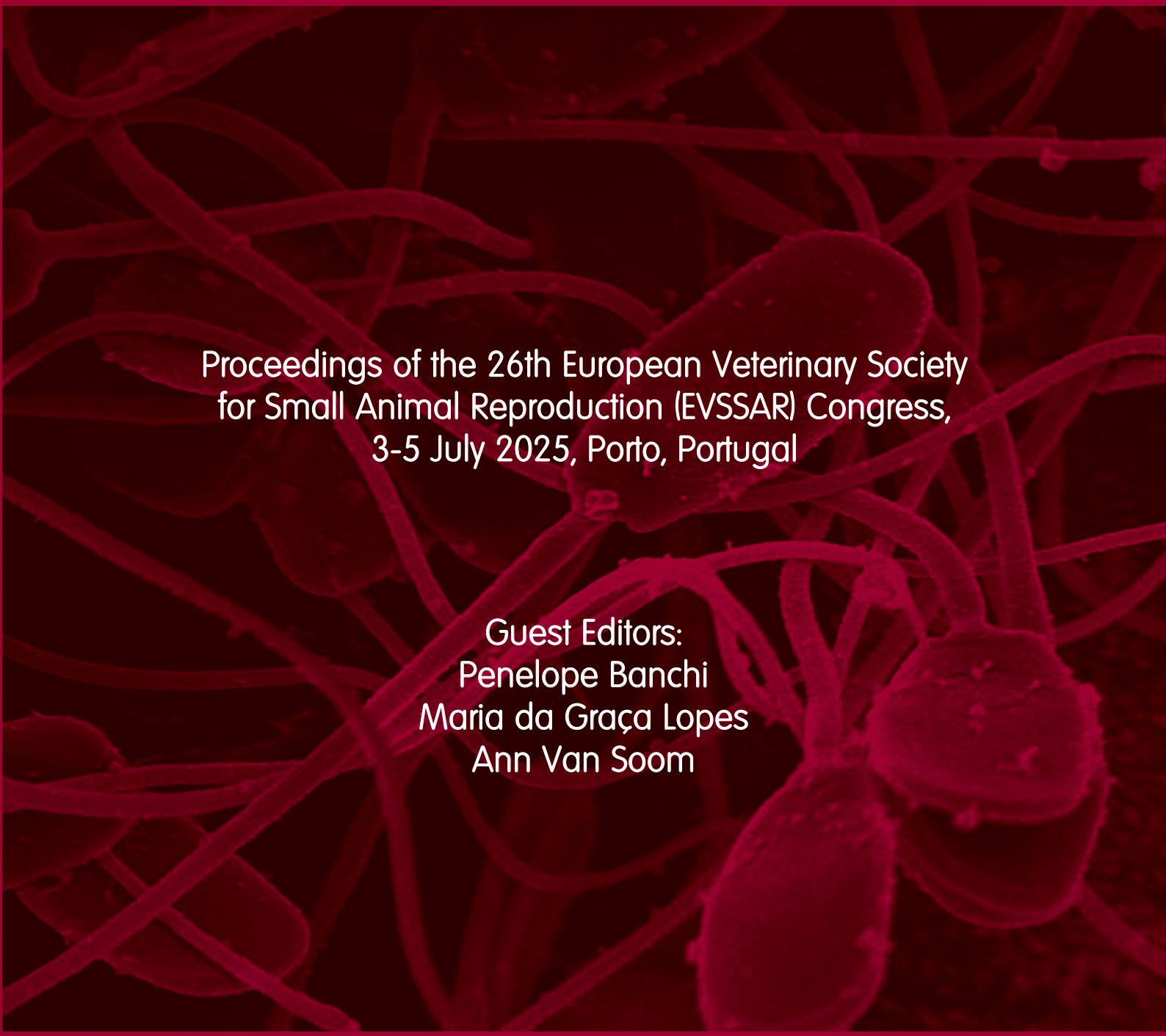

Proceedings of the 26th European Veterinary Society for Small Animal Reproduction (EVSSAR) Congress, 3-5 July 2025, Porto, Portugal

Guest Editors:
Penelope Banchi
Maria da Graça Lopes
Ann Van Soom

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Official Organ of
European Society for Domestic Animal Reproduction
European Veterinary Society of Small Animal Reproduction
Spanish Society of Animal Reproduction

Reproduction in Domestic Animals

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ABSTRACT

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ABSTRACT

EVSSAR

Dear EVSSAR Members, Estimated Colleagues and Friends, Dear Family,
On behalf of the EVSSAR Board, it is with great joy and a deep sense of fulfilment that we welcome you to the 26th EVSSAR Congress, taking place in the beautiful and soulful city of Porto, from 3 to 5 July 2025.

This congress is especially meaningful for us. It marks the culmination of 6 years of shared dedication, passion and teamwork that we—Chiara and Paulo—have devoted to the direction of the EVSSAR. It is our final congress together in these roles, and the last we have the honour of organising. After almost 20 years, the congress returns to Portugal—a symbolic and emotional milestone that makes this edition even more special.

These past years have been filled with many meetings, discussions and decisions—sometimes challenging, often demanding, but always guided by our deep affection for this society. Every step was taken with heart and soul, and above all, with genuine joy in contributing to the growth and strength of EVSSAR.

The scientific program, carefully shaped by our Scientific Committee, reflects the diversity and vibrancy of our field. We are delighted with the number and quality of abstracts submitted this year, and confident that the sessions will bring both inspiration and practical value to all attendees. From canine and feline reproduction to ARTs, neonatology, endocrinology, exotic species and beyond, there is something here for everyone.

We also warmly invite all EVSSAR members to join the General Assembly on Friday, 4 July. This year will mark an important transition: the departure of Chiara Milani, Paulo Borges and Cindy Maenhoudt from the Board. We shall welcome three new members who will bring fresh energy, new ideas and renewed enthusiasm to lead EVSSAR into its next chapter. It is a time to celebrate the continuity and evolution of our society.

Our heartfelt thanks go to the Local Organising Committee, the Scientific Committee, our abstract reviewers and our valued sponsors—your support has been essential. Above all, thank you to all of you who submitted your work, travelled to join us and continue to bring the EVSSAR spirit to life.

Porto welcomes you with open arms—ready to charm you with its warmth, culture, history and hospitality. We hope this congress will be a space to learn, reconnect and create memories that will stay with you long after it ends.

From the bottom of our hearts—welcome to EVSSAR 2025!

With Warmest Regards,
Chiara Milani—EVSSAR President
Paulo Borges—EVSSAR Vice President

On behalf of the EVSSAR Board

Welcome to the 26th EVSSAR Congress

Dear Friends and Colleagues,
We are honoured to extend a warm welcome to participants of the 26th EVSSAR Congress, in the beautiful city of Porto, Portugal! Whether you are a long-time EVSSAR attendee or joining us for the first time, we are delighted to have you with us.

Over the next 2 days, we will engage in a rich and stimulating program that continues the EVSSAR tradition of excellence. The congress will feature keynote lectures and parallel sessions, offering a diverse range of talks and discussions that span key topics in small animal reproduction—including fertility, contraception, pathology, neonatology and the evolving role of artificial intelligence in this field. The program includes invited speakers as well as presentations by delegates and PhD students, reflecting both the depth of expertise and the emerging talent within our field. In addition, more than

100 e-posters will be available for viewing at four interactive digital stations throughout the venue. We warmly encourage you to explore these contributions and connect with the authors. As in previous years, awards will be given for the best oral and poster presentations in both student and non-student categories. Be sure to join us for the closing ceremony, where we will recognise and celebrate the outstanding work of our colleagues!

We would like to express our profound gratitude to our sponsors and exhibitors for their invaluable support. Their dedication to advancing small animal reproduction plays a key role in making this congress a success. Do not forget to visit them during the breaks—you might come across a new idea or product that enhances your work.

Few cities could provide a more charming and inspiring setting for our gathering than Porto. From the colourful facades along the Douro River to its renowned Port wine and genuine hospitality, Porto offers a perfect balance of scientific focus and cultural richness. Whether you find yourself wandering its cobbled streets, savouring local cuisine or immersing yourself in its history, we hope you come to love this city as much as we do.

On Thursday (3 July) afternoon, we will come together for our traditional pre-symposium, which will focus on *Microbiota development in puppies*. The session will take place at ICBAS—School of Medicine and Biomedical Sciences—a leading academic institution of the University of Porto, located in a beautifully modernised historic building in the heart of the city. Later, we will meet for the welcome reception at the World of Wine (WOW), a vibrant cultural district in Vila Nova de Gaia, offering breathtaking views over the river and the historic city centre of Porto. As you sip a glass of local wine and catch up with colleagues, take a moment to explore this unique venue dedicated to the heritage and flavours of Portugal.

The main scientific program of the Congress (4 and 5 July) will be hosted at Alfândega do Porto—a stunning 19th-century customs house turned congress centre, situated right on the riverbank. Combining classical architecture with modern facilities, it offers an inspiring atmosphere for learning, sharing, and connecting.

On Friday (4 July) evening, we invite you to join us for the gala dinner at the magnificent Palácio da Bolsa, a neoclassical 19th-century palace once home to the city's stock exchange. With its majestic halls and the iconic Arabian Room, it promises to be a memorable evening of celebration, fine dining and camaraderie.

We are so happy to welcome you to Porto and to be part of this amazing community. Have a fantastic congress and enjoy every moment!

Warmest Wishes,
Graça Lopes—President of the LOC
Paulo Borges—Vice-President EVSSAR

The Local Organising Committee (LOC)
Ana Celeste Bessa (LOC)
Luísa Mateus (LOC)
Luis Montenegro (LOC)
Rita Payan-Carreira (LOC).

26th EVSSAR Congress

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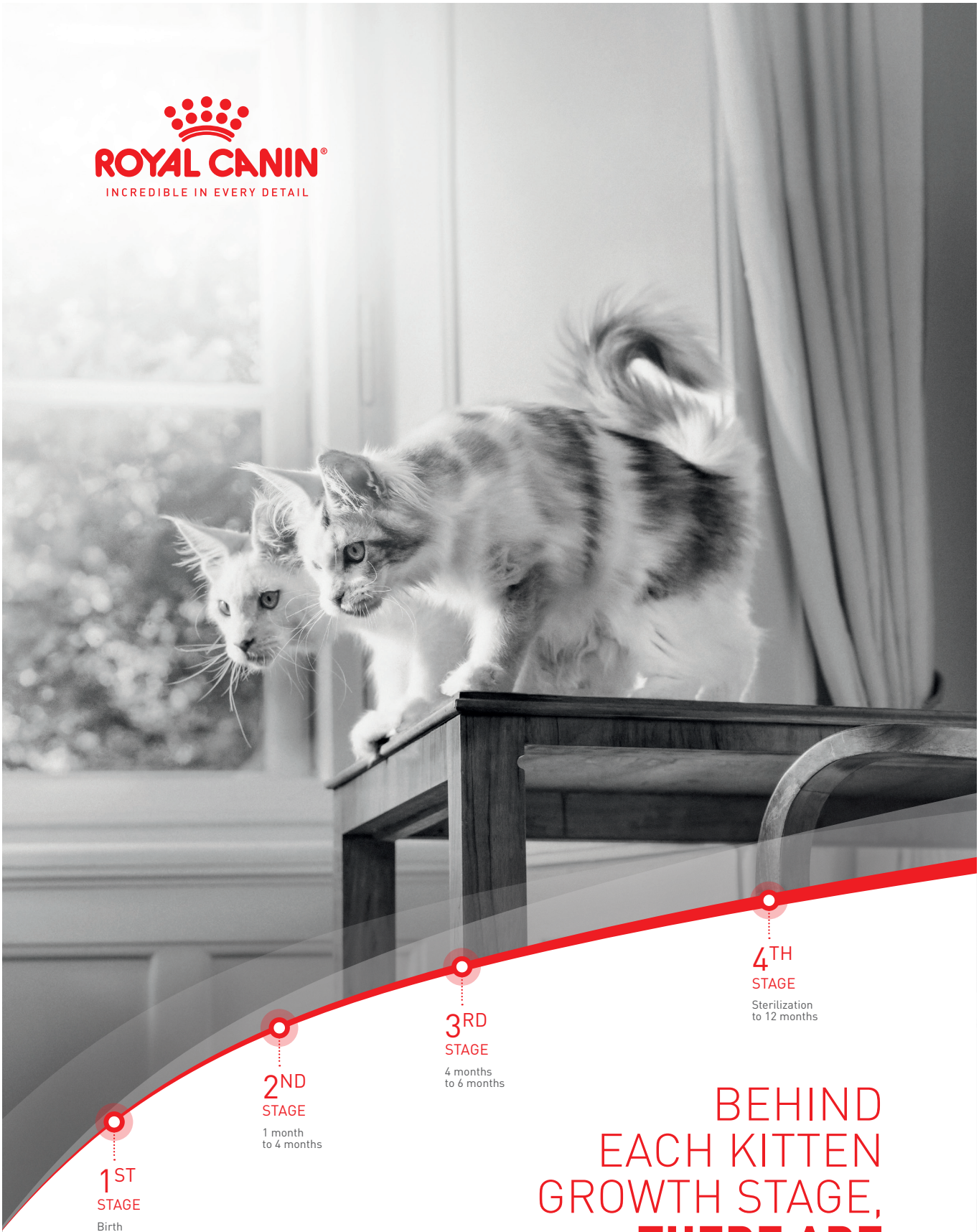
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Reproduction in Domestic Animals

ABSTRACT

Invited Speakers

Management of chronic endometritis in bitches

P. Borges[1,2]

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Chronic endometritis in bitches represents a persistent inflammatory condition of the endometrium, often associated with subtle clinical signs but with possibly severe reproductive implications. Unlike acute endometritis, which could be characterised by evident clinical signs such as purulent vulvar discharge and systemic illness, chronic endometritis tends to be more insidious and may go unnoticed until infertility or repeated pregnancy loss occurs. The growing understanding of its pathophysiology, diagnostic challenges and therapeutic approaches has emphasised the importance of early recognition and comprehensive management. Chronic endometritis may occur as a sequel of repeated or inadequately resolved episodes of acute endometritis, subclinical uterine infections or following repeated oestrous cycles without pregnancy, which promotes endometrial senescence and immune dysregulation. The condition may coexist with or precede cystic endometrial hyperplasia (CEH), contributing to a complex syndrome with overlapping clinical and histopathological features. The pathogenesis of chronic endometritis is believed to result from a multifactorial interaction involving hormonal imbalances, microbial invasion and immune system dysregulation. Prolonged progesterone exposure during the dioestrus phase is surely a central element, leading to endometrial glandular proliferation, decreased uterine contractility, cervical closure and suppression of local immune defences. These changes may promote the persistence of pathogens and establishment of chronic inflammation. Furthermore, repetitive progesterone impregnation without the possible anti-inflammatory resetting effect of pregnancy exacerbates the degenerative process of the endometrium. In older bitches or those with prolonged breeding inactivity, the accumulation of senescent endometrial tissue may create a pro-inflammatory microenvironment, characterised by infiltration of lymphocytes, plasma cells and macrophages. Recent studies also highlight the role of biofilms in chronic uterine infections. Certain pathogens, such as *Escherichia coli*, may form biofilms that protect them from host immune responses and reduce the efficacy of antimicrobial treatment, thereby perpetuating inflammation and contributing to therapeutic failure. Chronic endometritis often presents as subfertility or infertility, repeated embryonic losses, early resorption or small litter sizes. Rarely, a serosanguinous or mucoid vaginal discharge may be observed. Given the subtlety of clinical signs, diagnosis relies heavily on history and eventually complementary diagnostic examinations. Ultrasonography is a non-invasive cornerstone in the evaluation of the reproductive tract. While CEH may be visualised as a thickened, cystic endometrium, chronic endometritis alone may not yield overt ultrasonographic changes. However, increased endometrial echogenicity, slight fluid accumulation in the uterine lumen, especially during dioestrus, may be suggestive of this disease. Endometrial cytology obtained via low-volume lavage or uterine biopsy may reveal inflammatory cell infiltrates, particularly neutrophils and lymphocytes. Endometrial biopsy remains the gold standard for diagnosis, allowing histopathological grading of inflammation and identification of structural changes such as fibrosis, glandular atrophy and periglandular inflammation. Uterine swabs or lavages for aerobic and anaerobic culture are essential to identify causative pathogens and guide antimicrobial therapy. Nevertheless, due to the potential involvement of biofilm-forming bacteria or fastidious organisms, traditional cultures may yield false negatives. In such cases, molecular methods like PCR can improve diagnostic yield. Recent research has focused on the evaluation of local cytokine expression (e.g., IL-6, TNF-α, IL-1β) as potential biomarkers of chronic endometritis. Although not yet in routine clinical use, cytokine profiling may offer future diagnostic or prognostic value by reflecting the inflammatory status of the endometrium. Successful management of chronic endometritis in bitches involves addressing the underlying inflammation, eradicating infection if present, restoring uterine health and optimising future fertility. An individualised approach is crucial, particularly in valuable breeding animals. Systemic antibiotics remain a mainstay in chronic endometritis management, especially when culture and sensitivity data are available. Commonly used antibiotics include amoxicillin-clavulanate, fluoroquinolones and cephalosporins.

Treatment duration typically ranges from 10 to 21 days, depending on severity and response. However, the presence of biofilms may reduce treatment efficacy, necessitating alternative or adjunct strategies. Hormonal therapy and uterine lavage aim to modulate uterine contractility and enhance bacterial clearance. Antiprogestins (e.g., aglepristone) may be used to antagonise progesterone's inhibitory effects on uterine immunity. Aglepristone, in particular, has shown promising results in reversing progesterone-induced immunosuppression and promoting uterine clearance, without the systemic effects of PGF2α. However, repeated treatments may be necessary, and side effects should be monitored. Intrauterine lavage with sterile saline or antiseptic solutions could assist in removing inflammatory debris and biofilm, although they are quite defiant to perform. Caution is advised to avoid epithelial damage or ascending infection. Immune modulators have been studied, for example lactoferrin, a glycoprotein with antimicrobial and anti-inflammatory properties, has also been investigated as an intrauterine adjunct in other species. In cases refractory to medical management or where the uterus is severely damaged, ovariohysterectomy remains the definitive treatment. However, this option needs to be cautiously addressed with breeders, especially for high value breeding animals. The prognosis for fertility recovery depends on the extent and severity of endometrial damage, chronicity of inflammation and success in eradicating infection. Bitches diagnosed early and treated appropriately may return to fertility, especially if pregnancy is established soon after treatment to benefit from the anti-inflammatory milieu of gestation. Nevertheless, repeated episodes or long-standing chronic endometritis may lead to irreversible endometrial fibrosis and glandular dysfunction, significantly impairing conception and implantation. Regular monitoring and pre-breeding evaluations are essential and a fertility scoring should be thoroughly developed for dogs. Preventing chronic endometritis could involve reducing the number of non-conceptive cycles, promoting reproductive strategies, thus, minimising uterine contamination. Strategic breeding management may include:

- Early and accurate ovulation timing to reduce mating-related infections;
- Use of artificial insemination with proper techniques and materials;
- Post-breeding uterine evaluations in high-risk or previously affected bitches;
- Considering the use of progesterone antagonists during diestrus in non-pregnant cycles to prevent progesterone-mediated immune suppression;
- Consider the use of immune-mediators or anti-inflammatory agents in the peri-breeding period.

Additionally, breeding bitches should be evaluated annually with ultrasonography and in specific cases, cytological/histological or culture-based assessments to detect subclinical endometrial pathology. Current research in canine reproductive medicine is increasingly focusing on the molecular mechanisms of chronic endometritis, the role of uterine microbiota and the development of targeted therapies. Studies investigating endometrial gene expression, inflammatory pathways and immune cell populations aim to elucidate the pathophysiology of chronic endometritis and identify novel diagnostic biomarkers. Advanced imaging modalities, such as contrast-enhanced ultrasonography or endoscopic uterine evaluation, may offer improved visualisation and grading of endometrial pathology. Once it has been advocated that high expression of COX2 with abnormal prostaglandin generation contributes to the pathophysiology of uterine diseases, regenerative therapies, including stem cell therapy and platelet-rich plasma, are under preliminary investigation for endometrial chronic inflammation. The integration of these approaches into clinical practice requires validation through controlled studies, but holds promise for enhancing fertility outcomes in breeding bitches. Chronic endometritis in bitches remains a challenging yet increasingly recognised cause of infertility. Its diagnosis requires a high index of suspicion, particularly in bitches with unexplained reproductive failure. A thorough diagnostic work-up, including imaging, cytology, microbiology and histopathology, is essential for appropriate therapeutic planning. Management should be individualised and multimodal, aiming to resolve inflammation, eliminate infection and preserve endometrial function. Early intervention and preventive strategies are critical to maintaining reproductive health and success in breeding programs. As research continues to unravel the complexities of this condition, new diagnostic and therapeutic tools are expected to refine the management of chronic endometritis and improve long-term reproductive outcomes.

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Substances to avoid during pregnancy and lactation: Ensuring maternal and neonatal health in carnivores

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Administration of any substance during pregnancy requires specific attention due to possible modifications of pharmacokinetics associated with this physiological stage, the potential effect on pregnancy maintenance (through the induction of uterine contractions and/or luteolysis) and the transplacental passage leading to an embryofetal exposure, with possible disturbance of the organogenesis and/or differentiation processes. After parturition, the administration of any substance to the dam potentially exposes the offspring to adverse effects through milk transfer. Not only drugs prescribed by the vet practitioner, but also all substances administered by the owner on its own have to be cautiously controlled in case of pregnancy or lactation. To date, the production of an exhaustive list of at-risk molecules in canine/feline pregnancy/lactation is unreachable since data are most often lacking in these species per se. Toxicity trials on pregnant bitches and female cats are limited by funding and ethical restrictions. Drug toxicity for these species is extrapolated from data obtained in other species, namely humans, rodents and rabbits. Nevertheless, differences in placental structure and enzymatic activity (resulting into different drug

metabolisation) between species may result into differences in drug toxicity, meaning that lists available in human medicine have to be used cautiously in carnivores (1). Given the distinct pharmacological contexts—transplacental transfer during pregnancy versus transmammary transfer during lactation—safety considerations differ between the two conditions. But whereas literature provides lists of drugs with the associated risk for direct administration to neonates, none considers the risk for neonates when drugs are administered to the lactating bitch or queen, leaving neonates being exposed through milk transfer. Table 1 reports a non-exhaustive list of molecules for which the toxicity during pregnancy and/or lactation is established in at least one mammalian species (2–5). As a precaution, these drugs have to be avoided during pregnancy and/or lactation unless any life-threatening condition affects the dam. The effective risk also depends on the duration of treatment (difference between one shot administration and long-term treatment). In addition to pharmaceuticals, pregnant and lactating bitches and queens may be exposed to various non-drug substances that can impact maternal or neonatal health. These substances are usually used out of the control (and even knowledge) of the clinician. This includes for example essential oils, whose safety, either topical or systemic, is most often not evaluated. Ketone and lactone oils are known to induce abortion and oestrogen-like oils (e.g., *Salvia sclarea* and *officinalis* or *Mentha* × *piperita*) have to be avoided during pregnancy. Topical corticoids should probably be discontinued as much as possible, and especially during the first month of pregnancy. Supplements introduced into the diet during pregnancy are also to be checked, including calcium supplements (increasing the risk for eclampsia during lactation) and raw food (depending on the origin, quality, method for freezing/thawing, the risk for bacterial contamination and subsequent abortion has to be evaluated).

TABLE 1 Drugs with known or suspected toxicity after administration during pregnancy and/or lactation (2–6).

Drug class	Drug	Risks during gestation	Risks during lactation
Antibiotics	Tetracyclines	x	x
	Aminoglycosides (gentamicin, kanamycin, neomycin, streptomycin)	x	x
	Quinolones		x
	Metronidazole	First month of pregnancy	x
	Sulphonamides	First month of pregnancy	
	Trimethoprim	x	
Anti-inflammatory substances	NSAIDs	x	x
	Corticosteroids	x	
Immuno-modulators	Methotrexate	x	
	Vaccines	Live attenuated	
Antifungal	Griseofulvin	x	
	Ketoconazole, fluconazole	x	
Anticonvulsants	Phenobarbital	x	x
Anti-emetic	Maropitant	x	x
Angiotensin converting enzyme inhibitors	Enalapril, atenolol	x	
Anaesthesia/behaviour	Lithium salts	x	
	Acepromazin	x	
	Xylazin	x	
Hormonal treatments	Aglepristone	x	
	Cabergolin	x	x
	Deslorelin (long acting GnRH agonists)	x	
	Prostaglandins F2 alpha	x	
	Long acting progestins	x	
	Micronised progesterone	Potential	
	Antithyroid drugs (methimazole – thiamazole)	x	x

Apart from the substances whose negative effects are documented in pregnant/lactating bitches and female cats, toxicity for dam and offspring (pre and postnatal) for the vast majority of xenobiotics (chemical substances that are foreign to animal life, such as plant constituents, drugs, pesticides, cosmetics, flavourings, fragrances, food additives, industrial chemicals and environmental pollutants) is not well evaluated. The general principle is then to limit the administration of any substance during pregnancy and lactation, depending of the expected benefit/risk balance. The period at higher risk is the embryonic phase, because of active organogenesis (until 35 days of pregnancy). Noteworthy, any mated or inseminated female is to be considered by default as pregnant, especially before any accurate pregnancy diagnosis can be implemented (2–3 first weeks after mating). Drugs with published reproductive safety profiles (as indicated in the Summaries of Product Characteristics) have to be prioritised. If the expected benefit for the dam is significant, the administration regimen follows the lowest effective dose for the shortest duration. When appropriate, topical treatments allow to reduce systemic exposure. The calculation of drug dosages for pregnant animals is generally recommended to be based on the pre-gestational body weight of the female, particularly for drugs with a narrow therapeutic index. Indeed, gestational weight gain includes increased body fat, uterine contents and fetal weight, which do not necessarily correspond to an increased volume of distribution or altered drug clearance

in a predictable way. Control of patient response to drug administration then allows to adapt the dosage. Finally, during the course of any treatment to the dam, offspring monitoring is to be implemented, by ultrasonographic examinations during pregnancy and by comprehensive clinical examination including weight follow up in neonates. As for antibiotics, for which prudent use prevails, the use of drugs (or more generally of any xenobiotics) is not forbidden but is to be evaluated by the clinician in light of the balance between maternal health needs and fetal/neonatal safety (best treatment for the mother, less risk for the fetuses and newborns). Owners should also be made conscious about the risk associated with any substance, especially non-medicinal, in case of pregnancy or lactation of their dam.

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Three-dimensional systems for cat oocytes and follicles: Advances in culture and cryopreservation

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Three-dimensional (3D) cultures have revolutionised cell science, with a huge impact on biological and biomedical research. Compared to traditional two-dimensional (2D) systems, 3D cultures offer the advantage of being more physiologically relevant, since they mimic the spatial organisation, intercellular interactions and extracellular matrix composition of native tissues (1). Thanks to their ability to replicate in vivo-like conditions, they are useful platforms for the study of cellular behaviour, differentiation and function, and they have gained importance in several fields, including cancer research, tissue engineering, drug discovery and regenerative medicine. Although with fewer investigations, 3D systems have also been employed in cryopreservation with promising results. It is thought that 3D cryopreservation can support the precise handling of delicate cells or tissues and improve the stability of the preserved products (2). Moreover, it could provide a matrix that is directly employable for downstream applications, such as in vitro 3D culture of preserved cells, combining the benefits of both the techniques. In reproductive medicine, 3D systems can be a useful tool to deepen our knowledge on the development of reproductive cells, allowing us to study processes such as folliculogenesis, oocyte maturation, ovulation and embryo development, but they also hold promises to improve the outcomes of in vitro assisted reproductive technologies (ARTs), including standard procedures like in vitro embryo production or to aid in the development of more innovative fertility preservation strategies, such as in vitro gametogenesis. For female gametes, 3D culture environments are particularly useful to prevent the unnatural adhesion of cumulus cells to the substrate. In contrast, traditional 2D culture systems (e.g., drops of medium in Petri or multi-well dishes) disrupt communication between somatic and germinal compartments and cell-to-cell orientation and alter the diffusion of paracrine signals, thereby affecting the polarity and secretions of cumulus-oocyte complexes (COCs) and cumulus cells (3–5). After the first application on murine gametes (6), 3D systems were applied on both oocytes and follicles. Beyond preserving cellular structures, 3D systems allow follicular and oocyte growth, granulosa cell proliferation and development of oocytes capable of producing viable progeny (3, 7). An enhancement of in vitro outcomes could be especially beneficial for domestic cat oocytes, since in vitro maturation (IVM) and embryo development rates are still low, especially if compared to other species (8). Maturation rates can reach approximately 60%, but only around half of the cleaved embryos become a morula or a blastocyst (9). Production of embryos is even more challenging if cryopreserved gametes are used, since maturation and cleavage rates drop dramatically, with sporadic blastocyst formation in vitro (10). Moreover, successful in vitro growth of gametes, that would allow the development of ovarian follicles at early stages, especially the abundant primordial and primary ones, into fertilisable oocytes, would allow a significant increase in the number of potentially usable gametes. Development and optimisation of these protocols in the domestic cat would be of paramount importance for a better use of feline female germplasm in biodiversity preservation and conservation programs, since the techniques developed in this species could be adapted and transferred to related wild endangered species (11). This extended abstract explores the advancements in the use of 3D systems for culture and cryopreservation of oocytes and follicles, focusing on those applied in the domestic cat model. Several biomaterials have shown their suitability for reproductive cells. Among them, alginate is frequently chosen to support follicle growth and promote oocyte survival and maturation (12), as well as being an excellent candidate for 3D cryopreservation (2). Indeed, most of the work on 3D culture of cat oocytes has been done with alginate matrices. Alginate is a natural anionic polymer derived from alginic acid, a component of cell walls and intercellular spaces of brown algae from the genus Laminaria. Alginate hydrogels provide stable and reproducible culture systems that do not interfere with cellular functions (13) and are biocompatible, cost-effective and have low toxicity (14). In the last decade, alginate was often used in our laboratory for domestic carnivore oocytes, especially low competence ones, which could particularly benefit from enriched, in vivo-like, culture conditions. At first, we studied the effect of group culture of cat oocytes in alginate capsules. Denuded oocytes showed an increase in viability when co-cultured with COCs in 3D microcapsules, although no variations were observed in meiosis resumption (15, 16), while the embryo development of companion COCs benefitted from the use of this system (15). When a similar system was used for vitrified cat oocytes, whose development might be hampered by cryopreservation injuries, the use of the 3D system alone during IVM was not enough to improve the developmental outcomes of vitrified oocytes, but its combination with companion fresh COCs seemed beneficial for blastocyst formation (17). Following this finding, the creation of 3D follicle-like structures, consisting of granulosa cells encapsulated in alginate microcapsules,

was also attempted, with the creation of a suitable microenvironment for the IVM of vitrified cat oocytes that gave similar outcomes to IVM performed with 2D granulosa cells monolayers (18).

More recently, single encapsulation of cat fresh oocytes was experimented in smaller beads (around 1 mm diameter) formed with different concentrations of alginate and a different encapsulation technique, and IVM in 1% alginate significantly increased full maturation rates (19), allowing us to proceed in the optimisation of 3D IVM of feline oocytes. As for oocytes, alginate is often the biomaterial of choice for the culture of isolated follicles. After isolation of the follicles from the surrounding tissue, preferably with mechanical methods (20), follicles are encapsulated in alginate and cultured in suitable growing medium. In cats, hydrogel formed with 0.5% alginate proved to be the most suitable for the culture of two layer preantral follicles (21). Follicles cultured in 2D were reported to lose their shape, with granulosa cells migrating towards the follicle periphery and general flattening of the structure on the culture dish, while 3D cultured follicles grew in diameter and developed into multilayer secondary follicles (21). The same concentration of alginate was used in other studies on cat follicles (secondary and early antral), where different additives were tested to stimulate follicular growth. An increase in diameter was observed with eCG in follicles at different stages of development (22), and similar effects were observed with insulin (23). Short hypertonic exposure allowed greater growth of cultured follicles, probably also due to the longer culture time, and led to the isolation of meiotically competent gametes that could achieve full maturation in vitro (24). When follicles at earlier stages of development, such as primordial and primary, need to be cultured, different approaches are used. In situ culture, or culture of ovarian tissue fragments, where follicles are embedded in the ovarian cortex, has been experimented in several species, also using 3D matrices. Although encapsulation of whole fragments inside a hydrogel is possible (25), most of the studies are based on the so-called air-liquid interface (ALI). Air-liquid interface culture is an advanced technique often used to grow and maintain cells that are cultured at the interface between air and a liquid. This setup mimics physiological conditions and boosts the interaction of the cells with both the medium components and the gases, as well as maintaining the appropriate matrix stiffness and cell polarity (26). Fresh and cryopreserved fragments of cat ovarian cortex have been cultured in ALI on agarose gels that better supported follicle viability compared to the 2D control (27). Most follicles remained at the primordial stage during culture, with low expression of proliferative markers (28), but supplementation with growth factors such as epidermal growth factor or antioxidants such as melatonin might improve survival (29) or stimulate follicular activation (30), needed for progression to more advanced developmental stages. The literature suggests that a multi-step approach, where culture of ovarian fragments is followed by isolation of preantral follicles and their in vitro development until collection, growth and IVM of enclosed COCs (31), might be beneficial, but such combination remains to be tested in the domestic cat. Although 3D cryopreservation has previously been investigated for somatic cells and ovarian follicles, our studies on this topic with cat oocytes started recently, and this is its first application on mammalian oocytes. Despite never being tested in the domestic cat, so far, both slow freezing and vitrification were successfully used for 3D cryopreservation of preantral follicles. Viability and ultrastructure of human vitrified follicles were maintained after warming, and growth along in vitro culture was observed without differences from fresh controls (32). Similar findings were also reported in human follicles undergoing slow freezing with dimethyl sulfoxide (33). The structure of these preantral follicles, containing a small oocyte surrounded by one or few layers of cumulus cells, might somehow resemble that of the COCs, even if the fully-grown oocyte is likely to present more challenges during cryopreservation due to its size, the higher water content and the abundance of cumulus cells. Thus, the structure of the 3D matrix employed for cryopreservation, as well as its porosity, is crucial. Even though it is reported that alginate has high permeability, it is also known that it can be influenced by hydrogel concentration and gelation (34), and this must be considered for permeation of CPAs, required step for successful cryopreservation. Starting from successful vitrification of alginate capsules without encapsulated cells (19), our group is currently investigating the 3D vitrification of cat COCs. After encapsulation and exposure to CPAs solutions, capsule-enclosed COCs are loaded on a Cryotop and plunged into liquid nitrogen. The influence of different exposure times is being optimised to preserve the morphological and functional integrity of the oocytes. While standard exposure times to CPAs resulted in cell death in all preserved oocytes, increases in exposure times up to 2.5-fold gave better results. Although post-warming viability was lower than in standard vitrified oocytes, full maturation rates of viable oocytes were significantly higher in 3D vitrified oocytes than in conventionally vitrified ones, and no differences were observed in actin distribution, which is crucial for both oocyte maturation and further embryo development. This will be the focus of the next experiments, aiming to assess whether the enhanced maturational competence of viable 3D-preserved oocytes will be matched by improvements in embryo development and quality. The use of 3D systems might be an effective strategy for the in vitro culture and cryopreservation of cat female gametes. While some beneficial effects were already proved, such as improvements in the maturation potential, further investigations are needed to optimise encapsulation, culture and cryoprotectant exposure and to tailor the protocols to the species-specific requirements of cat gametes. This could allow better use of female germplasm in feline species and contribute to wild felid gamete rescue.

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When dermatology meets reproduction

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Dermatology is a relatively autonomous and independent specialty. However, dermatological conditions can sometimes be a consequence of more systemic or metabolic diseases, often related to hormonal imbalances. Although the endocrinopathies most frequently associated with altered skin homeostasis involve thyroid hormones and cortisol, sex hormones also exert significant effects on the skin. In this lecture, we will explore conditions related to sex hormones—particularly when they are present in excess—as well as dermatological diseases that affect the genital area. We will begin by examining the physiological role of sex hormones in the skin, then review the main dermatological disorders linked to your area of expertise, and finally, we will look at the current evidence regarding the involvement of sex hormones in Alopecia X. What is the role of sexual hormones on the skin? We know that in the dog there are oestrogen and androgen receptors in the skin at different levels: keratinocytes, hair follicles (in particular in the dermal papilla), sebaceous glands, fibroblasts, etc. However, the distribution of these receptors is not uniform across all skin regions, and their sensitivity and biological response upon activation can vary significantly, as has been well demonstrated in humans. Under physiological conditions, the role of sex hormones in maintaining normal canine skin and hair coat appears to be minimal. As stated in standard references of small animal dermatology: "The vast majority of adrenalectomised and ovariectomised dogs maintained on only mineralocorticoids have normal skin and haircoats, indicating that oestrogens or any other sex steroids are not necessary for normal skin and haircoats in dogs." However, it is well known by breeders that some dogs develop coat changes after castration, especially in specific breeds like Irish Setters. The coat of these animals may become "fluffy". Unfortunately, peer-reviewed information about this condition is scarce. What becomes clear is that the excess of sexual hormones has severe consequences on cutaneous homeostasis. Oestrogen for example, suppresses the anagen phase of the hair follicle, promotes telogen transition and exerts an atrophic effect on sebaceous glands. In intact male dogs, hyperestrogenism is most commonly associated with Sertoli cell tumours. These tumours are more frequently observed in cryptorchid dogs, with a higher prevalence in middle-aged to older individuals. In female dogs, although less frequently observed, hyperestrogenism may result from ovarian follicular cysts or granulosa cell tumours, both of which can cause persistent oestrogen secretion. Although the cutaneous signs of hyperestrogenism overlap with those observed in other endocrinopathies like hyperadrenocorticism and hypothyroidism, there exist some particularities to this condition:

- Symmetrical, non-inflammatory truncal alopecia, often beginning on the perineum and flanks and progressing cranially, secondary to progressive telogen transition of the hair follicles and inhibition of anagen phase.
- Macular hypermelanosis: it is common to have darkening of the skin in alopecic dogs (as effect of sun exposure). However, what appears to be quite characteristic of hyperestrogenism is the fact that the hyperpigmentation appears more commonly in the inguinal and perianal region and forms clearly defined macules.
- In male dogs, a suggestive sign of hyperestrogenism is linear preputial dermatosis, characterised by a well-demarcated area of hyperpigmentation and erythema along the prepuce (from scrotum to prepuce opening).
- Atrophy of the penile and testicular tissues, gynecomastia and pendulous prepuce may accompany the dermatological changes.
- Enlargement of the vulva and persistent oestrus signs may be observed in bitches.
- Chronic oestrogen exposure may also impair the skin barrier and immune defences, predisposing dogs to secondary pyoderma or *Malassezia* overgrowth (although in my experience this is not frequently observed).

In addition to dermatological changes, systemic signs such as bone marrow suppression must be investigated, as they can be life-threatening. In most cases, cutaneous lesions regress gradually following removal of the oestrogen source. However, owners should be informed that hair regrowth is slow and it may easily take 3–4 months to see significant changes. An increasingly recognised cause of hyperestrogenism is accidental dermal exposure to topical oestrogen-containing creams used by their owners (e.g., for menopausal hormone therapy). Affected dogs, often small breeds, may present with non-inflammatory alopecia (mainly in ventral areas of the body, most commonly exposed to owner skin while being on their arms). The diagnosis relies on asking the owner this uncomfortable question about topical oestrogen use. Resolution typically occurs following cessation of the owner's topical hormone use or ensuring preventive contact measures. Although the scrotum can be affected in different skin conditions, it is rarely affected solely. When only the scrotum presents with skin lesions, the most common disease is contact dermatitis (whereas it is irritative or allergic reaction). Scrotal skin is thin and with few hair follicles and is therefore very susceptible to this disease. Both primary and secondary lesions (erythema and erosions, ulcers, crusts, hyperpigmentation, depigmentation, respectively), associated with pruritus are

noted, whilst secondary complicating factors such as bacterial folliculitis or yeast dermatitis (*Malassezia*) may be present, both of which are frequently reported in the literature. The diagnosis of contact dermatitis is made by results of avoidance and/or provocation tests although this is rarely performed for obvious reasons. The author has seen many cases of scrotal dermatitis at the University Hospital on animals that are hospitalised and whose scrotum is in contact with on the typical blue or green pads used in the cages. It is not known whether the scrotal disease is the consequence of the contact with the coloured pad or with the products that are used for cleaning it. Treatment includes the use of a booster collar to avoid excessive licking. Mucocutaneous lupus erythematosus is a form of cutaneous lupus erythematosus in which German Shepherds, their crosses and Belgian Shepherds are notably overrepresented in the literature. The condition shows a predisposition in females, with lesions commonly appearing around a median age of six years. Clinically, lesions are mostly located on or around the mucosa of the anogenital region—particularly the vulva or prepuce—but can also involve the lips, eyelids and nasal planum. The lesions are typically ulcerative and depigmented, yet they tend not to result in scarring. Affected dogs may exhibit pruritus or discomfort and are often observed licking the affected sites persistently. The differential diagnoses include mucocutaneous pyoderma, mucocutaneous junction pemphigoid and erythema multiforme. Careful histopathological and immunohistochemical evaluation is essential to distinguish between these conditions. It is recommended to administer systemic antibiotics for at least two weeks prior to skin biopsies to control any local bacterial infection and limit the inflammation caused by it on the skin. Skin biopsy and histology to confirm the diagnosis of lupus is recommended.

Lesions associated with mucocutaneous lupus erythematosus usually respond well to immunosuppressive doses of glucocorticoids, with clinical remission frequently achieved within the first month of treatment. However, due to the tendency for relapse upon tapering corticosteroids, adjunctive immunomodulatory therapy is often required to maintain long-term disease control. To date, there is insufficient evidence to determine the most effective second-line immunomodulatory agent for this form of lupus. Treatment choices are therefore typically guided by clinician's experience, drug availability and individual patient response. Agents such as azathioprine, cyclosporine, mycophenolate mofetil and hydroxychloroquine have been employed anecdotally, but prospective studies evaluating their efficacy in mucocutaneous lupus erythematosus are lacking. Another disease called stud tail, also known as tail gland hyperplasia, is a condition primarily affecting intact male cats and, less commonly, male dogs. It is characterised by sebaceous gland hypertrophy located in the supracaudal area, typically at the dorsal base of the tail. This region contains a high concentration of hormonally responsive sebaceous glands, which are particularly sensitive to androgen stimulation. Clinically, affected animals present with greasy, waxy exudate, comedones, alopecia and occasionally secondary bacterial folliculitis in the affected area. While more common in intact males, neutered individuals may also be affected, suggesting that other factors, including individual gland sensitivity or androgen-independent mechanisms, may play a role. Treatment typically involves castration, which often leads to resolution of the condition, alongside topical antiseborrheic therapy or systemic antibiotics if secondary infection is present. Another dermatological problem is Alopecia X: it is a non-inflammatory hair cycle disorder affecting most commonly Pomeranians. Different disease names were used for Alopecia X in the past, such as adult-onset hyposomatotropism, growth hormone-responsive alopecia, pseudo-Cushing's disease, castration responsive alopecia, biopsy-responsive alopecia, adrenal hyperplasia-like syndrome, all reflecting the yet unknown pathomechanism of the disease. The strong predisposition of the disease for breeds with a fluffy undercoat, together with pedigree analysis of affected dogs, and the onset of the disease at a relatively young age suggest a hereditary background. Initially an adrenal steroid hormone imbalance was suggested as the underlying cause, similar to the congenital adrenal hyperplasia-like syndrome in humans, caused by a mutation in the *CYP21A2* affecting the steroid 21-hydroxylase. However, a genetic variant in this gene as the cause for Alopecia X in dogs was excluded and to date the mode of inheritance and the underlying pathomechanism of the impaired hair growth remain to be elucidated. Clinically the dogs develop truncal alopecia and hyperpigmentation of the skin. Systemic illness is not associated with this disease. Histologically, kenogen and telogen hair follicles predominate, whereas anagen follicles are sparse. Although this disease is considered by many as a “cosmetic disease”, the truth is that many of this animals develop secondary bacterial infection as a consequence of skin exposure to the environment and the animals are more sensitive to low temperatures. Multiple therapies, including melatonin, mitotane, trilostane, androgens, microneedling, laser therapy and surgical castration have been used with variable results in different dog breeds and dogs of varying sexual status. Cost of medication and laboratory monitoring, safety, availability and lack of efficacy can be obstacles to using these treatments. One of the most compelling arguments implicating sex hormones in Alopecia X is the documented hair regrowth observed following gonadectomy. In intact male dogs, surgical castration or chemical sterilisation using gonadotropin-releasing hormone (GnRH) agonists, such as deslorelin implants, has resulted in partial to complete coat regrowth in a substantial proportion of cases. This response supports a hypothesis in which circulating sex steroids—or their altered metabolism—play a contributory role in the disease process. Furthermore, the use of deslorelin implants can be also of interest in neutered animals showing also hair regrowth after the initiation of the therapy (Lucilene Bernardi, Dip.ACVD, Montreal University, personal communication). The mechanism behind this phenomenon is not yet fully elucidated. It is hypothesised that in affected dogs, hair follicles may exhibit increased sensitivity to sex steroids, or there may be a dysregulation in the enzymes involved in local cutaneous steroid metabolism. Supporting this, one study demonstrated altered expression of steroidogenic enzymes, including 3β-hydroxysteroid dehydrogenase and aromatase, in the skin of dogs with Alopecia X, compared to healthy controls. These findings suggest that the skin may act as a peripheral endocrine organ, with local dysregulation of sex hormone conversion contributing to follicular cycle arrest. Furthermore, sex hormone receptors, including androgen and oestrogen receptors, are present in canine hair follicles. Aberrant expression or activity of these receptors might also influence the anagen–telogen transition, leading to hair follicle miniaturisation or prolonged telogen phase. However, the exact hormonal profiles of dogs with Alopecia X do not consistently show elevations in serum sex

hormones, implying that the pathogenesis may be driven more by local cutaneous metabolism rather than systemic endocrine dysfunction.

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"To neuter or not to neuter? That is the question"... Dealing with "aggression" diagnosis and other behavioural problems

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Aggression is a natural behaviour that serves as a defence mechanism, enabling an animal to protect itself when it feels threatened or has no means of escape (1–3). This response can be essential for the animal's survival, depending on the context in which it occurs (2). When an animal resorts to an attempt at repulsion ("aggression"), this is its way of responding to a situation that it interprets, whether correctly or not, as a potential threat (3). As a result, many veterinary behavioural specialists now prefer the term "repulsion" over "aggression." The animal's primary goal is to increase its safety distance, using signals to prevent escalation. However, if these signals fail, the animal may resort to more intense defensive behaviours, ultimately displaying what is commonly labelled as aggression—despite its efforts to avoid doing so. Nevertheless, a dog may exhibit aggression in situations deemed inappropriate, with varying degrees of severity depending on the type of behaviour displayed and the response of the victim (2, 4). The classification of aggression is subjective, due the fact there is no standard method for categorising it (2). Different authors use their own terminology, varying in the number of categories they define. Some classify aggression based on the target—such as a caregiver, a stranger, a familiar person or another animal—while others categorise it according to the situation that triggered the behaviour (3, 5). For this reason, aggression should be viewed as a sign or symptom that requires investigation rather than simply being treated as an isolated issue. The goal is not just to address the symptom but to understand and treat its underlying cause. Therefore, accurately addressing the problem requires consideration of the context, the animal's motivation (2) and the emotional state driving the behaviour. "Aggression" is multifactorial, influenced by various factors such as breed (genetics), gender and management practices—including socialisation, physical and mental health, learning experiences, family environment and surroundings (2, 6). Recent research found significant differences in aggressive behaviour towards other dogs among breeds, such as "Huskies" type of dogs and Bulldogs (7). Repulsion ("aggression") is not limited to biting; it also includes various threat displays that may precede or occur independently of a bite, such as growling, barking, snapping and intense staring (3, 6, 8). However, the media often misuses the term "aggression", creating the misconception that it always refers to violent behaviour intended to cause harm (3). In reality, aggression is a significant and complex public health concern, with numerous reports worldwide of dogs attacking people or animals, whether family members or strangers. Victims of such incidents may endure physical and psychological suffering, as well as financial burdens, including medical expenses (2). Meanwhile, aggressive animals often face physical or verbal punishment, and while some receive care from veterinarians specialised in Behavioral Medicine, the majority are abandoned or euthanised (3). The possible impact of orchiectomy and ovariohysterectomy (OVH) on aggressive behaviour in dogs has been a topic of debate for many years. However, few studies have been conducted solely to evaluate this relationship, leading to numerous hypotheses from various authors, but few definitive conclusions on this subject. In females, the main behaviours that are inhibited are associated with hormonal changes in the oestrous cycle (9), while in males there is mainly a loss of interest in the opposite sex (10). Furthermore, research has shown that surgery can reduce behaviours such as roaming, territorial marking and mounting. However, individual differences must be considered as not all animals show the same reduction in behaviours post-gonadectomy, particularly territorial marking and mounting. The success of this type of approach does not appear to be related to age or pre-gonadectomy experiences, leaving the reasons behind these individual variations unclear (11, 12). When it comes to the neurology of aggression, "it is generally assumed that aggressive behaviours are regulated by testosterone. However, after a century of research it has become clear that it is a complex network of neuroendocrine pathways that regulate aggressive behaviours" (13). This network includes steroid and peptide hormones that influence aggression based on seasonal, reproductive and social factors. Some key agents include testosterone, oestrogens, dehydroepiandrosterone, glucocorticoids, serotonin, noradrenaline, oxytocin and prolactin (13–15). Aggressive behaviour has been the subject of several neuroendocrine studies, mainly because it varies with sex and reproductive season. It is now known that in most species, males are more aggressive than females and that aggression is often greater during the reproductive season (13). It is important to note that most studies were carried out on rats (*Rattus norvegicus*), which are generally docile, and on birds, so some caution is needed when extrapolating these conclusions to dogs (13). Gonadectomy is sometimes recommended as a treatment for canine aggression. However, its effect has not yet been proven, with some studies even contradicting this practice. In females specifically, O'Farrell and Peachey (1990) (16) found that OVH does not help control aggression. Instead, they observed that gonadectomy is associated with an increased aggression toward family members, especially in females spayed before one year of age who already exhibited aggressive tendencies. Similarly, Kim et al. (2006) (17) noted that after OVH, female German Shepherds became more reactive, potentially predisposing them to aggressive

situations. Pérez-Guisado and Muñoz-Serrano (2009) (18) also found spayed females become more aggressive, although males appeared to become less so. Messam et al. (2008) (19), in a study that focused on dogs that had bitten people, found that intact animals were more likely to bite and therefore to show aggression (RR of 2.56 and 3.22, intact males and females, respectively). In turn, Bennet and Rohlf (2007) (20) did not detect any differences associated with reproductive status. Caution is needed when interpreting these results. Many studies report higher numbers of sterilised animals displaying aggression, but this may be due to the practice of neutering aggressive dogs as a behavioural management strategy and does not mean that gonadectomy increases aggression. In a study that evaluated problematic behaviours in dogs, sterilised animals were more likely to growl and bite. However, caregivers were not asked about the reason for the surgery, so it is not possible to establish a causal relationship between these two variables (4). Podberscek and Serpell (1996) (21) further demonstrated this fact: while their initial data suggested gonadectomy increased aggression, after removing cases where animals had been neutered due to aggression, they eliminated this effect in males, though it remained in females. It is important to remember that castration drastically reduces the concentrations of circulating reproductive hormones; however, as previously mentioned, these hormones are not the sole factors influencing aggressive behaviour. Therefore, neutering should not be expected to reduce aggressive behaviour in all dogs, nor to eliminate it completely (22). Moreover, there are still concerns regarding the neutering of dogs exhibiting fear or anxiety, particularly when such emotional states manifest as aggressive behaviour (i.e., fear-induced aggression). Several studies suggest that neutering, in both male and female dogs, may be linked to an increase in fear- and anxiety-related behaviours. Analysis of a large sample of pet dogs using the Canine Behavioral Assessment and Research Questionnaire (C-BARQ) has shown that gonadectomy is associated with a higher likelihood of developing specific fears, including fear of noises, unfamiliar surfaces and heights, as well as increased reactivity to social stimuli such as unfamiliar people and other dogs (23). These behavioural changes appear to be particularly pronounced when neutering occurs early, especially before six months of age (23, 24). Similar findings have been reported in Vizsla dogs, where neutering has been associated with a higher incidence of phobias, fear-related aggression and separation-related problems (25). More recent research in German Shepherds further supports the idea that early neutering may increase susceptibility to anxiety disorders (26). Therefore, current evidence highlights the importance of carefully weighing the potential risk of exacerbating fear- and anxiety-related behaviours when considering neutering in dogs. In conclusion, before choosing castration for behavioural reasons, it is advisable to carry out a thorough emotional diagnosis with a veterinarian specialist in behavioural medicine, to evaluate the advantages of this procedure. One of the main problems is that we can create false expectations that castration alone will resolve a behavioural issue. If, after the emotional evaluation, castration appears to offer potential benefits, a trial using chemical castration with a hormonal implant may be considered. As Hart and colleagues concluded in their editorial for Frontiers in Veterinary Science (2024) (27), the decision to spay or neuter dogs has become increasingly complex as more knowledge arises on interactions with diseases, behaviour, welfare and overall canine health. The current approach emphasises evaluating the dog's lifestyle and individual circumstances to determine whether, and when, neutering is the right option (27).

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AI and vets in reproduction

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Artificial intelligence (AI) is transforming the landscape of veterinary medicine, including the field of small animal reproduction. From clinical diagnostics to communication, documentation and even personal productivity, AI tools are becoming increasingly integrated into the daily workflows of veterinarians. This article provides a broad, practical overview of AI and explores how it can assist veterinary specialists in canine and feline reproduction, both professionally and personally. What is AI? Artificial intelligence refers to the simulation of human intelligence by machines programmed to perform tasks such as reasoning, learning, problem-solving and decision-making. Machine learning (ML), a subset of AI, enables computers to detect patterns in large datasets and make predictions based on experience. Deep learning

(DL), a further refinement, is especially useful for interpreting complex data such as medical images. Generative AI tools, powered by large language models (LLMs), can produce human-like text, summarise documents and generate content based on short prompts. However, it is important to recognise that these models do not "think"; they simulate understanding through statistical prediction. This limitation is often described as the "black box" nature of AI—impressive performance without transparent reasoning. Because it generates outputs based on data and probability, it is powerful, but also fallible. Veterinarians working in canine and feline reproduction deal with complex decision-making processes involving timing, diagnostics and communication. AI can offer support in several areas:

- Clinical Decision Support: AI algorithms can help interpret hormone assays, cytology or ultrasound images, enhancing the precision of ovulation timing and pregnancy diagnosis.
- Cycle and Data Tracking: Wearable devices and apps integrated with AI can monitor activity, behaviour and temperature, assisting in oestrus detection.
- Semen Analysis: AI image analysis tools are increasingly capable of evaluating sperm morphology and motility with speed and consistency.
- Predictive Models: ML models have been developed to forecast outcomes such as whelping success, neonatal survival or disease risk based on clinical and historical data.
- Documentation: Voice-to-text tools like Whisper or Talkatoo enable real-time transcription of clinical notes, saving time and improving accuracy.

Beyond clinical diagnostics, AI can streamline practice operations and improve professional efficiency and communication:

- Client Interaction: LLM can generate personalised discharge instructions, appointment reminders or breeding recommendations. Translation tools facilitate communication with international clients. Chatbots and Virtual Assistants can answer common breeder questions, provide scheduling support and triage client concerns.
- Practice Management: AI tools can assist with inventory forecasting, billing optimisation and appointment scheduling based on prior patterns.
- Literature Summarisation: Chatbots trained on academic content (e.g., ChatGPT, ScholarAI) can help digest the latest research, generate summaries and even suggest exam questions.

AI can also improve the veterinarian's personal life:

- Task Management: AI-powered planners and note-taking tools help organise daily routines and manage complex schedules.
- Travel and Communication: AI-assisted travel planning and live translation break down barriers in global veterinary engagement.
- Lifelong Learning: Veterinarians can use AI to translate articles, summarise texts or generate quizzes for self-assessment.
- Mental Health and Well-being: AI-guided meditation, relaxation apps, health tracking systems and digital assistants can support stress reduction and work-life balance.

AI in veterinary medicine raises essential ethical questions:

- Transparency: AI outputs should be explainable. Veterinary professionals must understand and verify AI-generated results.
- Privacy and Data Security: Protecting client and patient data is crucial. Ensure compliance with data protection laws.
- Bias and Reliability: AI models must be trained on diverse datasets to avoid biased or inaccurate outcomes.
- Accountability: Final clinical responsibility always lies with the veterinarian, not the algorithm. AI supports, but does not replace, the veterinarian's expertise and accountability.

The use of AI in reproduction-specific tools—such as oestrus prediction models, semen shipping logistics and neonatal risk calculators—is expected to grow rapidly. The future will likely bring more integrated platforms that combine diagnostics, case tracking and client communication into a single AI-enhanced interface. As leaders in their specialty, theriogenologists have a unique opportunity to guide the responsible adoption of AI tools in small animal reproduction. This includes collaborating with developers, participating in validation studies and integrating AI literacy into educational programs. AI is not a replacement for clinical expertise—it is a powerful support tool. Used thoughtfully, it can enhance diagnostic accuracy, improve client satisfaction and ease the administrative burdens that often weigh on veterinary professionals. The most effective use of AI in reproduction will come from a hybrid approach: skilled human judgment supported by intelligent digital assistance. Veterinarians who embrace AI with curiosity, critical thinking and ethical mindfulness will be best positioned to thrive in this evolving landscape. Below, we list an AI toolkit for theriogenologists, with examples of practical tools to support work in canine and feline reproduction. List is not comprehensive and was created on 31 March 2025.

Clinical applications:

Use case	AI tools/examples	Notes
Ovulation timing	Predictive models, cycle tracking apps	Analyses hormone trends, behaviour patterns
Ultrasound interpretation	Image-recognition software (e.g., Vetology)	Early pregnancy detection, abnormalities
Semen analysis	AI plug-ins for motility/morphology, AI slide scan	Smartphone-compatible microscope kits
Oestrus detection	Wearable devices + AI monitoring	Detects changes in temperature/activity/behaviour
Neonatal risk scoring	Custom ML models (clinic/research built)	Combine APGAR, birth weight, litter data

Documentation & Workflow:

Use case	AI tools/examples	Notes
Voice transcription	Whisper, Talkatoo, Speke	Dictate examinations, histories or discharges

Use case	AI tools/examples	Notes
Case summaries	ChatGPT, ScribeNote, ScribbleVet	Draft SOAP notes or breeding reports
Client Instructions	ChatGPT, Docus.ai	Generate customised post-op or breeding guidance

Client communication:

Use case	AI tools/examples	Notes
Chatbots for breeders	Tars, Intercom + ChatGPT plugins	Automate FAQs about breeding cycles, timing, care
Language translation	DeepL, Google Translate	Communicate with international clients
Educational content	Gamma, Canva + ChatGPT	AI-generated breeder handouts or slide-decks
Podcasts	NotebookLM, Monica AI	Analyses text or audio input and generates podcast-style audio

Practice management & efficiency:

Use case	AI tools/examples	Notes
Inventory forecasting	SmartVet, Vetstoria AI tools	Analyses usage to plan for hormones, supplies
Billing/code suggestions	Shepherd, Digital AI tools	Detects missing charges, suggests service codes
Emai prioritisation	Gmail AI, Microsoft Copilot Apple Mail	Helps filter breeder or urgent client messages

Learning & personal productivity:

Use case	AI tools/examples	Notes
Literature summaries	ChatGPT, ScholarAI, Elicit	Summarises new research, generates Q&A
Translation and reading	DeepL, ChatPDF, PDF GPT tools	Read papers in any language, extract insights
Task and time management	Notion AI, Motion, Reclaim.ai	Organise your day, automate routines

Feline reproduction: What do we know and what we don't know yet?

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It is common place to say that cats have become the animals of the 21st century. Cats are increasingly popular in European households. In France, for example, a survey in 2024 revealed that 39% of families owned at least one cat and that the number of cats as pets in the country was 16.6 million, compared with just 9.9 million dogs (1). In addition, the number of pet cats has been rising steadily for over 20 years, while the number of dogs has stagnated or even decreased. Similarly, the breeding of pedigree cats—for a long time mainly popular in Central and Eastern Europe—is developing also in Western Europe, generating specific expectations from breeders regarding reproduction (management of infertility cases, artificial insemination, housing advice, etc.). Veterinarians involved in small animal reproduction have to adapt to these new and specific needs.

On the other hand, free-ranging domestic cats, from owned pets to feral cats, impact bio-diversity through predation, fear effects, competition, disease and hybridisation (2). It is vital and urgent to find and develop reliable techniques for controlling the reproduction of stray cat populations. This has an ethical impact, because, unfortunately, the number of cats abandoned in rescue shelters is still very high. However, knowledge of feline reproductive biology and pathology has been slow to emerge. For a long time, in veterinary medicine publications, the cat's specific features were relegated to the end of articles or chapters, and compared to the dog as if the cat were a « little dog ». In the proceedings of the first international symposium on canine and feline reproduction, held in Dublin in July 1988 (printed in the J. Reprod. Fertil. Suppl.39 in 1989), there were only 4 articles specifically dedicated to cats (out of a total of 42), plus two others devoted both to dogs and to cats. In the comprehensive review book written by Shirley D. Johnston, Margaret V. Root-Kustritz and Patricia N.S. Olson and published in 2001 (3), 387 pages are devoted to the dog, while 159 pages deal with feline reproduction, which is nevertheless a notable advancement. It was only in 2022 that Aime K. Johnson and Michelle A. Kutzler edited a book for veterinarians, devoted entirely to feline reproduction (4). Unfortunately, there is still a lack of in-depth data in many areas of feline reproduction, whether it be the control of reproduction, the physiology of reproduction, which is still surprisingly poorly understood, the causes of infertility or pregnancy arrest, specific genital pathologies or many other areas. The challenge of controlling feline reproduction is that we are dealing with two very different populations: stray cats and pet cats. The objectives are very different in these two cases, and so are the solutions to be found. As far as feral cats are concerned, gonadectomy (castration of males and ovariectomy of females) is very time-consuming and has unfortunately shown its limitations, with rather limited effects on reducing the number of stray cats. How best to control this population is controversial and has ranged from culling, relocation and « trap neuter return » (TNR) methods. Data support the success of TNR in reducing cat populations, but to have a large impact it will have to be adopted on a far greater scale than is currently practised (5). Some authors claim that better results in terms of reduction of population may be obtained by Trap-Vasectomy/Hysterectomy-Return (TVHR) (6). Surgical epididymectomy has been introduced as a new and potentially effective technique, but we do not yet have enough experience to judge (7). From a medical point of view, contraceptive vaccines offer hope, but their duration of action in the field is still limited (8). In one study, one fourth of vaccinated

queens did not cycle during 5 years (9). Recently, gene therapy has brought new hope for the future of reproductive control in cat populations, but research must continue and we are still a long way from large-scale use (10). While progress has been made in controlling the fertility of male pet cats, in particular with the use of subcutaneous implants of GnRH agonists, when it comes to controlling the reproduction of female pet cats, there is still a long way to go. It is highly paradoxical that the only in-label molecules are those developed in the 1970s, progestogens derived from 17OH-progesterone. To counter their potential undesirable effects on health, the use of much lower doses than traditionally recommended has been advocated, but there is not yet enough hindsight to confirm the safety of this practice (11). Other progestins occasionally used in cats, such as altrenogest, levonorgestrel or etonorgestrel, could have fewer side effects, but only very basic information is available (12). Deslorelin subcutaneous implants are not devoid of any secondary effects in queens; they are commonly used, but off-label. There is some hope with melatonin, but it is not clear why some cats respond better than others and why there seems to be reduced efficacy with repeated administration (13). Certain ideas persist in veterinary education, such as the fact that female cats are induced ovulators, as a result of vaginal penetration. In a recent study, incidence of spontaneous ovulation in queens was consistently >30%, tending to show that cats should not be considered strictly induced ovulators, but as a species in which ovulation can be either spontaneous or induced (14). The conditions leading to these spontaneous ovulations are still poorly understood. There may be breed effect, as oriental cats are supposed to ovulate spontaneously more often, which could explain the high incidence of pyometra in oriental cats (15). Unfortunately, the physiology of reproduction in cats has mainly been studied in domestic shorthair cats, and breed-specific features have received little attention so far, which limits helping breeders effectively. Another as yet little-studied specificity is the endocrine role of the placenta during gestation in the queen (16). In some cats, it seems to be sufficient at the end of gestation to maintain gestation until term even after ovariectomy (17). But why in some queens and not in others? Could this placental hormonal supply also explain, in this species, the rather lower efficacy of late-term termination of pregnancy using aglepristone (18)? There is also the myth of superfetation, which is the simultaneous presence of offspring of greatly differing gestational age in the pregnant uterus. It never has been definitively demonstrated to occur in domestic cats. Although it is difficult to conceive how it is possible physiologically, some cat breeders claim to have encountered this situation (19). Purebred cat breeding is growing throughout the Western world, and cat breeders, faced with fertility problems, expect their veterinarian to have the same expertise, knowledge and understanding of the cause of problems as they do for purebred dogs. Unfortunately, the causes of feline infertility are not well-known (20). In this respect, uterine problems may well be among the major causes of infertility in pedigree cats. Sub-clinical endometritis has been demonstrated to play a role in infertility or pregnancy arrest in dogs, but it has been poorly studied in cats (21). No specific markers of uterine inflammation are available. There are few publications reviewing the role of infectious agents in feline infertility. Especially, the role of respiratory viruses, such as feline herpesvirus (FHV) or calicivirus, remains unclear, although there is evidence in the literature of an impact on reproduction (20). The role of imbalance in the vaginal flora is probably underestimated. Very few studies have been performed on the genital microbiome in cats (22). Interesting approaches to detect « at risk » pregnancies have been done, such as the use of Doppler ultrasound to measure uterine artery resistance index (23). But, for the moment, studies are limited to the follow-up of normal pregnancies. Regarding male fertility, most studies describing semen quality and sperm characteristics in cats were performed on Domestic Shorthair cats, and data regarding semen quality in purebred cats are very scarce. Regarding these data, it seems that azoospermia/oligospermia and teratozoospermia often cause fertility problems in pedigree cats. According to some authors, this area of feline reproduction is still relatively undeveloped (24). Some diseases are unfortunately described in cats as if they occurred in the same way as those observed in female dogs. For example, mucometra is more often found in young queens, while it is mainly observed mostly in older female dogs, where it is sometimes considered as a subclinical stage of pyometra (25). Another unclear clinical description concerns mammary fibroadenomatosis in the queen. In practice, two clinical conditions may be found: one is a diffuse mammary hypertrophy mostly diagnosed in young queens with a solid fibrous content, that is commonly treated with success using aglepristone. Another form is a cystic aspect of the mammary glands, which appear full of liquid with often a blue aspect of the skin. This second form develops often in older queens and does not respond very well to the use of aglepristone. Is it the same disease (26)? To the best of the author's knowledge no study about the exact cause of feline fibroadenomatosis (increased sensitivity or presence of progesterone receptors...) has been done. Uterine pathologies, other than pyometra, and ovarian pathologies, are mostly described as case reports in the literature and there are very few academic or educational reviews (27). Disorders of the male genital organs are not well known, probably because most pet tomcats are castrated (only 27 pages in the book "Feline Reproduction" which contains 278 pages) (4). Some areas, such as feline obstetrics and neonatology, are better known nowadays, thanks to recent dedicated research (28). Assisted Reproduction Techniques (ART) are used less in cats than in dogs. Few cat breeders are using artificial insemination. Shipments of refrigerated or frozen semen, which could allow genetic exchange of pedigree cats between countries, are almost non-existent. In vitro fertilisation and in vitro embryo production are mastered but embryo transfer is still a challenge. Although improvements have been made, the overall success rate of ART remains low (29). Finally, advancements in understanding the conditions necessary for effective reproductive management in breeding catteries have enhanced efforts to optimise feline health and well-being and can contribute to building a fruitful veterinarian-breeder collaboration (30).

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Training small animal (dog & cat) reproductive techniques using low-cost models*

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Veterinary schools have to ensure that their graduates master the foundational skills required on day one of practice. In small animal reproduction, the day-one skills essential competencies required for new graduates include understanding reproductive anatomy, performing a clinical reproductive examination (male and female), and being able to perform routine surgical procedures like ovariohysterectomy and orchiectomy (1). For many years, clinical training in veterinary medicine has relied on the use of cadaver tissue before students were introduced to live animals, but some hands-on skills were performed first on live animals, having trainees' observing first and then performing procedures under expert's supervision in live animals (2, 3). However, significant ethical concerns and stricter animal welfare regulations have driven many changes in training practices. The European Association of Establishments for Veterinary Education emphasises the "never the first time on a live animal" policy, thus promoting a reduced use of live animals in training. Additionally, the mental burden experienced by students when performing procedures on live animals raises concerns about its impact on their confidence and training experience (1–3). Furthermore, difficulties in sourcing cadaver tissue, along with increasing class sizes and the rising costs of using animals and cadavers, have underscored the need for innovative approaches in veterinary education, that should balance ethical considerations with effective training (2, 3). Low-Cost Models for reproductive clinical and surgical techniques/procedures One solution to these challenges relies on integrating simulation-based training into veterinary curricula, using models that mimic real-life scenarios. This approach allows students to learn and gain hands-on experience in a standardised and safe environment without the use of live animals, while enhancing their skills development (2). In recent years, there has been a significant increase in the use of models for teaching in veterinary medicine, as a complement to more traditional methods. As a result, many veterinary schools have set up the so-called "skills laboratories" with a range of models covering different areas of veterinary education. These models range from simple or low fidelity and low-cost models to high fidelity and high-cost manikins (2–6). Currently, there are several ready-to-use commercial models available for training various reproductive clinical and surgical techniques in small animal veterinary medicine, which provide a high-fidelity and realistic experience, often highly expensive. Buying such models in sufficient numbers for all students to practice several times requires a considerable financial input from most institutions, especially if single use/replacement parts are needed (2, 3) Conversely, numerous studies have found that when models meet the educational goals, there is little to no difference in the performance or skills development between low- and high-fidelity models (2,4). As a result, many institutions have begun developing their own models using locally available resources. This approach reduces costs, while facilitating repeated practice—critical for mastering most techniques—and maintaining a safe learning environment (2, 4). It should be noted, though, that the development of educational models and the collection of pedagogical evidence required to validate them is a time-consuming process, that may require significant investment of time and staff resources to ensure successful implementation and delivery (2–4). To date, there have been some published studies, which describe and validate low-cost models for the learning and training of small animal reproductive surgical and clinical examination techniques. One of the most common procedures featured in the published literature regarding the creation and validation of a low-cost/low-fidelity veterinary models is small animal neutering surgery (ovariohysterectomy/ovariectomy and orchiectomy). This might be due to the fact that these procedures are invasive and considered "day-one skills" for a veterinarian. The majority of these articles are studies describing and evaluating the efficacy of low-cost models for teaching and training ovariohysterectomy/ovariectomy in dogs and cats (6). These models range from very simple cloth/ plastic and even built-your-self models to low-cost models with higher degree on reality/psychologic fidelity (5, 7–16). Less studies can be found on canine orchiectomy low-cost models (17, 18) and, to the best of our knowledge, only one feline orchiectomy model has been described in the literature (5). Likewise, to the best of our knowledge, no research article has been published describing a small animal c- section model, yet. In a survey among vet schools, one institution reported the use of a small animal c-section surgery model (6) Publications describing low-cost models for use in training of reproductive clinical examination techniques in small animals are scarce. This might be due to the fact that these are generally less invasive techniques raising less concerns in terms of animal welfare. Capilé et al. (19) described a canine prostate palpation low-cost model that allowed students to perform a clinical examination on a simulator. A vaginal cytology simulation model made by 3D printing and soft silicone accurately replicated the canine female reproductive tract, being reported as effective for learning the vaginal cytology procedure (20). A low-cost dog pregnancy ultrasound model was also described and evaluated in terms of enabling students to understand the use of imageology

in the live animal and to give context to what they see on the ultrasound screen (21). In the already mentioned survey regarding the use of models in the theriogenology curricula, a respondent reported on the use of a canine transcervical insemination model, made intra-institutionally and that its use slightly improved students' skills (6). Overall, studies using low-cost models to train students in small animal reproductive techniques have showed that models make students feel more confident and less anxious about performing the tasks on a live animal. Despite studies showed an improvement of students' skills, to some degree, it should be highlighted that they do not replace completely the training with live animals (2, 3, 14). During this presentation, we will provide a brief overview of low-cost models used in veterinary training of day-one skills on small animal reproductive medicine. Since many models used in veterinary schools may have not been published in literature, we will facilitate a brainstorming moment in order to share experiences and generate ideas for improving low-cost models for training the small animal reproduction skills. Additionally, we will explore strategies for improving the training programs in order to better prepare students for the growing complexity of reproductive care in modern veterinary practice.

*Models/Manikins/simulators

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From pathologist to clinician: An integrated approach on canine mammary tumours

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Canine mammary tumours (CMTs) are the most common neoplasms in intact female dogs, comprising approximately 50% of all tumours in this population. In regions where elective ovariectomy is not routinely practiced, CMTs may account for an even higher proportion of neoplastic diagnoses. About 50% of these tumours are malignant, with a peak incidence observed between 8 and 11 years of age. Although the protective effect of early spaying is a topic of controversy (1), tumours expressing oestrogen (ER) and progesterone receptors (PR) tend to be better differentiated and less aggressive, and their expression is associated with improved survival. However, as tumours become more malignant, ER/PR expression typically diminishes, reducing potential responsiveness to hormonal modulation (2). Regarding parity, studies have not shown a consistent protective effect of pregnancy or lactation. Intact bitches that have been bred continue to carry a significant risk of developing mammary tumours, particularly if spaying is delayed. Thus, breeding is not considered a preventive measure. The transition from pathologist to clinician in the context of CMTs presents both challenges and opportunities, yet a significant gap often exists between these two professions. CMTs represent a substantial proportion of cancer diagnoses in bitches, and they often present as multifocal and heterogeneous masses. Their complex cytological and histopathological profiles, along with variable prognostic implications, frequently pose significant diagnostic and therapeutic challenges. One of the primary challenges lies in interpreting pathological results—both cytological and histological—where morphological variations can substantially impact clinical decisions. Histology is considered the gold standard for accurately diagnosing canine and feline mammary tumours. In contrast, fine-needle aspirates (FNA) and cytological evaluation are generally considered less reliable for differentiating benign from malignant tumours, particularly in dogs. In fact, both FNA and core-needle biopsy (CNB) have low diagnostic accuracy for canine mammary tumours, with reported accuracies ranging from 47%–68% for FNA to 63%–73% for CNB (3). However, FNA remains a useful technique for identifying the general type of lesions that may arise in the mammary gland and abdominal region. It is especially valuable for distinguishing between neoplastic and inflammatory lesions or identifying round cell tumours (e.g., mast cell tumours) as opposed to epithelial cell-origin tumours. Cytology may also play a pivotal role on the pre-surgical identification of lymph nodes metastasis, an important diagnostic and prognostic factor. In human medicine, there are standardised protocols for the cytological evaluation of mammary tumours and the level of agreement between cytology and histology is higher, ranging from 65% to 74%. Interestingly, using the same cytological criteria applied to human medicine, the cytological diagnosis in canine mammary tumours is reported to increase to 68%–86% (4, 5). The histological classification of canine mammary tumours classifies lesions into hyperplasia/dysplasia, benign and malignant categories based on their cellular origin and morphology (6, 7). Among benign CMTs, adenomas, consisting of well-differentiated glandular cells, complex adenoma, with a combination of epithelial and myoepithelial cells, and benign mixed tumour with mesenchymal components such as cartilage or bone are relatively common. Fibroadenomas are less common, while benign tumours arising from mammary ducts are more frequent. Of note, the surgical excision of benign tumours results in an excellent prognosis. Malignant CMTs are most common in older, unspayed females, with carcinomas being the most prevalent type. Simple and complex carcinomas are common, while mixed carcinomas

containing mesenchymal elements like cartilage or bone are less frequent. Although sarcomas represent a smaller portion of malignant CMTs (about 5%–10%), they include types like fibrosarcoma, osteosarcoma and chondrosarcoma. Carcinosarcomas, which contain both carcinomatous and sarcomatous elements, are also rare but known for being quite aggressive. Another particularly severe form is inflammatory carcinoma, characterised by rapid progression and poor prognosis. There are also some rare types, such as squamous cell carcinoma and anaplastic carcinoma, the latter being highly aggressive and poorly differentiated (6–8). Malignant mammary tumours, in general, exhibit a higher degree of cellular atypia, infiltrative growth patterns and metastatic potential. Pathologists analyse tumour architecture, cellular differentiation and, when necessary, biomarkers expression, generating a report that typically includes a description, a morphological diagnosis and a comment. However, this structured report is not always utilised to its full potential.

The description, often detailed and comprehensive, is frequently overlooked despite being rich in prognosis-related information such as tumour size, infiltrative growth, histological subtype, grading, specific histological patterns (e.g., micropapillary), vascular invasion and the presence of regional or distant metastasis, all of which are critical for assessing tumour aggressiveness and determining appropriate treatment and prognosis. On the other hand, the morphological diagnosis, which usually contains the definitive diagnosis and often the grading, is the most consulted part of the report, but it should be viewed as a concise synthesis of the description rather than a complete representation. Additionally, the comment section is often limited to offering generic recommendations, and in the current era of personalised medicine, where prognosis and therapy are increasingly tailored, such general advice may lack relevance. The prognosis of CMTs is influenced by various clinical and pathological factors, including histological subtype and grade, tumour size (with lesions < 3 cm associated with better outcomes), lymphovascular invasion, lymph node involvement and distant metastasis. Additional prognostic indicators include surgical margin status, hormone receptor expression (ER/PR positivity being favourable), Ki-67 proliferation index and the expression of molecular markers such as HER2 and PARP-1 (8, 9). Molecular classification is increasingly used to guide prognosis and therapy decisions. Triple-negative, HER2-enriched and basal-like subtypes generally carry worse outcomes, like patterns observed in human breast cancer. Staging of CMTs adheres to the modified WHO TNM system and includes thoracic radiography or CT, abdominal ultrasound and cytology or histopathology of lymph nodes. The Brazilian Consensus recommends nodal assessment even if nodes are not enlarged. Advanced imaging improves surgical planning and detection of subclinical metastasis. Surgery remains the primary therapeutic modality for CMTs. The extent of excision ranges from lumpectomy for small, localised masses to radical unilateral or bilateral mastectomy for extensive or multicentric disease. Achieving clean margins is essential to reducing local recurrence. Regional or radical mastectomy may be indicated for extensive lesions or recurrent disease. Concurrent ovariectomy is advised in intact females at the time of surgery, especially for ER/PR-positive tumours. Some studies suggest spaying at the time of tumour removal improves prognosis in malignant cases without distant metastases (10). Adjuvant chemotherapy may be considered for high-risk patients (e.g., tumours > 3 cm, lymph node metastasis, high histologic grade, lymphovascular invasion). Doxorubicin ± Cyclophosphamide or Carboplatin are the most studied agents. The true benefit of chemotherapy remains controversial (8). Despite ER/PR expression in many CMTs, hormonal therapy has not proven clinically effective. Tamoxifen and related agents show limited efficacy and carry risks in intact females (e.g., pyometra, vulvar swelling). Therefore, hormonal therapy is not routinely recommended (2). The field of targeted and novel therapies is evolving. PARP-1 has been identified as a potential therapeutic target, particularly in triple-negative tumours, which are aggressive and lack ER/PR/HER2 expression, supporting the rationale for PARP inhibitor trials in veterinary patients (9). Additionally, HER2 and COX-2 are frequently overexpressed in aggressive subtypes. HER2-positive tumours may benefit from targeted strategies under investigation. COX-2 inhibitors like piroxicam may provide adjunctive benefits in certain high-grade or inflammatory tumours (2). Precision oncology approaches—including receptor profiling and emerging molecular markers—are essential for optimising future treatment protocols. Canine mammary tumours remain a significant clinical challenge, especially in regions where elective sterilisation is delayed. Surgical excision is still the primary treatment, while systemic therapies—such as chemotherapy and novel targeted agents—are becoming more advanced. Optimal outcomes rely on early spaying, accurate diagnosis and staging and individualised treatment guided by molecular and histopathological features. Strengthening communication between pathologists and oncologists and fully integrating detailed pathological reports into clinical practice is essential for improving diagnostic accuracy, guiding therapeutic decisions and enhancing patient outcomes—making this collaboration critical to advancing care.

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Nutrition and puppy growth: From weaning until adulthood

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The growth phase is nutritionally over demanding due to the high nutrient and energy required to support the organ development, muscle synthesis and skeletal growth. In comparison with adult dogs, growing dogs are at higher risk of suboptimal nutrition. Malnutrition can negatively impact the puppy's health and well-being but also negatively affect its adult life and potentially

reduce its lifespan. The energy and nutrient requirements vary throughout the growth phase, with the post-weaning period corresponding to a more rapid growth, which gradually slows down over time. Since dog breeds exhibit a broad range of mature body weight (BW), from approximately 1.5 kg to over 100 kg, they also have highly variable weight gain rates and reach maturity at different ages (1). This gives an extra dimension to the challenge of providing adequate amounts of energy and nutrients throughout their growth. Large breed puppies are particularly challenging due to their higher relative growth rate when compared to puppies from smaller breeds, that is, they gain more weight per kg $BW^{0.75}$ over the same time period. The National Research Council (NRC), (2). of the National Academies of Sciences has provided nutritional guidelines, based on this information and more recent research, the European Pet Food Federation (FEDIAF, (3)) has published nutrient profiles for all life stages, including the growth phase, to be used by the pet industry to produce nutritionally adequate pet foods. These references are also used to check if any type of food can be considered an option for the growth stage. These guidelines specify the minimum recommended concentration for all essential nutrients and the maximum, for the nutrients where this value is known. The guidelines also provide different nutritional profiles for the more intensive early growth phase and for the later period of growth. There are also specific recommendations for large and smaller breeds regarding calcium, phosphorus and their ratio, because the higher growth intensity and longer growth period of the large and giant breeds modifies their requirements. The assessment of the alignment between the food nutritional profile and the Nutritional guidelines is only the initial step of adequacy control. The amount of food fed will determine the amount of nutrients a puppy will ultimately ingest as well as the amount of energy intake. Both, NRC (2) and FEDIAF (3), provide energy recommendation guidelines. These guidelines serve only as a starting point and have to be adjusted to the individual puppy at every step of its growth. This means the nutritional recommendation for a puppy requires constant checks and adjustments. It is crucial to recognise that the nutrient recommendations provided by our most reliable guidelines are not fixed values but estimations which include safety margins of varying degrees. They should not be applied without critical assessment and should be adapted as new research emerges. Due to the adverse effects that inadequate levels of energy, calcium and phosphorus intake have on puppy's health, and the more recent findings related to these nutrients recommendations, they will be the focus of further discussion in the following sections. Energy: Accurately estimating the Metabolisable Energy (ME) requirements for dogs is crucial, as it determines the amount of food to be fed and, by consequence, defines the adequate nutrients concentration in the food. Therefore, equations estimating the ME requirements for pet dogs must be highly accurate especially for growing puppies. It is important to keep in mind that calculations based on these equations are going to define the initial feeding dosage of the pet food offered to the majority of the puppies, as they are the basis for pet food packaging recommendations. Similar to nutrient guidelines, NRC (2) serves as the standard reference to calculate energy recommendations. However, these energy requirements were mainly derived from studies with laboratory dogs and their applicability to pet dogs has been questioned. In 2016, Thes et al. (4) demonstrated an overestimation of the NRC (2) ME requirements for adult pet dogs. Three years earlier, Dobenecker et al. (2013) (5) also found that NRC (2) equations overestimated the ME requirements for laboratory puppies in the early growth phase. Later, Klein et al. (2019) (6) confirmed similar findings for growing pet puppies, in particular when puppies are less than 26 weeks of age. As a result of this later study, this research team proposed a more accurate model to estimate energy requirements of pet puppies, now endorsed by FEDIAF (3):

$$\text{ME requirements (kcal)} = (254.1 - 135.0 \times [\text{actual BW/expected mature BW}]) \times \text{actual } BW^{0.75}$$

Even if the proposed equation is accurate and reliable for most puppies, the individual differences of ME requirements exhibit high variability across dogs with similar characteristics and in similar conditions (4–6). Therefore, after estimating energy requirement and calculating the initial food dosage, continuous checking of the recommendation is essential to ensure a healthy growth for each puppy. Excessive energy intake is a well-documented risk factor for accelerated growth and lead to developmental orthopaedic diseases (7, 8). Contrary to what happens in adult dogs, in growing puppies the excessive energy intake may not result in an immediate increase in Body Condition Score (BCS) (9). This is an obstacle to the detection of the overfeeding, since the puppy looks healthy. While excessive BCS is a well-recognised problem during growth, the excessive growth rate itself is a silent risk to the healthy development of puppies. This risk is particularly high is large and giant breeds due to their high growth rate. The prevention of an excessive growth rate will prevent high BCS during growth, but preventing a high BCS alone does not prevent an unhealthy growth. The most effective way to prevent either high energy intake, that would lead to excessive growth rate and eventually high BCS or low energy intake leading to delayed growth rate and low BCS, is the regular (ideally weekly) plotting of the weight of each puppy against its healthy growth curve. This methodology ensures continuous adjustment of the energy intake to the specific energy requirements of each puppy. For this statement to be true in practice, it is necessary to have reliable equations to estimate the healthy curves. To date, the most accurate predictive curves for dogs older than 8 weeks are the logarithmic curves based on recommendations of the GfE (1989) and Meyer and Zentek (1992) (3) (Table).

TABLE Equations for growth curves modified after GfE (1989) and Meyer and Zentek (1992), valid from weaning age (8 weeks) to 1 year (3).

Expected mature BW (kg)	Growth curve
≤7	% of expected matureBW = 36.92Ln(W) − 43.57
> 7–15	% of expected matureBW = 36.86Ln(W) − 48.22
> 15–27.5	% of expected matureBW = 39.88Ln(W) − 60.7

Expected mature BW (kg)	Growth curve
> 27.5–47.5	% of expected matureBW = 36.96Ln(*W*) − 56.18
> 47.5	% of expected matureBW = 36.61Ln(*W*) − 62.39

Abbreviations: BW, body weight; W, age in weeks.

Calcium and phosphorus are key nutrients for growing dogs, as deficiency, excess or unbalanced ratio of these essential minerals can lead to disorders in skeletal development (10). Young dogs, face a greater challenge because, unlike adults, their intestinal calcium absorption is not regulated (11). Excess calcium during growth is particularly detrimental when combined with rapid growth (12) or elevated calcium to phosphorus ratio. Phosphorus deficiency can also hinder growth and impair musculoskeletal development (13, 14). Puppies of large and giant breed receive heightened scrutiny due to their susceptibility to developmental orthopaedic disorders (15, 16). However, also beagle puppies exhibited subclinical premature closure of growth plates in long bones as a result of calcium excess, despite being a smaller breed receiving a restricted energy supply (17). Recent studies in adult dogs indicate that excess calcium may downregulate bone turnover (17). The risk of skeletal disorder is particularly high when excess calcium (slow bone turnover) is combined with rapid growth due to energy oversupply (12, 19). These two can easily simultaneously occur in case of overfeeding. The NRC (2) calcium and phosphorus requirements for post-weaning puppies are widely recognised as standard guidelines. The requirements were derived by extrapolation of feeding trials with varying dietary calcium and phosphorus, using a trial and error approach. However, they were primarily based on large breed puppies, leading to a clear under representation of small or medium-sized breeds. This raised concern about a potential overestimation for these minerals for puppies from small and medium-sized breeds. A recent study Böswald et al. (20) challenged the NRC (2) recommendations for growing puppies using the factorial method. The key advantage of this method, in addition to providing an alternative approach, is that accounts for differences in growth rates between small and large dogs. Compared to NRC (2) recommendations, Böswald et al. (20) found significantly lower requirement of calcium and phosphorus for miniature and medium size puppies (5–35 kg mature BW). Additionally, the study recommends a calcium/phosphorus ratio of 1.4/1 in the food of growing puppies.

A final note to vitamin D which is essential for the modulation of calcium homeostasis and bone metabolism. Insufficient amounts of vitamin D results in bone mineralisation dysfunction even when dietary calcium and phosphorus intake is adequate (21). In conclusion, NRC and FEDIAF (2, 3) provide scientific guidelines for nutritional and energy recommendations during growth phase of puppies. Nutritional demands are elevated during growth, making this period critical. Malnutrition can compromise a puppy's health during growth and have long-term consequences in adulthood. The nutritional requirements vary throughout growth and depend on breed size. Regularly weight monitoring against validated growth curves is essential to prevent excessive growth rate and inadequate BCS. Unbalances in calcium and phosphorus intake can lead to developmental orthopaedic disorders. Large breed puppies are particularly susceptible, while NRC (2) recommendations for small and medium- sized breed puppies may overestimate their calcium and phosphorus needs and require adjustment.

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Ovarian ageing: Use of animal models

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Reproductive ageing is a complex and multifaceted process, characterised by a gradual decline in ovarian (1) and uterine function (2) and dysregulation of the hypothalamic-pituitary-ovarian (HPO) (3–4). These changes eventually lead to female infertility and adversely affect overall health. Physiological changes during reproductive ageing involve significant hormonal alterations at both the hypophyseal level (FSH and LH) and the ovarian level (anti-Müllerian hormone, oestrogen, progesterone and inhibin). Additionally, disruptions in gonadotropin-releasing hormone secretion from the hypothalamus further exacerbate hormonal imbalances. The contributions of the ovary, uterus and HPO axis to reproductive ageing vary across species. These

interspecies differences underscore the importance of a "One Health" approach that integrates insights from diverse animal models to deepen our understanding of reproductive ageing and to enhance the development of species-specific interventions, including those for humans. By comparing the contributions of the ovary, uterus and HPO axis in various species, researchers can uncover both shared and unique pathways, enabling the design of more effective strategies for preserving reproductive health and addressing age-related dysfunctions. Invertebrate and vertebrate models, particularly mammals, offer valuable opportunities for studying ageing. Invertebrates, with their short lifecycles, high fertility rates and evolutionary conservation of genes, are practical for examining ageing mechanisms (5). Rodents such as rats and mice show reproductive decline related to abnormal gonadotropin release patterns. Similar hypothalamic-pituitary dysfunctions often precede gonadal failure in women (6). While rodents are indispensable for studying reproductive ageing, they present limitations, including shorter lifespans, rapid reproductive processes and genetic uniformity (7). Consequently, mammalian models that are genetically closer to humans or share similar environmental exposures hold promise. Domestic livestock and companion animals, in particular, offer unique insights. Integrating data from these diverse animal models will provide a holistic understanding of reproductive ageing within the "One Health" framework, bridging human and animal health research to inform strategies for preserving reproductive health and managing age-related challenges. In humans, reproductive ageing is primarily marked by a gradual decline in ovarian function, including a reduction in follicle numbers and age-related declines in oocyte quality. Women rarely give birth after the age of 45 years and will cease to cycle, at menopause, around the age of 50 years, after almost two decades of fertility decline (8). Menopause has also been described in rhesus monkeys (*Macaca mulatoo*) (9) and in two other species, killer whales (*Orcinus orca*) and short-finned pilot whales (*Globicephala macrorhynchus*), both of which having comparable post-reproductive lifespans as humans (10). The process of ovarian ageing is influenced by a combination of genetic, environmental and lifestyle factors, with systemic repercussions. Ageing oocytes exhibit inefficiencies in meiosis, leading to chromosomal abnormalities such as nondisjunction, disrupted microtubule apparatus and shortened telomere length (6). However, the underlying mechanisms remain complex and are not yet fully elucidated (5). The use of human biological material to study reproductive ageing presents significant ethical constraints and practical challenges. Nonetheless, understanding the mechanisms driving ovarian ageing is critical, especially as the average age at first childbirth has risen exponentially over recent decades and now exceeds 30 years in industrialised countries. This highlights the necessity of utilising suitable mammalian models to investigate reproductive ageing, with a focus on the ovary. The effects of maternal age have been extensively studied in domestic species, more specifically in large animal models. In general, age-related reproductive decline in these species is characterised by irregular oestrous cycles, lower conception rates, fewer neonates per birth, increased embryonic mortality, abortion, congenital anomalies, stillbirths, dystocia and other complications stemming from diminished gamete quality and challenges in maintaining pregnancy to term. Among livestock, bovine ovarian physiology has been extensively studied for its translational value. Bovine reproductive physiology and morphology share many similarities with humans, including mono-ovulation, regular oestrous cycles (unless pregnant), a similar gestation length and comparable ovarian physiology, including follicular and hormonal dynamics (11). However, unlike humans, cows do not experience menopause but rather a gradual decline in fertility culminating in infertility around 13–16 years of age (12). In cattle, maternal age affects oocyte quality; for example, cows older than 180 months exhibit reduced mtDNA content in their oocytes, which compromises their cleaving ability (13).

Mares have also been studied as models for human reproductive ageing, particularly as they are often maintained into advanced age. Research on mares has provided valuable insights into follicular function, oocyte maturation and age-related reproductive decline (7). Advanced age in mares is associated with reduced fertility and increased pregnancy loss due to declining oocyte quality (14). Similarities between mares and humans include shortened cycles with elevated FSH levels, prolonged cycles with intermittent ovulations, and increased FSH and LH concentrations. Both species experience oocyte quality decline, reduced viability and meiotic arrest over decades of ageing (7). In addition, advanced age in mares correlates with altered mitochondrial function in oocytes (15) and uterine dysfunction, such as angiopathies and impaired cleansing mechanisms, which further contribute to reduced fertility (16). However, mares present some limitations as models for human reproductive ageing, including their seasonal reproductive patterns, reduced responsiveness to superovulation and the relative scarcity of biological material compared to cattle (12). Ovine and caprine models have gained attention as valuable alternatives for studying reproductive ageing. Research on these models has revealed significant reductions in follicular reserves in adult animals compared to juveniles (6 months old) (17). Sudan black staining has identified multinucleated giant cells (MGCs), which are distinctive markers of ovarian ageing previously documented in small animal models. These cells, formed by macrophage fusion, are believed to assist in tissue debris removal (18). These findings highlight sheep and goats as promising models for understanding the effects of ovarian ageing on fertility. Also companion animals can serve as models for aging. Over the past few decades, the number of aging dogs and cats has increased significantly, mirroring trends in human populations. This makes them suitable models for translational gerontology studies and aligns with the One Health approach (19, 20). The increased longevity of companion animals is attributed to advancements in veterinary medicine, improved preventive care and more comprehensive management of different life stages (21). Research into animal ageing is critical not only for improving the health and quality of life of these animals but also for their potential as models for human ageing (22). Despite differences in lifespan, dogs offer unique insights into ageing due to notable parallels with humans, including naturally occurring diseases that closely resemble human conditions (23). Their close association with humans and shared environmental exposures influencing reproduction and fertility further underscore their value as research subjects (24). Moreover, the rising number of companion animals and their enhanced medical monitoring highlight the importance of understanding their reproductive ageing. This is particularly relevant given current trends to reduce early gonadectomy; ensuring reproductive health is maintained over their lifetimes. The manifestation of reproductive ageing in dogs is influenced by several

factors, including breed, adult body size, genetics, exposure to injury and disease and nutritional status (25). This decline is often associated with a reduced conception rate and smaller litter sizes. Advanced maternal age at the first litter—beyond six years—has been linked to smaller litter sizes and a higher risk of perinatal mortality (26). Additionally, older first-time mothers are more likely to experience obstetrical complications and stillbirths, often attributed to single-puppy pregnancies, uterine disorders and prolonged labour (27). Reproductive pathologies are another critical aspect of reproductive ageing in dogs. These conditions not only compromise fertility but may also result in systemic health issues. While some lesions can occur at any age, others, such as ovarian pathology, cystic endometrial hyperplasia and pyometra, are more prevalent in older dogs (28). Similarly, the risk of undetected ovarian tumours increases with age (29). Notably, the co-occurrence of ovarian and endometrial pathologies often results in persistent subfertility, even when the affected ovary is removed (30). Despite considerable investigation into reproductive disorders in dogs, research on the intrinsic mechanisms of ovarian ageing remains limited. In assisted reproductive technologies, advanced age appears to impact the total number of cumulus-oocyte complexes (COCs) collected, even if the morphological quality of the COCs remains unaffected (31). Little is known about the contribution of the ovarian and follicular microenvironment on ovarian ageing in domestic species. Physiological ageing is associated with chronic low-grade inflammation, with effects at several levels (32). Chronic low-grade inflammation induces prolonged oxidative stress, increasing levels of reactive oxygen species, which will activate cytokines involved in tissue fibrosis (33). Still, the role of inflammation in physiological ovarian ageing is not clear. However, some hypotheses seem likely, such as the long-term accumulation of oxidative stress leading to deterioration of the ovarian microenvironment, apoptosis of granulosa cells and follicular atresia; the role of cellular senescence and the senescence-associated secretory phenotype, which is increased in aged mice and impairs oocyte-somatic cell communication; and the ovarian deposition of extracellular matrix remodelling leading to stromal fibrosis (34). Our previous studies have begun to uncover markers of canine ovarian ageing, focusing on the accumulation of multinucleated senescent-like cells associated with lipofuscin deposition at earlier stages of ovarian ageing (35). Lipofuscin accumulation, together with a reduction in the primordial follicle pool, appears to serve as an early marker of ovarian ageing in dogs, prior to the decline in fertility. Additionally, histopathological evaluations of the uterus in middle-aged dogs have revealed uterine pathologies that may further impair fertility. In cats, preliminary results suggest a different situation, with a scarce presence of multinucleated cells and less marked decline of primordial follicle pool (36). Further studies should focus on the differences of ovarian microenvironment in companion animals and, using a mouse model, to evaluate the use of antioxidants in the modulation of age-dependent ovarian inflammation. In conclusion, understanding reproductive ageing in non-human mammals provides crucial insights into both human reproductive ageing and the broader ageing process. Increased longevity in animals, much like in humans, is a direct result of advances in housing, nutrition and medical care. For companion animals, this extended lifespan has strengthened bonds with their owners, who increasingly view them as family members and prioritise their health and well-being. Comparative studies across species, particularly companion animals and livestock, offer valuable insights into the complexities of reproductive ageing. By leveraging these models, researchers can develop interventions to extend reproductive health and improve overall well-being in both humans and animals. Companion animals, in particular, hold promise for bridging gaps in knowledge due to their shared environments and lifespans closely mirroring those of humans.

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Alternatives to antibiotics in semen extenders: A "One Health" approach

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Use of antibiotics in human and veterinary medicine has been under scrutiny for a number of years. There is a growing realisation that resistance to antimicrobial substances places a huge and increasing economic burden on society in terms of untreatable bacterial infections and even deaths (1). Considerable efforts have been made to restrict administration of antibiotics to therapeutic use only, that is, where a bacterial infection has been diagnosed and the susceptibility of the causal organism to the antibiotic to be prescribed has been established (2). Such recommendations for prudent antibiotic stewardship would seem to be self-evident, yet there are still some cases of antibiotic usage that fall outside these specifications. The addition of antibiotics to semen extenders for artificial insemination is a case in point (3). Artificial insemination (AI) was developed initially as a means of controlling disease in farm animals. Semen is collected from animals that are shown to be free of infectious diseases, and the gametes are transferred from donor to recipients without any contact between animals. Farm biosecurity is improved by transporting semen between farms, rather than live animals for breeding (4). It quickly became apparent that there is an added advantage in being able to use semen from superior sires, thus enabling rapid genetic progress to be made in production traits such as milk yield or carcass quality. Artificial insemination is used in other animals as well, such as companion animals and for conservation breeding of wild animals, transcending the geographical and chronological limits imposed by natural mating. The downside of AI is that it would also be an efficient method of spreading disease (5), since one ejaculate provides insemination doses for several females (or up several hundred semen doses in cattle). Genes for antimicrobial resistance could also be transmitted in this way via bacteria in semen, since until recently all commercial semen doses were required to contain antibiotics (6). Although semen collection is performed using strict hygiene measures, it is very difficult to avoid some bacteria in semen, even from healthy donors. The mucosa of the reproductive tract has its own microbiota (7) and some of these bacteria are transferred to the semen on ejaculation (8). Furthermore, the semen can become contaminated with bacteria from the animal's skin and hair, from the semen collector and from the environment (9). Some of these bacteria can have a detrimental effect on sperm quality or may be capable of causing disease in inseminated females. Therefore, antibiotics are included in semen extenders, as specified by various governmental regulations for trade in animal products. The problem is that in the last decades viable bacteria have been cultured from semen containing antibiotics (e.g., (10)). In other words, bacteria are able to survive and grow despite the presence of these antibiotics. Moreover, the inseminated liquid exits the animal into the environment via backflow (11), exposing bacteria in the caudal reproductive tract and in the environment to these antibiotics. Changes in the microbiota of the reproductive tract were detected in inseminated sows (12), mares (13) and cows (14), indicating that exposure to even low concentrations of antibiotics can affect the resident bacteria. Moreover, changes in the microbiota may be associated with an adverse effect on fertility. Personnel taking care of the animals are also potentially exposed to resistant bacteria and hence to resistance genes. Thus, there is a clear need to be aware of the inclusion of antibiotics in semen extenders as being a non-therapeutic use of antibiotics, and, crucially, to find alternatives to inhibit bacterial growth in semen. The first secretions to pass through the urethra are likely to contain the most bacteria (15). Therefore, allowing the male to make several "false mounts" while preparing him for semen collection allows the urethra to be cleansed with fluid from some of the accessory glands, thus reducing the bacterial load and neutralising traces of urine before the semen passes through. One advantage of collecting semen in dogs and pigs is that the method used (digital manipulation) allows the collector to decide which fractions, and how much of each, should be included in the sample. Therefore, this could be a way of reducing the bacterial load. Bacteria are adept at surviving in unfavourable environments, a property that enables them to adapt to avoid attack by antibacterial substances (1). Even dead bacteria can pass on resistance genes to other bacteria (16), making their avoidance mechanisms even more effective. Thus, there is a risk that microorganisms can become resistant to any novel "antimicrobial substance" that is used to attack them. Some alternatives to conventional antibiotics, reviewed recently (17), include antimicrobial peptides, extracts of various plants or nanoparticles. Separating spermatozoa from bacteria by physical means appears, intuitively, to be a better solution than trying to kill the bacteria or prevent them from multiplying by applying antibacterial substances. Several separation devices exist that select the most robust spermatozoa from the ejaculate but also, importantly, separate them from seminal plasma with its bacterial load (18). The disadvantage is that these devices can cope with only small volumes of semen or low sperm concentrations, and are therefore better suited to preparing sperm samples for in vitro fertilisation (IVF) than for AI. Furthermore, evidence-based studies on the efficacy of such techniques for removing bacteria under field conditions are lacking. Another physical method of separating spermatozoa from bacteria is colloid centrifugation, which is a cell separation method traditionally used as a density gradient, that is, centrifuging the sample through several layers of colloid of different densities (3). However, this technique is not user-friendly for large volumes of semen or concentrated ejaculates, as are commonly found in animal semen. Therefore, the technique was modified to use only one layer of colloid, that is, Single Layer Centrifugation (SLC), preferably using species-specific colloid formulations. This modification has the advantage that it can be scaled-up to process large volumes of animal semen relatively quickly and efficiently, which is needed for dealing with the high throughput of ejaculates typical of a semen collection station. The effect of SLC on reducing bacterial load was reported for boar (19), stallion (20), dog (21) and bull semen (22), but a number of spermatozoa were lost in the process. Therefore, lower densities of colloid were tested, to achieve a balance between removing most of the bacteria while retaining most of the spermatozoa (23). Fertility trials are underway to test such sperm preparations in pigs (24) and horses (25). Thus far, there are no indications that any remaining bacteria can adversely affect sperm quality or fertility in the inseminated female.

The inclusion of antibiotics in semen extenders is relevant for small animals, too. Although most litters of puppies and kittens result from natural mating, AI is used occasionally in these species (26, 27) and antibiotics are included in the semen extender. Since they tend to share the same living accommodation as their owners (28), dogs and cats can serve as sentinel species for human beings; thus, monitoring resistant bacteria in dog semen could provide an indication of AMR in human habitation. The importance of studying the reproductive tract microbiota in healthy individuals is highlighted by the question of whether antibiotics in semen extenders might have an adverse effect on fertility. Studies in patients attending fertility clinics suggest that the success of the treatment is linked to the vaginal microbiota. Probably the latter is acting as an indicator that the environment in the reproductive tract is conducive to the establishment of pregnancy (29). What we need to establish is whether the inclusion of antibiotics in semen extenders alters the composition of the reproductive tract microbiota to the extent that it could interfere with fertility. However, without an in-depth knowledge of this microbiota in individuals with normal fertility, we do not know what effect dysbiosis might have, and therefore what effect we might inadvertently cause with antibiotics in semen extenders. It would seem to be prudent for us, our animals and the environment, to focus on finding alternatives to antibiotics to reduce the bacterial load in insemination doses.

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Treating benign prostatic hyperplasia in the breeding dog—Strategies for sperm quality improvement

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Benign prostatic hyperplasia (BPH) is the most commonly occurred pathological condition of prostatic gland in dogs (2,7,12,15). Testosterone produced by Leydig cells in testicles is converted by type II 5α-reductase enzyme into biologically active compound dihydrotestosterone (DHT) and stimulates multiplication of the prostatic cell number (hyperplasia) and cell size (hypertrophy) which results in 1) BPH related prostatomegaly (10) and 2) facilitates prostate inflammation and/or infection (6,15). Development of both conditions simultaneously is common, when infection is developing secondary to androgen-dependent BPH (7,15). Clinical manifestation of disease includes locomotory disorders, deterioration of body condition and gastrointestinal symptoms like constipation/tenesmus. Later, with the progressive enlargement of the prostatic gland a serous to sero-sanguineous discharge from urethra, hematuria and hemospermia, ribbon-like appearance of feces and sometimes rectal obstruction are observed (2,11). BPH could impair fertility due to decreased libido, physico-chemical changes of prostatic secretion, blood in ejaculate (3,14).

Treatment for BPH includes the suppression/prevention of androgen synthesis or action. The obvious method of devoiding of testosterone source is surgical orchiectomy. It results in decline of testosterone level in peripheral blood and thus cessation of its stimulatory effects on gland, shrinkage of prostate and disappearance of clinical signs associated with prostate pathology (2,4,7). However, removal of gonads excludes male dog from reproductive use.

Nowadays, pharmacological control of prostatic diseases is frequently demanded. It is indicated to avoid trauma considering welfare implications and health status, in working animals, in old individuals and in animals intended for breeding. In breeding dogs, treatment with pharmacological compounds inhibiting the production or activity of androgens is preferred in order to maintain fertility.

BPH pharmacological treatment should not be applied in cases of prostatic and testicular neoplasia. In cases of BPH complicated by prostate infection effective antimicrobial therapy should be applied before reproductive use of particular male. Several methods of management of BPH in stud dogs have been described in literature. Their effectiveness regarding 1) preserving/improvement of fertility as well as 2) the level of prostate shrinkage is variable. Non-surgical methods of control of prostate pathologies include the use of progestogens (19), 5-α-reductase inhibitors and inhibitors of steroid receptors (1,3,16,21), GnRH agonists and antagonists (5,8,9,13,19), antioestrogens (e.g., aromatase inhibitors) (15) and others. Synthetic progestins exert antiandrogenic action. Many such drugs have been used for decades in the past. Their administration is sometimes considered when short and low-cost therapy is required. Progestin administration results in testosterone concentration drop and shrinkage of prostate gland. Data regarding their effects on sperm quality are contrary (cit. after 15,19). Considering possible risks of hypothyroidism, hyperadrenocortisism and diabetes mellitus, these

drugs currently are not used as commonly as in the past. Inhibitors of 5α-reductase may be classified into two types according to their distinctive mechanisms of action: competitive—represented by finasteride—and non-competitive—represented by episteride. Finasteride administered orally and daily effectively reduces prostate size. The degree and rate of prostate reduction vary with dosage and duration of treatment (15,19). It did not cause detrimental effects on semen quality, however it also did not result in its improvement. Percentage of progressively motile and morphologically normal, live sperm cells did not change during/after treatment. Semen volume may be diminished after administration of this compound but total sperm counts remained unchanged (19). Reports about the clinical use of episteride are scarce. Osaterone Acetate (OA) impairs uptake of testosterone by prostate, inhibits 5-α-reductase and blocks androgen receptors. Due to biliary drug recycling it has very long half-life (> 200 h) after oral administration (23,24). Pills containing different dosages for different weights of dogs are given for 7 consecutive days in BPH dogs (1). OA administration results in decrease of prostate volume by > 30% within a few days and immediate disappearance of prostate-associated symptoms of BPH. Therapeutic effect lasts for 5 months. The next OA may be used again after this period of time. OA may modify composition of seminal plasma (3) and may diminish its volume but it does not change spermatogenesis, libido and sperm characteristics, and dog treated with this drug may be used in reproduction (14,17). Flutamide, a pure androgen receptor blocker, inhibits androgen uptake and binding to the nuclear androgen receptor. Flutamide has been positively tested as a therapeutic drug in BPH cases. However, in most countries, flutamide is not approved for use in veterinary medicine, although it appears safe and effective (15). GnRH analogues are used for temporary reversible pharmacological castration of male dogs (4,5,21,22). Subcutaneous implants containing 4.7 mg and 9.4 mg of deslorelin acetate allow for controlled substance release over a long period of time. Initially after implantation *flare up* effect is observed as a form of exacerbation of reproductive function. Due to continuous drug release, the *down regulation* effect is observed, which results in decrease of the number of GnRH receptors in hypophysis and quiescence of hypophysis-pituitary hormonal axis with drop of testosterone concentration. Fertility is temporary lost. Implantation of BPH and BPH/prostatitis dogs with 4.7 mg DA results in mild *flare up* effect and within 14 days testicles and prostate start to shrink. Prostate-linked symptoms disappear within 2–3 weeks (17). The drop of prostatic volume is more profound in comparison to OA. After 3–4 months, volume of BPH prostate is appr. 35% of pretreatment, initial prostatic value. Volume and echogenicity of prostate observed after implantation resembles prostate of prepubertal dogs (17). Colour Doppler ultrasound reveals lowering of blood perfusion (16,18). Intraprostatic cysts disappear or are significantly smaller. Prostate is diminished in size for 10–12 months post implantation. Thus, this treatment is appropriate in severe cases of BPH. Obviously, dog losses fertility for 6 months when 4.7 mg implant is used (14,17). However, male may be used for reproduction after 6 months after implantation considering, that the therapeutic effect of DA regarding prostate is maintained for nearly one year. The author proposes to use DA in treatment of BPH cases in dogs. If BPH is complicated by prostatitis administration of antibiotics is necessary. To avoid *flare up* effect the administration of OA before implantation is advisable. It facilitates quick improvement of condition of BPH dogs and later DA implantation will enhance and maintain therapeutic effectiveness of therapy for the period of 10–12 months. Then decision should be made regarding next implantation (20) of surgical castration. In breeding dogs, successful reproductive use is possible beyond 6 months after implantation with 4.7 mg implant. GnRH-antagonists used in human medicine as acycline and linzagolix were tested for BPH treatment. Both compounds were effective and appr. 40% decrease of prostate volume was observed after administration. However, there is lack of comprehensive study on their influence on semen quality (15,19). Tamoxifen competitively blocks oestrogen receptors with a mixed antagonist–agonistic effect. Tamoxifen was successfully used for the treatment of prostatic diseases in dogs. Administration of the drug significantly decreased testicular dimensions, libido, peripheral testosterone concentrations and the prostate size accompanied by a reduced ejaculate volume (cit. after 15). Impairment of sperm motility and morphology was observed. Therefore, it should be avoided in dogs used for reproduction. The aromatase inhibitor anastrozole reduced prostate gland volume by more than 20%. Compared to tamoxifen, anastrozole produced less pronounced decreases in libido, testicular consistency and scrotal diameter. In dogs treated with anastrozole ejaculate volume, sperm count, sperm motility and morphology remained unaltered throughout the study. Administration of anastrozole, similarly to tamoxifen, seemed to be efficient in improving prostatic echogenicity to a normal pattern. Taking into account the potentially estrogenic effects of tamoxifen and scarce clinical data on the use of anastrozole, cautious use is advisable. Common problems regarding BPH dog semen include deterioration of motility/morphology (oscillations, looped, coiled tails) originating from physicochemical changes of semen plasma features or erythrocyte admixture. In cases of decreased motility/coiled tails variety of method of sperm cells selection may be used. Separate sperm-rich fraction of ejaculate collected directly into collection tube filled with 1–3 mL of prewarmed diluent enhancing motility may be helpful. Swim-up technique or single layer centrifugation or simple centrifugation and sperm sediment resuspension with TRIS buffer or other medium works efficiently. It should be bear in mind that BPH-prostatitis dogs should be treated with antimicrobial drugs before reproductive use. Other prospective and worthy to study drugs used in human medicine and tested initially for BPH treatment in dogs include eplerenone—an aldosterone receptor antagonist, α1A-adrenergic receptor antagonist silodosin, tadalafil—a phosphodiesterase (PDE)-5 inhibitor, a non-secosteroidal vitamin D receptor (VDR) agonists, mepartricin-semi-synthetic polyene macrolide complex produced by *Streptomyces aureofaciens* NRRL 3878, *Serenoa repens, Urtica dioica* and other extracts. In conclusion, many protocols of pharmacological treatment for BPH in dogs have been described. In stud dogs, the maintenance of fertility is a challenging task. When fertility of BPH dogs should be preserved, the best and safe option is the use of osaterone acetate or finasteride. Deslorelin acetate may be considered in severe cases of BPH when postponement of fertility by at least 6 months is acceptable. BPH cases complicated by prostatitis should be treated with antibiotics before semen is collected for insemination. The new prospective strategies for fertility improvement in BPH dogs include potential use of GnRH antagonists in the future. New methods of treatment for human BPH-related symptoms investigated recently need further research in dogs.

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Laparoscopic reproductive surgery in exotic animals

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Definitive diagnosis in exotics is not always easy due to the diversity of symptoms specific to these species, which can lead to frustration for the clinician. To obtain this definitive diagnosis, the identification of characteristic lesions and the etiological agent is necessary. Laparoscopy is not only a very powerful diagnostic technique, but also a means of performing minimally invasive surgeries that allow for better patient recovery. This presentation aims to provide a non-exhaustive overview of the most commonly used laparoscopic techniques for exotics. The basic equipment for performing a laparoscopy includes a lens and its working channel, a camera and monitor, a light source and a fibre-optic cable, an insufflating system and surgical instruments. Although lenses are available in various sizes, the most frequently used instrument for exotics is the lens with a 2.7 mm diameter, measuring 18 cm in length, and featuring a 30°. This lens transmits the image to the camera. The 30° angle allows for visualisation of small cavities by rotating the lens. The lens is protected by a protective sleeve with a working channel allowing the insertion of 1.7 mm diameter instruments. To visualise the interior of the body cavities, light must be applied. The LED or xenon light source produces this powerful light, which is transmitted to the lens via a fibre-optic light cable. A camera is connected to the eyepiece of the optics and to an image-processing unit, which is in turn connected to a monitor that allows the surgeon to visualise his or her actions. The image-processing unit can be connected to a video acquisition unit, allowing photos and videos of the procedure to be recorded. Finally, a CO_2 insufflator is required to "inflate" the cavities to be explored. This procedure separates the organs from each other and provides the necessary perspective to provide sufficient field of view to explore the cavity. If an insufflator is not available, a syringe can be used for small animals. Sterile saline is preferred for aquatic species, as the use of gas can lead to poor flotation. In birds, since laparoscopy is performed through the air sacs, insufflation is not necessary. There are portable endoscopes that combine a light source, image acquisition and insufflator in a single device. There is a large number of instruments that can be used in different situations. Among the most commonly used, atraumatic forceps are used to mobilise organs and remove parasites, and biopsy forceps are used to collect samples for histological analysis. During laparoscopic surgery, two surgical ports are positioned to create a triangulation for tissue manipulation. Grasping forceps, electrocoagulators, scissors and all other instruments necessary for the surgery are positioned through these ports. Birds are the animals in which laparoscopy is the simplest to perform. Due to their single cavity, like in reptiles, we are speaking about coelioscopy instead of laparoscopy. The camera is positioned in the air sacs, eliminating the need for insufflation. Endoscopy has been used routinely in birds for about twenty-five years as a diagnostic tool (to sex birds) but also for surgery (such as castration), which makes it possible to overcome the need for a very small surgical approach due to the size of the keel. In addition to anaesthetic contraindications, it is recommended not to perform laparoscopy in obese birds or in those suffering from ascites. The most commonly used approach is the left lateral approach through the left caudal thoracic air sac. The bird is anaesthetised and positioned in right lateral recumbency, with its wings held together with a vetrap. The leg is pulled forward and tied, again using a vetrap. A few feathers are plucked at the junction between the last rib and the caudal part of the leg. The incision point is located at the intersection of the last rib and the cruris medialis muscle. A 3mm incision is made on the skin using a cold blade. A fine haemostatic forceps is used to perform a blunt dissection through the muscular plane and enter the caudal thoracic air sac. This approach allows the lungs to be visualised, then bypassing into the thoracic air sac (cranially) to observe the most cranial part of the lungs, the heart and the liver. The optic is then returned to its initial position and moved caudally in order to pass into the abdominal air sac by piercing the thin membrane of the air sac with the optic. The reproductive system, the urinary system, intestines and adrenal glands can be observed in the abdominal air sac. A cross stitch is performed on the muscle incision, and a U-shaped stitch is made on the skin to close the surgical wound with a single-strand, non-absorbable suture. While the technique described above allows for biopsy via the working channel of the protective sheath, for proper endosurgery, a multi-entry technique is required. The first cannula is positioned as previously described for the optic, the second cannula is inserted just posterior to the pubic bone ventral to the cruris medialis muscle, and the optic is placed just cranially to the pubic bone ventral to the cruris medialis muscle. This three-port endosurgical technique allows for sterilisation or mass resection. Laparoscopy in reptiles is also possible. More recently, a number of techniques have been described for lizards, snakes and chelonians. In the latter, endoscopy is particularly useful because it avoids the need for a plastrotomy (cutting and temporary removal of the ventral part of the carapace providing access to the viscera). For lizards, entry points can be made paramedially, just behind

the last rib, or medially, behind the pelvic veins. A 3 mm incision is made between two scales using a cold blade. The scales and muscle wall are lifted using forceps and fine blunt scissors (e.g., Stevens scissors). These allow for dissection of the muscle wall and perforation of the coelomic cavity without damaging any organs. The optic is then inserted into this orifice, and a purse-string suture is made using a single-strand thread around the optic to allow insufflation. A CO_2 insufflation of 3–5 mmHg is sufficient. In iguanas, this approach allows visualisation of the reproductive tract, urinary tract, the lungs, liver, pancreas, small intestine, large intestine and fat pads. Coelioscopy is less commonly performed in snakes because their adipose tissue is diffusely distributed throughout the coelomic cavity, and because most organs cannot be visualised via a single approach. However, the approach can be made between two ribs opposite the target organ. The incision point is chosen using organ-positioning tables based on the snake's length. In chelonians, coelioscopy is more and more used, as the carapace causes a certain amount of interference, reducing the quality of images obtained by radiography and ultrasound. The most commonly used approach is through the prefemoral fossae. The animal is positioned in right lateral recumbency; the pelvic limb is pulled back and taped to the table. After disinfection, a 3mm craniocaudal incision is made in the middle of the prefemoral fossae. The subcutaneous tissue is dissected with blunt scissors down to the coelomic fascia. The coelomic fascia is incised, and the optic can then be positioned. The brownish-red liver is the most easily visible organ in the coelomic cavity. Other organs such as the gonads, salpinx, kidneys, heart, stomach, pancreas, duodenum, bladder, caudal parts of the lungs, can be visualised. All of these organs can be biopsied. This approach can be used to perform endoscopically assisted surgeries. The incision at the level of the false prefemoral is widened in order to be able to exteriorise organs visualised and captured by endoscopy. Thus, ovariosalpingectomy can be performed without having to perform a plastrotomy. Laparoscopy in small mammals is not widely used today, unlike gastroscopy, otoscopy, rhinoscopy and stomatoscopy. However, the use of laparoscopic techniques can replace laparotomy to perform ovariectomy, ovario-hysterectomy or castration (for rabbit and rodents), while being much less invasive, resulting in better recovery for the animal. To explore the abdominal cavity, the approach is made through a 3mm incision at the umbilicus. The skin and linea alba are incised by lifting the muscle wall to avoid perforating an abdominal organ. After inserting the scope, a purse-string suture is placed to maintain a seal and allow for insufflation. While in rabbits and ferrets, insufflation is performed with CO_2, in smaller individuals, a syringe can be installed on a port of the protective sleeve and air can be insufflated. For laparoscopic surgery such as ovariectomy, it is necessary to add two ports for the instruments (grasping forceps and electrocoagulator). A first port is positioned 2–5 cm cranially to the navel and a second 2–5 cm caudally to the navel. The ovary is then grasped with the forceps; the ovarian pedicle is cauterised with the harmonic sealing forceps and then cut with scissors. The ovaries are extracted through one of the ports. The three incisions are closed with a muscle stitch and a skin stitch using a single-strand absorbable suture. In conclusion, laparoscopy is the future of coelomic/abdominal cavity exploration and surgery. While these techniques require investment in both equipment and training time, progress is relatively rapid, and the procedure can always be converted to a conventional laparotomy in the event of a complication. Ultimately, these techniques are comparable in duration to conventional surgery but offer faster and improved recovery outcomes.

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A journey through feline pregnancy – Insights and mysteries

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Cats are different from dogs, this concerns among others the sexual cycle, regulation of follicle selection and growth (1), the prerequisites for establishment of pregnancy, and the endocrinology of pregnancy and parturition. In cats, as in dogs, no feto-maternal signalling leading to establishment of pregnancy is known. At the uterine level, information on changes in lymphocyte subsets, feto-maternal crosstalk and other mechanisms leading to local immunosuppression, allograft acceptance, implantation, decidualisation and the physiology of parturition is scarce. This review shall provide an overview on what is known about establishment and maintenance of feline pregnancy while bridging scientific findings with practical applications. In cats, while spontaneous ovulations are described (2), mating as well as vaginal stimulation and petting, may induce an increase in serum-LH concentrations finally leading to ovulations and formation of corpora lutea (CL). Serum-progesterone concentrations increase during the first week after mating. During the first half of pregnancy, progesterone is produced by CL; later, production and secretion of progesterone are performed by the placenta while CL regress (3, 4). Peak values of P4 are reached between day 11 and 30, with a gradual decline from day 38–39 on until parturition and becoming basal immediately after parturition (5–7). Progesterone serum concentrations are higher in pregnant cats than in non-pregnant cats, especially during the first half of gestation (8); however, this cannot be used as a pregnancy test. Disturbances of pregnancy in the second half of gestation may be accompanied by decreasing progesterone values, which may be used for monitoring of disturbed pregnancies. In pregnant cats, an increase of oestradiol after day 5 and until day 15 post copulation was observed with concentrations staying elevated until parturition; the placenta is an additional source of oestradiol secretion (3, 7). The serum oestradiol concentration varies considerably between individuals, rendering this steroid hormone unsuitable for timing of parturition. In the placenta, relaxin is produced by villous trophoblast cells (9); it is the only pregnancy specific hormone, and the serum concentrations show a similar course as in dogs (10). Measurement of serum relaxin concentration can be used as a pregnancy test from day 20 on, with 100% sensitivity from day 29 on (11). Similarly, urinary relaxin was found to indicate pregnancy (12). The relaxin receptor (RXFP1) is mainly expressed in the pregnant uterus and placenta (9). Intrauterine auto- and paracrine functions of relaxin are currently under investigation; participation in decidualisation is supposed (13). Prolactin is secreted by the anterior hypophysis and concentrations increase towards parturition,

staying elevated during lactation. Prolactin affects luteotropic from mid-gestation on; the dopamine-agonist cabergoline antagonises the effect, causing abortions accompanied by decreasing progesterone values. Dopamine-agonists are less effective during late gestation (14, 15). Prolactin and its receptor might play a pivotal role in decidualisation and placenta development; however, in cats, data are preliminary (13). Prostaglandin (PG) F2α secretion from the placenta also increases towards term, which is mirrored by an increase in the concentration of its metabolite PGFM during the three weeks before parturition, rising functionally during the last week (16). PGFM may be useful as an indicator for approaching parturition, provided a practical test is available. Expression of corticotropin releasing hormone (CRH) mRNA and protein were detected in the feline placenta, in larger decidual cells and foetal trophoblast cells, between days 21 and 35 of gestation. The hormone was in addition detected in maternal blood, increasing from early to mid-pregnancy and reaching values resembling human concentrations during the early third trimester (17). Expression of the specific receptor within the placenta and local functions of CRH warrant further investigation; the question remains, whether in cats, CRH is necessary for establishment and maintenance of pregnancy, as in humans. In the first half of pregnancy, follicle formation despite existing corpora lutea is a physiological observation (18); these follicles may undergo ovulations. In the pregnant cat, as in other mammals, the glycoprotein anti mullerian hormone (AMH), is produced in ovarian preantral and early antral follicles, and supposedly in corpora lutea (8). In one study, serum AMH concentration increased significantly until day 32–40, stayed elevated until day 46, and decreased significantly towards term. AMH serum concentrations were found to be higher than in non-pregnant cats (8). A recent study found that supraphysiological serum concentration may lead to pregnancy loss (19); the function of AMH on the hypothalamus-hypophysis-gonadal axis, the corpora lutea and/or placental steroid hormone secretion warrants investigation. At present, measurement of AMH serum concentration is mainly useful for detection of gonads, whereas the use of AMH for fertility control is under investigation. The mechanisms leading to immunotolerance of the foetal allograft are not sufficiently investigated in cats; embryo and uterine secretion products were supposed to participate (20–22). One study found that a balance in the Treg and TH17 cells is important (23). Recent in-vitro studies investigated the secretion of extracellular vesicles (EV) by maternal and embryo tissue; these exosomes and microvesicles contain DNA, RNA, proteins and other substances for cell-cell communication (24, 25). Whereas, in cats, embryo and uterine EV are not sufficiently investigated, it is known that the feline oviductal epithelium secretes EV, influencing sperm function and fertilisation and probably embryo development (26). Embryo development within the uterus is a few days quicker than in dogs; implantation is completed by day 20. Cats have a zonary, endotheliochorial placenta; however, an incomplete zonary placenta was found in 62.5% of pregnant cats (27). Even though the placenta type is the same as in dogs, mechanisms leading to decidualisation of placental stroma cells are different in detail; among others, markers of decidualisation differ and the nuclear progesterone receptor was not detectable in decidual cells or other placenta cells (13). This may have practical implications for medical care during disturbed pregnancies. Comparable to dogs, markers of endometrial receptivity were detected and upregulated in the feline placenta and uterus; such as transcripts for growth factors, cytokines and enzymes, contributing to degradation of the extracellular matrix, vascularisation, growth, immunomodulation and decidualisation of stromal cells (20, 21, 28). While clinical examinations, measurement of progesterone and relaxin may indicate whether the course of pregnancy is normal, sonographical examination of the pregnant uterus and its contents allows for visualisation of disturbances and assessment of embryo/foetal fitness. The sonographical appearance of the pregnant uterus, embryo/foetus, fetal membranes and placenta during defined stages was described in detail (29, 30). The embryo becomes detectable between day 15 and 17 post copulation, heartbeat becomes visible at approximately day 16, foetal membranes become differentiated around day 20, when the embryo is located in the centre of the gestational chamber. Length of the embryo was described to be 3.3 mm at day 17, 8.7 mm at day 20, 11.2 mm at day 22, 17.4 mm at day 25 and 30.1 mm at day 30 (31). The foetal membranes appear hypoechoic and become well visible after day 30. Inner organs can be differentiated from day 30 on; layered structure of the intestine is visible late, between days 52 and 56 (30, 32). A change in uterine and umbilical artery blood flow, as assessed by Doppler ultrasound, indicated pregnancy disturbances before clinical or ultrasonographical signs were detectable (33). In conclusion, manifold details on establishment and development of feline pregnancy were revealed, bringing up even more questions; however, only few parameters are useful for pregnancy monitoring. Some hormone measurements in serum and urine as well as sonographic examinations during early and advanced pregnancy are useful for diagnostic purposes.

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Canine pyometra and dystocia: Surgical versus medical approach

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Pyometra is a possibly life-threatening uterine infection commonly seen in intact female dogs. It typically occurs due to hormonal influences leading to cystic endometrial hyperplasia and bacterial infection. The condition can progress to systemic illness, sepsis and even death if left untreated. While medical management is an option in select cases, ovariohysterectomy (OHE) remains the definitive treatment for pyometra (Table 1).

TABLE 1 Clinical and management parameters taken into consideration while choosing the treatment option.

Clinical parameter	Medical therapy	Surgery
Use in breeding	Yes/No	No
Age	Young	Old
General condition	Good to excellent	Bad
Cervix	Open	Open/Closed
Other conditions	None	Peritonitis or organ dysfunctions

Aglepristone (a progesterone-antagonist marketed for induction of abortion in mismated bitches) is currently the medical treatment of choice for canine/feline pyometra. Aglepristone blocks the action of progesterone, and subsequently leads to cervical relaxation and promotes uterine contractions, aiding in the expulsion of uterine contents. Aglepristone is supposed to be given twice at 24-hr interval and then repeated after one week (calculated form the first injection, for example, day 0, 1 and 6) and then repeated again depending on need at weekly intervals. A protocol featuring injections on day 0, 2, 5 and 8 seems to be more effective than the day 0-1-6 protocol, and not associated with relapses, although more research is needed on this aspect. Dosage of aglepristone in bitches is 10 mg/kg (the same as for the induction of abortion). Side effects are none or very mild such as itching at the injection site. When using other drugs for treating pyometra, one needs to be concerned about emptying the pus-filled uterus as well as causing luteolysis. When the cervix is open, the uterus can be easily emptied by stimulating myometrial contractility. Of all the drugs causing contraction of the uterine musculature (prostaglandins, oxytocin, ergot derivatives), prostaglandins (PGF and PGE) are the most indicated for the clinical treatment of pyometra because of their more physiological action on the dioestrous uterus (Table 2). Side effects of prostaglandins should not be overemphasised as, when using, that is, doses of 50–100 mcg/kg of natural PGF2a, they are not observed in all bitches, tend to subdue during the course of the treatment and are significantly less common when using dosages of natural PGF2a < 50 mcg/kg. However, side effects of PGF2a compounds tend to be more evident in bitches with pyometra, perhaps because of the deteriorated physical conditions, which characterise the uterine disease. Efficacy of very low doses of natural PGF2a (50 mcg/kg) has been reported for bitches with pyometra. Such a treatment protocol is effective provided that prostaglandins are administered 3 times daily for as long as a vulvar discharge is present.

TABLE 2 Dosages of the most commonly used prostaglandin compounds in bitches to induce luteolysis and cause uterine contractility (PGF2α) or just uterine contractility (PGE).

Prostaglandin	Daily dose in the bitch	Administrations/ day
PGF2α, Natural compounds	Bitch – 50–80 mcg/ kg (0.05–0.1 mg/kg)	3–2, SC
PGF2α, Cloprostenol	Bitch – 1 mcg/kg (0.001 mg/kg)	1, SC
PGF2α, Alphaprostol	Bitch – 20 mcg/ kg (0.02 mg/kg)	2, SC
PGE, Misoprostol	Bitch – 10 mcg/ kg (0.01 mg/kg)	2, per os

Aglepristone will cause myometrial contractility but not immediately, only due to the removal of the P4-induced blockade—and in fact, myometrial contractility will start a few days after treatment onset—while prostaglandins act more rapidly. Therefore, aglepristone works well in conjunction with prostaglandins, and this association can be used whenever the uterus is filled with large quantities of pus (Table 3).

Using antibiotics—Antibiotic treatment should be specific as soon as culture results and antibiogram are available (a broad spectrum antibiotic such as ampicillin at 22 mg/kg 3 times/daily can be started in the meantime) and it should also continue for as long as a purulent vulvar discharge is present (which may persist for a few days after uterine diameter has gone back to normal again).

Length of treatment—In order for a pus-filled uterus to be emptied prostaglandin administration should be continued as long as a vulvar discharge is present. The decision on when to stop the treatment should be based on a) evaluation of uterine dimensions before, during and after therapy (in order to confirm that uterine diameter has gone back to normal) as well as b) disappearance of fluid within the uterine lumen detectable on ultrasound; in normal conditions the uterine lumen is not detectable, but it becomes distinguishable when liquid accumulates within the uterus.

Risk of uterine rupture—The administration of myocontractants may be dangerous if the uterus is fully dilated with pus and/ or the uterine wall is thin and atrophied or the cervix is only partially dilated, as this may cause either a) the pus to be forced backward into the uterine tubes and then into the abdomen or b) the uterine wall to rupture.

In general, the use of an Elizabethan collar is applied to prevent ingestion of vulvar discharge and fluid therapy is advocated to correct dehydration and treat toxic shock

TABLE 3 Example of a treatment protocol for a bitch with open-cervix pyometra treated for 15 days.

	Days of treatment														
Aglepristone	1	2						8							15
PGF2α/PGE			3	4	5	6	7	8	9	10	11	12	13	14	
Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Surgical treatment including an OHE is still considered as the safest and most effective treatment option in canine pyometra. Surgery results in the removal of the source of infection and bacterial products and also prevents the recurrence of the disease at a later age. However, despite being the safest and most effective treatment option, surgery permanently eliminates the breeding ability of the female. Surgery is the only recommended treatment option in cases of stump pyometra.

Key points in the surgical treatment of pyometra

1. An open OHE is the choice of surgical technique. Only dogs with mild to moderate pyometra (a uterine diameter of < 20 mm in dogs below 10 kg of body weight and < 40 mm in larger dogs) should be considered as good candidates for laparoscopic surgery. Open OHE should be the preferred surgical technique in dogs with perforations, peritonitis or significant uterine distension.
2. Adequate intravenous fluid therapy should be applied to the patient before, during and after the surgery to correct hypotension, hypoperfusion, shock, dehydration, acid-base balance and electrolyte abnormalities, coagulation disturbances and organ dysfunctions.
3. A standard OHE with slight modifications should be performed as urgent as possible due to the risk of endotoxaemia and sepsis when the uterus remains in situ. Spay hooks should not be used and great care must be taken not to rupture the uterus during the manipulations. In case of a rupture, the abdomen should be rinsed with several litres of warmed physiologic saline solution and a closed suction (or open) drainage should be considered.
4. The use of vessel sealing device may reduce the anaesthesia and surgery time significantly with similar intra-operative complication rates. After the uterus is removed, the uterine stump is cultured and then cleaned of any remaining discharge. Oversewing the uterine stump is not necessary and may increase the risk of granuloma or abscess formation.
5. The patients should be intensively monitored after the surgery and supportive care should be continued as needed. In uncomplicated cases, 1 day to 2 days of postoperative hospitalisation is usually sufficient. The need for continued supportive care and antimicrobial therapy is evaluated several times daily on case by-case basis.
6. Clinical and laboratory predictors of peritonitis and prolonged postoperative hospitalisation (≥ 3 days) in surgically treated cases include inappetence, fever/hypothermia, depression and pale mucous membranes, reduced packed cell volume, leukopenia, azotaemia, sustained or increased concentrations of C-reactive protein, serum amyloid A, procalcitonin and cell-free DNA.
7. The overall complication rate for surgically treated pyometra is reported to be up to 25%, the most common being peritonitis (12% of the patients).
8. The initial choice of antimicrobial drug should be effective against *E. coli*, which is the most commonly isolated pathogen in cases of pyometra. Thus, the primary and the secondary recommendations include the fluoroquinolones such as enrofloxacin or marbofloxacin and amoxycillin/clavulanate. In cases of life-threatening peritonitis, severe sepsis or septic shock a combination of antimicrobials is usually recommended instead of a single agent.

Dystocia, or difficult labour, is a common reproductive emergency in dogs, particularly in brachycephalic and toy breeds. Prompt intervention is crucial to ensure the survival of both the dam and the neonates (Table 4).

TABLE 4 Parameters to be considered while choosing the most appropriate treatment in a case of dystocia. Caesarean section (CS).

Parameter	Conservative approach	Surgical approach
Fetal presentation/ position/posture	Normal	Abnormal
Cervix	Open	Close
Birth canal	Wide enough No deformation	Narrow Deformation
Fetal size	Normal	Large
Number of fetuses in the uterus	2 (−3)	≥ 4
Fetal heart beat (per min/fetus)	> 200 – normal < 180 – fetal stress	150–170 (consider CS) < 150 – (immediate CS)
Duration of the second phase of birth	< 5.5–6 h	> 5.5–6 h
General condition of the mother	Good/Very Good	Bad/Good/ Very Good
Other	Last puppy is dead No obstruction	No success in the medical approach

The usual medical approach to a case of failure to complete parturition includes stimulation of spontaneous uterine contractions by exercising (walking) the bitch or by feathering her vagina, administration of ecbolic drugs (once foetal oversize/malpresentation and obstruction of the birth canal or uterine rupture are ruled out) and the use of glucose solutions. Manipulative treatment is generally successful in mild dystocia cases with normal size puppies. Oxytocin is frequently used in cases of non-obstructive dystocia caused by uterine inertia. However, oxytocin is generally believed to induce placental detachment, and therefore, its administration during dystocia is advised only if proper follow up care can be provided, due to risk of foetal death. Based on this assumption, several authors advise early surgical intervention if two or more injections of oxytocin administered at 20–30-min intervals fail to cause expulsion on the ground that placental separation may occur. Current recommendations are to start with doses of oxytocin of 0.25–4 IU SC and to proceed with multiple administrations of lower doses (0.25–0.5 IU) every 30 min. In most cases, one SC injection of a low dose followed by a second injection 30 min later are sufficient to allow expulsion of a pup and if not, a more aggressive (surgical) intervention should take place. Administration of calcium may help the myometrium to respond to oxytocin as calcium increases the strength of uterine contractions (as opposed to oxytocin increasing the frequency of contractions). Bitches not responding to oxytocin treatment often respond well to calcium, even if pre-treatment calcium concentrations are normal. A bolus of 10% calcium gluconate (0.2 mL/kg) should be administered slowly IV keeping the heart under constant auscultation to make sure maternal heart frequency remains normal during treatment.

Some authors recommend the administration of glucose to bitches with dystocia. However, blood glucose concentrations should be measured prior to this as hypoglycaemia is fairly rare in bitches with dystocia.

Surgical treatment consists of an episiotomie and a CS. Caesarean section is required in 59–65% of bitches presenting for dystocia. Caesarean section is classified as elective and emergency CS according to urgency and as normal or en bloc CS depending on the preservation/removal of the uterus.

Key points in the surgical treatment of dystocia

1. Anaesthesia should not be induced before the staff is familiar with their duties to maximise efficiency and the location of the equipment and the medications.
2. Intravenous fluids should be administered at the intraoperative rate (10 mL/kg/h) during preparation for surgery.
3. Preoxygenation using a facemask for five minutes prior to induction is recommended to replenish oxygen reserves before surgery. However, end the procedure if the bitch shows severe discomfort or signs of stress.
4. The abdomen should be adequately clipped and cleaned outside of the surgery room and before anaesthesia to reduce the induction-to-delivery time and thus reduce neonatal mortality.
5. Some authors recommend tilting the bitch 15° to 20° off midline to prevent supine hypotension syndrome. However, investigators failed to identify any change in systemic arterial blood pressure regardless of the position therefore dorsal recumbency is an acceptable position for the bitch during surgery.
6. Most commonly, a midline incision is made from cranial umbilicus to just above the pubis. The pregnant uterus should be exteriorised. However, in cases when the uterus is too large for manipulation, only one uterine horn should be exteriorised. Trying to exteriorise the entire uterus with large litter may result is rupturing.
7. The uterus should be isolated and packed off with surgical laparotomy sponges. However, small surgical sponges should not be used as the use of these may result in their unintentional retention. Either a single incision is made in the body of the uterus and all fetuses are manipulated in a milking fashion to this incision for extraction or more incisions should be performed depending on the number of fetuses.
8. After extraction, the fetal membranes are broken open immediately by the surgeon and placenta is removed. If unable to remove without risking significant haemorrhage, they may be left in situ to pass naturally.
9. Once all neonates have been removed, the uterus should be palpated and visually inspected from cervix to ovaries. The uterine incision may be closed with absorbable suture and a single or double-layer suture pattern.
10. Oxytocin administration after closing the uterine incision(-s) locally or systemically facilitates involution and the passage of any remaining placentas as well as significantly reduces the risk of uterine bleeding.
11. Following the surgery, antimicrobial and pain management protocols should be applied to prevent the risk of a post-surgical infection and to prevent the risk of milk letdown problems triggered by the post-operative pain of the mother.
12. Ideally, patients are discharged as soon as possible and do not have a prolonged hospitalisation due to the risk to the immune-naive neonates.

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Potential relevance of sperm and seminal plasma components for fertilization and early embryo development in small animals

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In humans, infertility affects one in six couples and may be primary (never conceived) or secondary (previously conceived but unable to now). Male factors contribute significantly to infertility cases, accounting for 30–35% of cases, similar to female factors, whereas 30–40% involve both partners, and approximately 20% remain unexplained. In farm animals, the selection of sires makes it more difficult to find an infertile individual, but subfertile males

exist. In companion animals, such as dogs and cats, infertility may also be diagnosed in males. One of the most important causes underlying male infertility is semen quality, which may be one of the leading causes explaining impaired fertility outcomes when assisted reproductive techniques are employed. While most of the research in this realm has been conducted on humans and livestock, compiling evidence may be applied to small animals. To understand the relevance of semen, one needs to first refer to its components, that is why the plenary lecture will approach them. Semen comprises sperm (cellular fraction) and seminal plasma (liquid fraction). With regard to sperm, they are highly specialised cells that are formed in the testes through spermatogenesis and mature during their transit throughout the epididymis. Three parts can be distinguished in the sperm cell: the head, which contains the nucleus and the acrosome; the connecting piece or neck; and the tail, where mitochondrial, principal and end pieces can be differentiated. During epididymal maturation, sperm undergo different changes, including plasma membrane remodelling, chromatin condensation and incorporation of proteins through extracellular vesicles. Upon ejaculation and deposition within the female, sperm travel—with a combination of active and passive transport—through its reproductive tract, encountering several selective barriers. In the uterus, sperm are known to trigger the formation of neutrophil extracellular traps (NETosis), which allows for the selective removal of excess and lower-quality sperm. Sperm that survive this removal continue to advance until they reach the utero-tubal junction, which also represents another selective barrier before entering the oviduct. Moreover, in the isthmus of the oviduct, high-quality sperm bind the epithelial, forming a reservoir until ovulation. Near ovulation, sperm detach from these oviductal epithelial cells, undergo capacitation and trigger the acrosome reaction necessary to penetrate the oocyte vestments. In the next paragraphs, the potential contribution of sperm components such as centrioles; integrity and condensation of chromatin; and the transcriptome, epigenome and metabolome is described. Centrioles are crucial organelles located in the sperm's connecting piece. Mature sperm contain proximal and distal centrioles that, in most mammals (including companion animals) except rodents, are inherited by the fertilised oocyte, which lacks its own centrioles. These paternal centrioles play a crucial role in the organisation of the zygote cytoskeleton, mediating pronuclei migration and driving cell cleavage. In humans, abnormal centrioles have been identified in sperm from infertile men and have been suggested to underlie the higher proportion of aneuploid embryos in humans compared to rodents. Sperm chromatin is formed by DNA and nucleoproteins. Unlike somatic cells, where DNA is bound to histones, 85–90% of histones are replaced with protamines during spermiogenesis. Thus, protamines represent the most abundant nucleoproteins in the sperm chromatin. This replacement transforms canonical nucleosomal chromatin into toroidal structures, providing greater DNA condensation and protection. Yet, insufficient replacement of histones by protamines (chromatin immaturity) is associated with an abnormal degree of protamination and chromatin condensation, which may lead to impaired embryo development. Furthermore, while all mammalian species have protamine 1, only some specific species, such as humans, horses and rodents, have another type of protamine (protamine 2). In these species, alterations in the ratio between protamine 1 and protamine 2, which is specifies-specific, are associated with male infertility and poor embryo quality. The other sperm chromatin component, the DNA, is also crucial for successful reproduction. Damage can occur in single or double DNA strands and is associated with male infertility, impaired embryo development and potentially adverse offspring health outcomes. Factors contributing to DNA fragmentation include lifestyle habits (smoking, poor nutrition), diseases (diabetes, obesity), advancing male age, altered histone-to-protamine ratio, insufficient chromatin condensation, abortive apoptotic changes and oxidative stress. While oocytes can repair some paternal DNA damage, their capacity is limited and is influenced by female factors, including age. Remarkably, unrepaired paternal DNA damage may pass to the embryo, potentially causing developmental arrest, implantation failure, miscarriage and reduced clinical pregnancy rates. The sperm proteome is another component that appears to play a significant role during gamete fusion, oocyte activation and potentially the very early stages of embryo development. Interestingly, in humans, differences between the proteomes of fertile and infertile men have been reported. There are particular sperm proteins that have been demonstrated to be involved in sperm-oocyte fusion. One of the most important is IZUMO1, which is located in the equatorial region, and interacts with JUNO, its oocyte membrane receptor. Other proteins like TMEM95 and tetraspanins CD9, CD81 and CD151 also seem to be crucial for fertilisation. Another sperm-specific sperm protein is phospholipase C Zeta (PLCζ), which activates the oocytes upon gamete fusion, triggering calcium oscillations and alleviating it from the metaphase-II arrest. Sperm lacking functional PLCζ often fail to activate the oocyte, which results in total fertilisation failure. As far as post-fertilisation events are concerned, evidence supports that sperm proteins such as desmocollin 3 (DSC3), which regulates cell adhesion in blastomeres before embryo genome activation, and choline-phosphate cytidyltransferase A (PCYT1A), implicated in blastocyst development and implantation, could also have an important function. With respect to the transcriptome, one must first note that sperm are generally thought to be transcriptionally silent, notwithstanding they contain residual mRNAs that may contribute to early embryonic development. In effect, various transcripts have been hypothesised to play roles in immunotolerance during implantation (PSG1, HLA-E) and early embryonic divisions (SSFA2, SESN1). Moreover, the levels of specific transcripts like protamine-1 (PRM1), protamine-2 (PRM2) and POU5F1 in sperm have been observed to be correlated with embryo development outcomes. Despite this, further research is required to ascertain the specific functions and degradation timeline of such mRNAs. The sperm epigenome encompasses DNA methylation, histone modifications and non-coding RNAs, all potentially contributing to embryo development. Sperm DNA methylation patterns are established during spermatogenesis, with active demethylation of paternal DNA occurring in early embryos before genome activation. Alterations in sperm DNA methylation profiles are linked to male infertility, impaired embryo development and miscarriage. The 10–15% of histones retained in sperm chromatin may carry epigenetic marks and are often associated with developmental genes. Non-coding RNAs in sperm, including microRNAs (miRNAs), small interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs), may regulate gene expression during fertilisation and embryo development. For instance, miR-34c plays an essential role during the first cleavage in mice, while circCNOT6L participates in the transition from zygote to 2-cell stage. Regarding the metabolome, previous studies performed in humans revealed significant differences between fertile and infertile patients. Infertile men

often show lower levels of L-carnitine, polyunsaturated fatty acids, certain amino acids and biogenic amines in seminal plasma. These deficiencies may impact energy production pathways and signalling mechanisms. The sperm metabolome also differs between young and aged men, with specific metabolites more abundant in older individuals. Moreover, studies in animal models further support that sperm metabolic profiles influence fertilising ability and embryo viability, suggesting that basal metabolism in mammalian sperm is relevant for successful reproduction. Apart from sperm, the component of semen, the seminal plasma, plays an essential role in reproductive success. Indeed, while the seminal plasma has traditionally been merely regarded as a transport medium for sperm, it actively interacts with the female reproductive tract. In the mouse, the seminal plasma modulates the expression of cytokines and regulatory T-cell genes in the endometrium, increasing maternal tolerance to paternal and fetal antigens and facilitating embryo implantation. Although other mammalian species, such as humans and cattle, ejaculate into the vagina rather than the uterus, the seminal plasma factors in those species could potentially influence the uterine environment by adhering to the sperm surface. In effect, studies performed in separate species suggest that exposure to seminal plasma can increase pregnancy rates after embryo transfer, supporting its role in priming the endometrium for implantation. A major component of the seminal plasma is extracellular vesicles, primarily epididymosomes and prostasomes, which transfer proteins and miRNAs to sperm or endometrial cells. For instance, epididymosomes, produced by the epididymis, fuse with epididymal sperm, transferring proteins that influence motility and capacitation. Prostasomes, produced by prostate epithelial cells, modify the sperm plasma membrane composition, regulate motility and capacitation and may protect sperm from the female immune system. In humans, seminal plasma vesicles have been suggested to fuse with endometrial stromal cells, induce decidualisation and increase prolactin secretion. In conclusion, semen factors, which include sperm and seminal plasma, play a crucial during fertilisation and early embryo development and also seem to influence the uterine environment and even implantation. Sperm components such as centrioles, chromatin condensation, DNA integrity, transcriptome, epigenome and metabolome appear crucial for what occurs during fertilisation and beyond. Furthermore, the seminal plasma contributes through immunomodulation and extracellular vesicles that shape both sperm function and the female reproductive tract. These findings enhance our understanding of paternal factors in infertility and suggest that further research is needed to fully comprehend their significance for embryo development, implantation and offspring health.

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New concepts in neonatology: A healthy gut is a healthy puppy

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The neonatal period is a crucial stage in puppy development, marked by rapid physiological changes and the establishment of the gut microbiome. The gut microbiota plays an essential role in immune maturation, digestion and protection against pathogens. Given that 10–25% of puppies experience gastrointestinal disorders in their first year, understanding the factors that shape neonatal gut health is vital. This abstract summarises key insights into maternal microbiota transmission, the impact of birth mode, early microbial colonisation and its long-term effects on health. The maternal microbiota undergoes dynamic shifts during pregnancy, involving the vaginal, gut and oral microbial composition. In humans, vaginal microbiota is dominated by *Lactobacillus* spp., which protects against pathogens (1) and reduces the risk of preterm birth. Canine studies indicate that maternal vaginal microbiota influences neonatal colonisation, with vaginally delivered puppies inheriting microbes from their mother's vaginal flora (2). In caesarean-section (CS) deliveries, neonatal microbiota is instead shaped by maternal oral bacteria, highlighting alternative microbial transfer routes (2). The maternal gut microbiota is believed to have a key role in maternal health and fetal development. It is a rich ecosystem of bacteria, viruses and fungi that undergoes significant modifications during gestation, with studies in humans associating a reduction in gut microbiota diversity with pregnancy complications such as preeclampsia and gestational diabetes (3). In dogs, the maternal gut microbiota is believed to play a critical role in transferring essential microbial populations to the offspring, influencing its long-term health and development. However, comprehensive studies on canine gut microbiota during pregnancy are limited, and further research is needed to elucidate the specific mechanisms involved. Given the recognised role of the maternal gut microbiota, any factor affecting its composition may also influence fetal development. Diet, antibiotic use, infections and stress significantly influence maternal microbiota. Dietary changes affect gut microbial composition, impacting neonatal immune and metabolic development, with high-fat or undernourished diets leading to metabolic dysfunction and restricted fetal growth in humans (4). Furthermore, short-chain fatty acids (SCFAs) regulate regulatory T cells (Tregs) and modulate cytokine levels that cross the placental barrier, shaping immune tolerance and response (5, 6). Furthermore, beneficial bacteria like *Bifidobacterium* and *Lactobacillus* reduce allergy and autoimmune risks in humans (7), while segmented filamentous bacteria (SFB) promote Th17 cell development for mucosal immunity in murine models, contributing to mucosal immunity and defence against pathogens (6). Given the conserved nature of immune system regulation among mammals, similar mechanisms may exist in dogs. The maternal microbiota is also involved in fetal metabolic programming by regulating maternal metabolism, nutrient availability and microbial metabolite production. During pregnancy, maternal gut microbiota undergoes compositional shifts that enhance energy storage and nutrient absorption, ensuring adequate fetal growth (8). SCFAs, which are produced by microbial fermentation of dietary fibres, serve as key signalling molecules that influence fetal metabolic pathways, including glucose and lipid metabolism (9). In humans, alterations in maternal microbiota, linked to conditions like gestational diabetes, can disrupt fetal metabolic development (10) by increasing insulin resistance, inflammation and lipid metabolism imbalances. Similarly, studies in animal models suggest that maternal microbiota contributes to fetal metabolic health through mechanisms such as microbial modulation of bile acid metabolism and vitamin synthesis (11). While direct evidence in dogs is scarce, it is reasonable to assume that maternal microbiota influences canine fetal metabolism in similar ways, although

species-specific differences likely exist. One notable distinction is that dogs have an endotheliochorial placenta, which limits direct microbial metabolite exchange between mother and fetus, unlike the haemochorial placenta found in humans (1). Consequently, while maternal microbiota influences fetal development in dogs, postnatal microbial colonisation via maternal grooming and environmental exposure may be even more critical (12,13). In humans, prenatal microbial exposure occurs mainly through the placenta and amniotic fluid (14), whereas in dogs, early microbial colonisation is additionally influenced by the whelping environment and maternal grooming behaviours (15). Furthermore, while probiotic supplementation and dietary interventions are commonly employed in humans to support gut health (16), these strategies are not as widely practiced in veterinary medicine. In conclusion, the maternal microbiota plays a crucial role in prenatal immune and metabolic development (17, 18), and by integrating human and animal research, we can optimise microbial composition to improve neonatal health across species.

Development of neonatal gut microbiome happens from colonisation throughout early life. Previously, it was believed that newborns were sterile at birth, with microbial colonisation occurring postnatally (6). However, recent research suggests that microbial communities exist in the placenta, amniotic fluid and meconium, indicating that colonisation may begin in utero (3, 19). Despite this, the most significant colonisation occurs at birth when neonates are exposed to the mother's vaginal and fecal microbiota (20). In caesarean deliveries, neonates acquire different microbial profiles, often resembling maternal skin or environmental bacteria. Colostrum and breast milk further contribute to microbiota development by supplying nutrients and bioactive compounds that promote beneficial bacteria and immune maturation (21). Microbial succession occurs as the gut matures, with competitive exclusion ensuring the dominance of beneficial species involved in digestion, nutrient absorption and immune function (22). Dysbiosis, or microbial imbalance, can have long-term consequences, leading to gastrointestinal and systemic diseases (23). Advances in DNA sequencing and metagenomics have improved our understanding of microbiota composition, but knowledge gaps remain regarding the specific microbial communities in puppies and their role in digestive and immune health (24, 25). The establishment of a stable gut microbiota in puppies begins immediately after birth and is crucial for immune maturation, digestion and energy extraction. Guard et al. (26) observed significant microbial changes from birth to 42 days, with diversity increasing over time. Initially, Firmicutes, including *Lactobacillus* and *Clostridium*, dominate the microbiota, supporting carbohydrate fermentation and gut homeostasis. As puppies mature, *Bacteroidetes* and *Fusobacteria* become more prevalent, reflecting dietary and environmental adaptations essential for metabolism and pathogen defence (17). Microbial shifts occur rapidly: microbial profiles at Day 2 differ significantly from those at Days and 28, suggesting long-term health implications for disease susceptibility, allergies and metabolic disorders (23). Maternal microbiota plays a direct role in shaping neonatal gut composition through birth, lactation and skin contact. Maternal milk provides essential oligosaccharides that promote beneficial bacteria like *Bifidobacterium* and *Lactobacillus* (27). Environmental exposure also affects microbial colonisation. Puppies raised in sterile conditions may develop a less diverse microbiome, weakening immune resilience, while exposure to diverse microbes fosters a robust gut microbiota, improving pathogen resistance (28). Other factors, such as birth weight, are associated with gut microbiota development. Garrigues et al. (29) found that low birth weight (LBW) puppies exhibit delayed microbiota maturation, with higher levels of *Escherichia coli* and *Clostridium perfringens*, while high birth weight (HBW) puppies have more beneficial bacteria like Faecalibacterium and Bacteroides, indicating a more mature gut microbiota (29). Nevertheless, although LBW puppies initially showed microbial immaturity, their microbiota gradually aligned with NBW and HBW puppies. Research on mammals suggests early microbiota imbalances can influence obesity risk, metabolic disorders and inflammatory diseases (30, 31). Early-life microbiota development is critical for long-term health, and understanding these processes can optimise rearing strategies, improve nutrition and reduce disease risks in adulthood.

Fading Puppy Syndrome (FPS) is a fatal neonatal condition characterised by weakness, reduced nursing and high mortality within the first two weeks of life. It has a multifactorial aetiology, including congenital anomalies, infections, environmental stressors and inadequate maternal care, with microbial dysbiosis emerging as a key factor. Dysbiosis is linked to gastrointestinal disturbances, systemic infections and failure to thrive in neonates (32). Puppies deprived of colostrum lack essential immunoglobulins and commensal microbiota, increasing their susceptibility to infections (12). Antibiotic use may further disrupt microbial balance, exacerbating FPS risks (33). FPS is associated with persistent diarrhoea, malabsorption, immune suppression and bacteraemia, often linked to microbial disruptions leading to systemic inflammation. FPS-affected puppies have reduced *Lactobacillus* and *Bifidobacterium* levels, with increased *Clostridium* and *Escherichia coli*, bacteria linked to septicaemia and mortality (34). Microbiome profiling suggests gut composition at birth may predict FPS-related mortality. Preventative strategies include optimising maternal health, ensuring colostrum intake, maintaining hygienic whelping conditions and using probiotics and prebiotics to stabilise gut microbiota (35). Future research should explore microbiome-targeted interventions to improve survival rates, leveraging metagenomic and metabolomic analyses to identify microbial biomarkers predictive of FPS risk. These insights may revolutionise neonatal care and FPS prevention strategies (29). Future research is essential to understand how early-life gut interventions impact lifelong immunity, disease resistance and metabolism in puppies. Investigating optimal timing and methods for microbiome manipulation could refine veterinary care. Personalised neonatal strategies, considering genetic and environmental factors, may enhance health outcomes. Advances in microbiome-targeted therapies, such as next-generation probiotics and precision nutrition, hold promise for selectively promoting beneficial bacteria and improving gut balance. Integrating these innovations into veterinary practice could enhance growth, immune function and disease resilience. Continued research will drive groundbreaking treatments, transforming neonatal care and ensuring better long-term health for canine populations. In conclusion, a healthy gut is vital for puppy growth, immunity and long-term health. Early microbiota development influences digestion and disease resistance, with disruptions increasing infection risks. Probiotics, prebiotics and proper nutrition support gut balance,

enhancing immune resilience. Prioritising gut health in veterinary care improves neonatal outcomes, prevents chronic diseases and ensures healthier, more resilient adult dogs.

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Reproduction in Domestic Animals

ABSTRACT

Oral Presentations

7422 | The microbiome of the ejaculate of male breeding dogs: A comparative analysis of culture-dependent and culture-independent analytical methods

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The 16S rRNA bacterial gene sequencing, an established next-generation sequencing method, allows for precise determination of the microbial composition of a sample and opens new opportunities for interpreting the influence of the microbiome on the ejaculate of breeding dogs, as shown in humans (1) and other animal species (2,3). The aim of this research was to investigate the composition, diversity and alterations of dogs' raw semen microbiome by combining culture-dependent and independent approaches for its characterization.

Privately owned male breeding dogs ($n=46$) with no pre-existing disorders of the reproductive tract were included in the study. Prospects were 1–8 years old (mean 4 years) with a minimum weight of 10 kg (mean 27.4 kg) belonging to 20 different breeds. All dogs initially underwent a general clinical examination followed by manual semen collection, concluded with an ultrasound examination of the reproductive tract. The semen-rich-fraction (400 µL) was used for microbiological examination: culture dependent investigation of bacteria and mycoplasma was done according to clinical standard methodology (4). The DNA was extracted with DNeasy PowerSoil Pro Kit, followed by PCR amplification of the 16s-region V3–V4. Library preparation and analysis were performed by using Illumina MiniSeq System and protocols.

A total of 44 different pathogens were isolated by bacterial culture, i.e. on average 2.8 per semen sample (range: 0–13). Mycoplasmas were detected in 76% of the samples ($n=35$). The most common pathogens were *Canicola haemoglobinophilus* ($n=24$), *Mycoplasma cynos* ($n=18$), and *Mycoplasma canisemінis* ($n=12$). Using culture independent methods, 69 different pathogens (>1% in the corresponding sample) were identified, of those 28 were identified in several samples. On average, 5.8 pathogens were detected per sample (range: 1–20) with an average of 22,793 reads per sample. Mycoplasmas were determined in 97.7% of the samples ($n=44$). The pathogens most frequently detected by 16s analysis were again *Canicola haemoglobinophilus* ($n=28$), *Mycoplasma cynos* ($n=23$) and *Ureaplasma canigenitalium* ($n=23$). Regarding the number of colony-forming units >100/mL, the agreement of the occurrence of pathogens among both diagnostic methods was 92.4%. Further statistical analysis showed no significant influences of age, breed, feeding, previous mating or subclinical changes in the prostate on the microbiome composition of raw semen.

To the best of our knowledge, in this investigation mycoplasma was detected via 16S rRNA bacterial gene sequencing in dog ejaculates for the first time. The results indicate that the autochthonous microbiome in the ejaculate of healthy stud dogs is diverse with a large proportion of mycoplasma. In contrast to studies of the microbiome of the male genital tract in other species, there is a great agreement in its composition determined by culture-dependent and independent approaches.

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6683/7815 | Teaching tip – A student-centered approach to teach ethics in small animal breeding

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A high proportion of dogs and cats are having health and welfare problems that are related to breed-specific conformation or

inbreeding. According to a survey among veterinary students they have severe concerns regarding the wellbeing of some breeds and a critical view on ethics in small animal reproduction. In addition, they request more teaching in this area (1).
Teaching concept and results: In the veterinary curriculum at our faculty four teaching hours of 45 min were used for a new teaching concept on "Ethics in small animal breeding" for 4th year students in March 2025. In total, 21 students preparing for their small animal clinical rotation participated in this project.
In a short (20 min) introduction information about animal protection law and a brief description of specific health issues was given. The new legislation in the Netherlands was explained and then a shortened version (8 min) of a video (http://www.youtube.com) with statements against this law was shown. Afterwards a discussion was initiated which was markedly intensive.
Then, students were tasked with preparing 5-min group presentations on a topic of their choice within 1 h. Some suggestions for topics were given. The selected topics included gene test results and strategic breeding, the Merle factor, Shar Pei, Rhodesian Ridgeback, Scottish Fold, and Feline Hypertrophic Cardiomyopathy. All these topics were well prepared and presented and led to questions and comments from the audience. In the final 10 min the role of veterinarians and a published self-declaration about reproductive medicine services of the university repro groups in Germany, Austria and Switzerland (2) were discussed. The session concluded with a call for collective, professional responsibility in ethical breeding practices.
A total of 16 students participated in the evaluation via mentimeter.com, though not all responded to every question. The students (15/15) appreciated that the topic was chosen and liked (15/15) the group work. The time for group work was graded as adequate ($n=8$) or too long ($n=8$). Some students mentioned their need for more fundamental information about diseases related to overtyping and inbreeding. The overall grade given by the students was very good ($n=2$), almost very good ($n=11$), and good ($n=3$). Lower grades were not given.
Veterinary students clearly appreciate the teaching of topics such as overtyping and inbreeding. To date it is not known if and how these aspects and problem-solving strategies are taught in the veterinary curricula. The presented concept can be used in small group teaching settings, engages students and fosters discussion of the topic.

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7106/7860 | Alcoholization as an alternative treatment for prostatic cyst and abscess in dog

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Prostatic abnormalities, including benign prostatic hyperplasia (BPH), cysts, and abscesses are prevalent in older male dogs. Current treatments for prostatic cysts and abscesses often involve surgical procedures like reduction and omentalization (1). Recently, ultrasound-guided drainage combined with platelet-rich plasma injection has emerged as a promising minimally invasive technique, though it remains rarely used in routine practice (2). This study describes the treatment of prostatic cysts and abscesses via ultrasound-guided percutaneous drainage and alcoholization (alcohol sclerotherapy), a technique first described in 1999 (3). The aim is to contribute to the limited number of cases available in the literature and to detail our experience regarding methodology, outcomes, and complications.
Forty-three dogs of various breeds, ages, and weights with a single prostatic cavity measuring 1–6 cm in diameter were enrolled. Based on symptoms, clinical and ultrasound findings, and cytological, bacteriological, and biochemical analysis of cavity fluid, the dogs were categorized into two groups: Group A (22 dogs with prostatic abscess treated via alcoholization) and Group B (21 dogs with BPH-associated cyst). Group B was further subdivided into three subgroups: Group B1 (7 dogs treated only with finasteride), Group B2 (7 dogs treated via cyst alcoholization), and Group B3 (8 dogs treated with finasteride followed by alcoholization after 60 days). Alcohol sclerotherapy was performed once, under sedation, through percutaneous drainage followed by ultrasound-guided injection of absolute ethanol equivalent to 1/3 to 1/4 of the aspirated fluid volume. After 25 min, the ethanol was drained and replaced by a smaller volume (1/10) of the aspirated fluid. Ultrasound assessments were performed at specific intervals: days 0, 5, 30, and 150 for Group A and days 0, 30, 60, 90, and 150 for Group B. Data were analyzed using the ANOVA test.
In Group A, 76.19% of abscesses resolved within 30 days; all were resolved by day 150. In Group B subgroups, statistically significant differences were observed in cyst outcome at day 150 and prostatic dimension. Notably, all dogs in Group B3 demonstrated cyst diameter reduction within 30 days, and complete resolution occurred in 62.5% of the dogs by day 150.
Discussion and Conclusion. Alcohol sclerotherapy proved effective for treating prostatic abscesses in Group A, supporting prior findings that this approach can resolve abscesses efficiently (3). In cyst cases, combining finasteride with alcoholization (Group B3) yielded superior outcomes compared to standalone treatments. Finasteride reduced prostatic parenchyma size while alcoholization targeted cyst resolution synergistically. Differences in treatment efficacy between groups may be explained by the fact that the cystic cavity is lined with epithelial cells, which could be more resistant to alcohol sclerotherapy. In conclusion, this study highlights alcohol sclerotherapy as a minimally invasive alternative to traditional surgery for selected cases involving single small prostatic cavity, well surrounded by prostatic parenchyma and not communicating with the urethra.

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7103/7859 | Hypoluteinism: Effects of progestin therapy and clinical implications

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Hypoluteinism is characterized by insufficient production and secretion of progesterone (P4) by the corpora lutea,

resulting from primary ovarian dysfunction. This condition can lead to embryonic resorption, fetal abortion, or premature delivery (1). Despite growing scientific interest in this pathology, its underlying mechanisms remain poorly understood, and diagnosis is challenging. Progesterone measurement remains a key diagnostic tool; however, the lack of a definitive progesterone threshold value correlated with gestational age complicates secure diagnosis, leading to potential overdiagnosis. Furthermore, to ensure the delivery of viable puppies, supplementary progestin therapy is indicated. However, such treatment may induce undesired side effects on both the mother and the fetus, highlighting the necessity for accurate diagnosis to prevent unnecessary treatment (1). The aims of this study were to evaluate the diagnostic accuracy of current methods, assess the side effect of progestin therapy as described in the literature, and analyze progesterone concentration during pregnancy.

A total of 28 pregnant bitches of various ages and breeds were retrospectively enrolled in this study: 23 were diagnosed with suspected hypoluteinism based on literature data [2], of which 20 were treated with progestins, and 5 were healthy. Each bitch underwent clinical and gynecological examination, ultrasound examination, and progesterone measurement at each control until parturition. Data regarding pregnancy, delivery and puppies were collected and analyzed. All data were presented using descriptive statistics and analyzed with Pearson's linear correlation and a one-way ANOVA test.

Results were generally consistent with the literature but differed regarding predisposition related to animal size, interestrous interval, and fetal development defect as side effect of the therapy. Key findings revealed breed predispositions (Bernese Mountain Dog: 25%, French Bulldog: 20%, Cavalier King Charles: 20%), uterine inertia requiring emergency C-sections in 33.3% of progestin-treated dogs, and transient lactation failure in 52.9% of early-treated cases, resolved via domperidone. Overall, in dogs treated with progestin, hypoluteinism was confirmed in 77.77% of the cases.

These findings suggest that therapy may not only prolong gestation but also lead to primary inertia due to uterine atony. Additionally, earlier therapy initiation increased the risk of lactation failure after parturition. While no data exist on the impact of progestin supplementation on prolactin secretion, and our study found no correlation between supplementation onset and lactation, we hypothesize that administering progestins during early prolactin secretion (35th–40th gestation day) may delay lactation onset. Furthermore, P4 concentration in both healthy and affected bitches were lower than previously reported in the literature, aligning with more recent study (2), underscoring the need to update reference values. The use of synthetic progesterone allowed measurement of endogenous P4, confirming suspected hypoluteinism in 77% of cases. Even if further research is needed, this data, despite uncertainty regarding the P4 threshold for diagnosing hypoluteinism, highlights the importance of accurate diagnosis through clinical and ultrasound monitoring combined with P4 measurement to optimize pregnancy management and avoid unnecessary hormonal treatments.

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6363/7758 | Prostatic changes negatively impact microbial diversity in canine semen

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Prostatic alterations are a common finding in adult male dogs, with prevalence increasing with age (1). These conditions, including benign prostatic hyperplasia (BPH), prostatitis, and prostatic neoplasia, can significantly affect reproductive function, general health, and quality of life. Despite the importance of the prostate in male reproductive health, the association between prostatic disease and the semen microbiome remains poorly understood. In recent years, the advent of next-generation sequencing (NGS) has enabled the characterization of microbial communities across different biological niches, including the male dog reproductive tract (2). In humans, changes in the semen microbiome have been linked to prostatic disease (3), supporting the hypothesis that microbial dysbiosis may contribute to prostatic pathology. However, no study has yet specifically investigated the semen microbiome in dogs with prostatic disease. This study aimed to characterize the microbiome of the prostatic fraction of semen collected from dogs with benign prostatic hyperplasia and healthy controls using 16S rRNA gene sequencing. Eighteen intact male dogs (benign prostatic hyperplasia BPH $n=8$ and healthy prostate HP $n=10$) were enrolled. All dogs underwent semen collection by digital manipulation using sterile collection funnels, obtaining three ejaculate fractions. The third fraction, representing the prostatic fluid, was used for microbial DNA extraction using the QIAmp Microbiome Kit (QIAGEN). Due to insufficient DNA yield, two samples were excluded from downstream analyses, leaving 16 samples (BPH $n=8$, HP $n=8$) for sequencing. Amplicon sequencing targeting the V3-V4 hypervariable regions of the bacterial 16S rRNA gene was performed on the Illumina MiSeq platform. Taxonomic classification, alpha diversity (within-sample diversity), and beta diversity (between-sample diversity) were assessed. Statistical analyses included Wilcoxon rank-sum tests for alpha diversity and PERMANOVA for beta diversity based on Bray-Curtis distance matrix. Differential abundance analysis was performed using Linear Discriminant Analysis Effect Size (LEfSe). Significance was set at $p<0.05$.

Alpha diversity analysis showed that AP samples exhibited significantly lower microbial diversity compared to HP ones, with Shannon index values significantly reduced in the BPH group ($p=0.0046$). Beta diversity analysis, however, revealed no significant differences in overall microbial composition between BPH and HP groups (PERMANOVA, $p=0.68$). Taxonomic profiling identified increased relative abundance of *Enterococcus* spp. and *Streptococcus* spp. in the BPH group, with these taxa significantly enriched compared to the HP group (LEfSe, $p=0.003$).

This study provides the first characterization of the prostatic semen microbiome in dogs with benign prostatic hyperplasia. The reduction in microbial diversity, combined with the enrichment of specific genera, suggests that prostatic disease in dogs may be associated with microbial dysbiosis. While causality cannot be established, these results highlight potential microbial contributions to canine prostatic health and disease.

Further research is warranted to clarify the functional significance of these changes and explore their diagnostic and therapeutic potential in veterinary and comparative medicine.

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6616/7937 | Launch of an app for breeders and puppy owners – User acceptance and future possibilities

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As digitalization advances in veterinary medicine, we have created an app for dog breeders and owners. It provides owners with access to medical information and recommendations, as well as enables the acquisition and analysis of research data. The app allows owners to record a wide range of information, including health data, heat cycle data, pregnancies, litters, and puppy development. This data is stored in a central database and can be used for analysis to gain insights into heat cycles, pregnancies and litters as well as puppy development from individual breeds and weight classes (1, 2). The app was released in August 2024 worldwide on iOS and Android in German and English language. Premium users have the option to store their data in the cloud and thereby consent to its use. Results: Within the first 7 months, the puppy-center app has approximately 10,000 downloads and 7000 active users worldwide. We have downloads in 71 countries, with more than half in the United States, followed by Germany, England, Austria, Australia, Switzerland and Canada in descending order. About 1700 users are logged in with their email address and by now around 500 breeders are using the premium version.

The goal to provide breeders with an intuitively designed platform for recording their breeding data and gain medically based information on all topics related to dog breeding met with high acceptance. And an increasing number of dog owners are willing to use digital tools to manage their data - providing us the opportunity to use data obtained in this way for future projects. The creation of a research database using an app for dog breeders offers an innovative solution for systematically recording and evaluating breeding data, including long-term data-driven research projects on dog breeding and puppy development.

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6417/7949 | Canine brucellosis unmasked: A serological study comparing analytical methods

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Brucella canis is a zoonotic bacterial infection, causing abortion and other canine reproductive disease. Direct contact such as mating is the main transmission route. Diagnosing the infection is challenging. A positive culture or PCR analysis is confirmatory, but sensitivity of these methods is often low, due to intermittent and low-grade excretion of the bacteria. Serology is used for screening and is the most common diagnostic choice. However, varying specificity between methods and false positive results contribute to uncertainty in interpretation, causing much concern for veterinarians and dog owners. The prevalence of *B. canis* in Sweden is unknown. The same is true for most other countries within Europe, but there are reports from several European countries describing an increasing number of canine cases, as well as two human cases during the last decade. Stray dogs from Eastern Europe and smuggled dogs are at higher risk of infection (1). There have been a few reports from Ukraine, suggesting the presence of the infection in certain populations (2, 3). The aim of this study was to compare two serological methods, and to estimate the seroprevalence within two subpopulations of Swedish dogs.

Serum samples that were available from 435 Swedish dogs were analyzed by two methods: indirect enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA). The risk group were dogs identified at the border control as suspected smuggled dogs, or dogs from Ukraine. The control group were healthy dogs, or with clinical signs not attributed to *B. canis*. Sera from two dogs previously diagnosed with *B. canis* were used as own controls. Bayesian latent class analysis was used for the statistical analysis. The risk group ($n = 88$) included 24 different breeds with a mean age of 4.5 (0.5–13) years, and the control group ($n = 347$) 108 different breeds with a mean age of 3.2 (0.25–10) years. Three samples (3.4%) tested positive using ELISA in the risk group and seven samples (2.0%) tested positive using ELISA in the control group. All samples were negative using the LFIA test. The own controls tested positive by both ELISA and LFIA.

Based on the results of the present and other studies, the prevalence of *Brucella canis* in Europe is low. A pan-European surveillance program, including more dogs, would be necessary to gain more power to be able to estimate the impact of the infection.

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6335/7796 | Ultrasonographic and elastographic assessment of cervical changes throughout the canine oestrous cycle

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The cervix plays a crucial role in the reproductive physiology of female dogs, acting as a dynamic barrier that regulates sperm transport, prevents ascending infections, and undergoes structural changes in response to hormonal fluctuations during the oestrous cycle (1). Despite its importance, real-time ultrasonographic monitoring of modifications of the cervix in the estrous cycle remains underexplored in veterinary medicine. Ultrasound elastography is an advanced imaging technique that evaluates tissue elasticity and stiffness, providing valuable insights into structural changes (2). In veterinary medicine, research on the standardization of elastography for tissue evaluation is relatively recent, and data on its application in assessing cervical biomechanics are absent. This study evaluated B-mode ultrasonography and ultrasound elastography (UEl) to detect structural and consistency changes in the canine cervix across the oestrous cycle.

The oestrous phase was determined using progesterone (P4) levels, clinical signs, vaginoscopy, and cytology (3). Thirty-five bitches were examined at different cycle stages: pro-oestrus ($n=7$), pre-ovulatory oestrus ($n=12$), peri-ovulatory oestrus ($n=15$), dioestrus ($n=17$), and anoestrus ($n=8$). Cervical length and diameter, along with elastographic parameters (Elastographic Index, ElI; Elastographic Ratio, ElR), were measured on both sides in longitudinal and transverse sections. ElI values were automatically generated by the ultrasound software based on cervical tissue deformation under compression. ElR was calculated by comparing cervical stiffness to that of a reference area in the abdominal muscular wall.

Results indicated that cervical length was highest during pre-ovulatory and peri-ovulatory oestrus (32.61 ± 8.02 mm and 31.93 ± 7.23 mm, respectively), significantly decreasing in anoestrus (19.38 ± 2.63 mm; $p < 0.05$). The cervical diameter followed a similar trend, with the widest value in peri-ovulatory oestrus (15.63 ± 4.50 mm) and a significant reduction in anoestrus (8.96 ± 1.51 mm; $p < 0.05$). When normalized for body weight (BW), the cervical lenght/BW and cervical diameter/BW ratios were highest in pro-oestrus ($33.92 \pm 6.07\%$ and $18.00 \pm 4.13\%$, respectively) and progressively declined through the cycle, reaching their lowest values in anoestrus ($19.50 \pm 10.25\%$ and $8.81 \pm 4.31\%$; $p < 0.05$). ElI values indicated increased cervical stiffness in dioestrus (3.02 ± 0.34) and anoestrus (3.00 ± 0.35) compared to pro-oestrus (2.51 ± 0.40) and pre-ovulatory oestrus (2.54 ± 0.28; $p < 0.05$). No significant differences in ElR were observed across phases. ElR did not show significant variations across cycle phases.

These findings underscore the utility of elastography in quantifying cervical tissue consistency and its correlation with hormonal influences, providing a novel diagnostic perspective for a more comprehensive understanding of reproductive health.

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6291/7775 | Prostatic urothelial carcinoma in an intact male French bulldog: Diagnostic challenges and clinical evolution

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A 9-year-old intact male French Bulldog was presented to the University Veterinary Hospital with a 36-h history of vomiting and inappetence, alongside progressive weight loss, abnormal gait, stranguria, and reluctance to walk. Clinical examination revealed an emaciated dog (BCS 3/9) with prostration and mild dehydration. Abdominal palpation identified a full, firm bladder. Based on the signalment and clinical signs, a presumptive diagnosis of prostatitis with urinary obstruction or prostatic abscessation was made—conditions commonly associated with intact male dogs presenting with urinary signs. Radiographs were performed initially, but notably, rectal palpation and abdominal ultrasonography were deferred, potentially delaying the identification of neoplastic disease.

Initial management included catheterization, fluid therapy, antibiotics (amoxicillin-clavulanate), maropitant, tamsulosin, and a urinary diet. Despite this treatment approach, the dog's condition deteriorated over four days, with persistent vomiting, inappetence, and worsening gait. A rectal examination was then performed, revealing an enlarged, firm prostate with a gritty texture, and a mobile mass was detected in the caudal abdomen. Subsequent abdominal ultrasound and repeat radiography were performed to further characterize these findings. Prostatic cytology was performed twice and revealed significant inflammatory infiltrate but was inconclusive for neoplasia—highlighting the diagnostic challenge when inflammation and neoplasia coexist. Hematology showed only mild neutrophilia initially, with progressive increases in hematocrit and RBC count, and emerging thrombocytopenia as the condition advanced.

Despite intensive supportive care, the dog's condition continued to deteriorate, developing respiratory distress with radiographic changes suggestive of pulmonary metastasis by the fifth day of hospitalization. Given the poor prognosis, euthanasia was elected. Post-mortem histopathology confirmed urothelial carcinoma originating from the prostatic urethra with secondary prostatic invasion.

This case underscores several important clinical considerations. Although prostatic tumors, particularly urothelial carcinoma, are more frequently associated with castrated males, this case demonstrates that such neoplasms can occur in intact individuals and should be included in differential diagnoses regardless of reproductive status. The case highlights how the signalment of an intact male can mislead clinicians toward inflammatory

prostatic conditions, potentially delaying appropriate diagnostic steps. The report emphasizes the importance of early and comprehensive imaging—particularly rectal palpation and ultrasonography—even when the clinical presentation seems consistent with more common conditions. Additionally, it illustrates the limitations of cytology in cases complicated by concurrent inflammation, where the inflammatory response may obscure neoplastic cells. Histopathology remains essential for definitive diagnosis in such challenging cases.

6405/7878 | Double mating in pure-breed dogs in France: Survey on breeders' motivations and practices

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Besides a great variety of dog breeds, increased selection has led to homogeneity within breeds and a reduction in genetic diversity. The rise in hereditary conditions linked to inbreeding has become an increasing concern for breeders. Since 2020, the French Kennel Club (SCC) has offered breeders the possibility of using two males during a mating (natural mating or artificial insemination). This study aimed to characterize the French breeders' motivations for carrying out a double mating and their practices based on an online survey.

A concise questionnaire was developed using the Sphinx software (Version 4.31, Sphinx, Roubaix, France), consisting of two parts. The first part, with 12 questions, targeted breeders who had used double mating to gather detailed information on their general data (number of double matings carried out, breeds involved, etc.), on their practice (mating methods, time interval between males, prolificacy of each male, etc.) and the reasons why they used it. The second part, with 5 questions, was for breeders who hadn't used double mating to gauge their opinions and knowledge on the subject. A questionnaire was distributed from August 31 until November 11, 2023 by the French Kennel Club (SCC) on their social networks (Facebook) whose page is followed by more than 56,000 breeders.

Among the 780 breeders who participated in the survey, 16.8% (131/780) practiced double mating in their facility, most of them between 1 and 5 times (97.6%; 125/128). The most frequent reasons cited were to maximize reproduction chances (34.4%), to preserve the genetic potential of older, less fertile dogs (23.0%; 30/131), and for genetic benefit (15.2%). In 77% of cases (97/126), breeding is carried out using the same method for both males with two main practices: either natural matings or artificial inseminations with fresh semen. In total, 68.7% (88/128) of breeders reported having a breeding order for males to obtain as many puppies as possible from their "favorite" male. Among them, 84.1% (74/88) reported putting their favorite male first. A total of 44.1% (56/127) of breeders performed the two matings less than 24 h apart: 64.3% (36/56) between 1 h and 12 h apart, and 19.6% (11/56) did it simultaneously. The pregnancy was confirmed in 96.5% (120/114) of the double matings with the majority (58.2%; 64/110) of them actually giving single-parent litters. In biparental litters, the average number of puppies from the first male was 2.4 ± 0.6 puppies per litter versus 3.2 ± 0.6 for the second male. Among the 83.2% (649/780) breeders who have never done double mating in their facility, 75.8% (488/644) considered that double mating is of interest for dog breeding. The most cited advantage for this practice was the increased genetic diversity for low-population breeds 33.1% (213/644).

Double mating is used by some French breeders to enhance reproduction and genetic diversity, though single-parent litters remain common. While not widely adopted, its potential benefits, particularly for rare breeds, suggest it could play a growing role in future canine breeding strategies and genetic management.

6406/7879 | Effect of double mating on prolificacy in pure-breed dogs in France: Retrospective study on data collected between 2020 and 2023 by the French Kennel Club

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Double mating, the practice of breeding a female dog with two different males during the same estrous cycle, has gained interest after its authorization by the French Kennel Club (SCC) in 2020 due to its potential effects on prolificacy and genetic diversity. While commonly observed in wild canids, its implications for domestic dogs remain underexplored. This study aimed to evaluate the association between the double mating and prolificacy declared by dog breeders to SCC between January 2020 and June 2023.

An anonymized database was built based on double mating and puppies' declarations received by the SCC from French dog breeders of all breeds. Data were available only on bitches having whelped. For each included bitch, the records of other available single matings were also registered. The data included 42 variables characterizing reproductive bitches and sires (kennel ID, animal ID, breed, breed size, age, inbreeding coefficient, bitch parity, sire origin), matings (single/double, male used), and litters (puppy count, paternity, sex distribution). After processing, complete data on 460 matings (164 double and 296 single) were available for statistical analyses. A generalized linear mixed model was used to assess the relationship between double mating and the number of declared puppies, including the characteristics of the animals used. The final model was selected based on the lowest Akaike Information Criterion.

Among all matings declared by SCC, 0.50% (777/156,143) were double matings. The final database included information of 157 bitches (5.8 ± 1.4 mating/bitch) of 65 different breeds (mostly Medium, 43.3%) coming from 130 breeding kennels (1.2 ± 1.5 bitch/kennel). There were 403 sires used for double or single matings (in average 1.5 ± 3.5 times). On average, the kennels carried out 1.3 ± 1.5 double matings, mostly on multiparous bitches (68.3%; 112/164), at the mean age of 3.9 ± 1.6 years. The breed with the most registered double matings was Siberian Husky (19.5% of all double matings; 32/164). In 47.6% (78/164) of double mating cases, both sires were from a different kennel than the bitch, whereas in 38.4% (30/78), they came from the same one. Among all the studied variables, only the breed size was significantly associated with the declared prolificacy ($p < 0.01$). On average, 6.4 ± 3.0 Giant, 7.0 ± 2.5 Large, 5.4 ± 2.1 Medium

and 4.1 ± 1.7 Small sized puppies were declared to the SCC per litter. The adjusted effect of double mating was insignificant ($p = 0.388$), with a mean declared prolificacy of 5.5 ± 2.1 puppies after double mating against 5.3 ± 2.4 puppies declared after single mating.

Our results do not highlight any significant effect of double mating on declared prolificacy. However, the absence of information on the practical application of double mating, such as the delay between ovulation and insemination or the quality of the semen used, greatly limits our understanding of the mechanisms behind this supposed compensation.

6456 | Does uterine aging process influence PTGS2 expression in canine endometrium?

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Infertility in female dogs is a complex issue that concerns both pet owners and breeders. Aging and uterine pathology such as pyometra play an important role in reproductive decline (1). Aging may also lead to chronic inflammation and fibrosis of the uterus (2). These process raises an important question about their impact on fertility. One potential marker involved in aging and inflammatory processes is prostaglandin endoperoxidase-2 (PTGS2) (3). This study aimed to investigate PTGS2 expression in both healthy young and old dogs, along with those suffering from pyometra.

In total, 39 intact nulliparous bitches were included in this study. The bitches were categorized into three groups based on their age and health status: healthy young dogs <6 years old ($n = 20$), healthy old dogs ≥6 years old ($n = 8$), and bitches with pyometra ($n = 11$). A full-thickness uterine sample was collected from the left uterine horn. Immunohistochemical staining with primary antibodies against PTGS2 (monoclonal mouse anti-rat PTGS2 antibody) was used. All layers of endometrium (luminal epithelium; LE, glandular epithelium; GE, and stroma; ST) were investigated. The expression of PTGS2 was evaluated with semi-quantitatively both the intensity of specific staining and the proportion of the cell that positively stained by following the immunoreactive score (IRS) method (4). To compare the score for each layer and group, Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner post hoc test were performed using SAS. Differences with $p < 0.05$ were regarded as statistically significant.

The mean PTGS2 expression in LE and GE of old and young healthy groups was significantly lower than pyometra group ($p < 0.01$). In dogs with pyometra, PTGS2 showed the highest expression in LE compared with GE ($p < 0.05$) and ST ($p < 0.01$). However, the PTGS2 expression between healthy old and young dogs was not significantly different ($p > 0.05$).

The high immunoreactive score in pyometra group indicated that the inflammatory condition caused by infection has more significant effect on PTGS2 regulation than the possible effects of inflammation due to aging. The absence of significant differences between young and old healthy dogs suggested that aging does not strongly influence PTGS2 expression, possibly due to compensatory mechanisms to prevent excessive inflammation or a gradual accumulation of changes that do not reach a critical threshold (5). The highest expression was found in LE of the pyometra group indicated its key role in the inflammatory response, potentially serving as the primary site of immune activation and prostaglandin regulation (6). These findings highlight the predominant influence of uterine inflammation due to pyometra over age-related changes in uterine health on PTGS2 expression. Further study should focus on the molecular mechanisms of canine uterine aging related to inflammatory responses.

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6293/7785 | Urogenital pathologies associated with early spaying in bitches

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Early spaying has been recommended in bitches for decades to avoid reproductive diseases such as pyometra and mammary tumors. Nevertheless, time has demonstrated that early spaying also has counterproductive effects. The literature extensively describes numerous negative effects such as higher incidences of other types of neoplasia, urinary incontinence, obesity and orthopaedic diseases among others. However, according to the authors' knowledge, no study about the deleterious effect of early spaying on urogenital pathologies, such as vaginitis and cystitis, has been performed.

In the present study, fourteen females were selected from the database of our Veterinary Teaching Hospital following the selection criteria of vulvoplasty surgery. Data on the species, hormonal status, age of spaying, and age and reason at consultation were collected and analyzed.

The selected patients were referred to the reproduction service for recurrent or long-lasting cystitis, vulvovaginitis or peri-vulvar dermatitis. Twelve out of the fourteen females had been spayed, 8 of them being spayed before or shortly after their first heat. When the pathologies were analyzed separately, vulvitis was present in 6 patients, recurrent cystitis was diagnosed in 6 bitches, and vaginitis and peri-vulvar dermatitis were diagnosed in 4 cases each. When analyzing the patients separately, peri-vulvar dermatitis, vaginitis and vulvitis were observed concomitantly in 3 cases, and concomitant peri-vulvar dermatitis and vulvitis were diagnosed in three other cases. In contrast, five females presented only recurrent cystitis. All the females

presented sunken vulva, and three of them, which were spayed early, had an infantile vulva too. Regarding the age at consultation, most of them came at an advanced age. Six out of fourteen were between 2 and 6 years old, and six others were between 7 and 11 years old. In addition, twelve bitches belonged to medium-large breeds, except for two cases that were Carlino's breed. Finally, after undergoing the vulvoplasty, the fourteen females successfully recovered, and only two of them needed antibiotic therapy a few weeks later.

The present results highlight that premature spaying is strongly related to the presence of a sunken vulva and the associated signs. Likewise, there is evidence that the incidence of this condition is higher in bitches of medium-large breeds and at an advanced age. A feasible explanation is that these conditions are chronic cases with long-lasting treatments where the underlying etiology, namely sunken vulva, goes undetected. Additionally, older animals tend to gain weight more easily, so perivulvar skin folds increase, and associated symptomatology is accentuated. Early spaying predisposes to a greater risk of suffering sunken vulva and consequent urogenital pathologies such as vaginitis, recurrent cystitis, peri-vulvar dermatitis and vulvitis.

7163 | Usefulness of vaginal speculum examinations in the bitch

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The usefulness of vaginal speculum examinations as diagnostic procedure stretches far beyond evaluating the nature and extent of disease in the vestibule and vagina of female dogs. The aim of this manuscript is to describe the many uses of a vaginal speculum in the bitch. Visual appraisal of all described techniques by means of illustrations, images and video clips are crucial to fully appreciate the full extent of use of vaginal speculums in aid of diagnostic, therapeutic and procedural events.

Many different speculums are available but the authors preference simple tubular Perspex speculum (8–18 mm inner diameter, dependent on the size of the bitch and 120–330 mm long) with a strong external cold light source shone into the lumen of the speculum. The speculum is guided over the pelvic brim upwards and then lifted and gently pushed forward. caution should be exercised to ensure that the operated place the speculum such that the vaginal vault is inspected avoiding pressing the end of the speculum against vaginal walls and thus distorting the texture of the mucosa by stretching it. The smooth surface of the speculum allows insertion without the aid of lubrication which may in some instances interfere with evaluation of the vaginal mucosal wall, patency of the cervix or be spermicidal if insemination is to follow speculum examinations.

Vestibulo-vaginal conditions may be diagnosed which include circumferential stricture (ring stenosis), dorsoventral band strictures, segmental narrowing of the vaginal vault, double vagina, vaginal septa, clitoral pathologies, papilloma, polyps, neoplasia, infectious and inflammatory conditions.

Estrus monitoring may be achieved through a speculum evaluating the macroscopic appearance of the vaginal mucous membrane (Lindsay et al. 1988). Whilst progesterone assays remain the gold standard optimizing time of breeding and estimating the LH peak, speculum examinations may help guide the veterinarian when to start progesterone assays thereby limiting number of costly progesterone assays as well as indicating vaginoscopic diestrus. Vaginal smears are commonly collected using earbuds or cotton tips by parting the vulva using fingers, but this may result in contamination of skin cells and cells sloughing from the vaginal vault which may be avoided by taking smears from the cranial vagina through a speculum. Procedures that are facilitated by the use of a vaginal speculum are vaginal douches for vaginitis, draining vaginal vault when vaginal pooling occurs during estrus, urethral catheterization, correct placement of insemination pipettes beyond the paracervix whilst avoiding inadvertent inserting insemination pipettes into the urinary bladder.

Parturition management. Digital and unaided visual examination of the bitch's reproductive tract is complicated by its anatomic features and dimensions. Without visual inspection of the vagina, through an endoscope or speculum, cervical examination is impossible in bitches (Schweizer & Meyers-Wallen 2000). Visual inspection of the vagina through a vaginoscope is a valuable tool in examinations of the vagina and cervix (Lulich 2006) but can also be achieved using a speculum. Determining readiness for caesarean section is greatly assisted by the use of vaginal speculum examinations to observe for the presence of cervical dilatation.

7041 | Computed Tomography (CT) features of canine mammary tumours – A retrospective study

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Mammary tumours are common neoplastic diseases primarily affecting bitches. It has been shown that benign mammary tumours tend to become malignant with the progression of time and malignant mammary tumours often metastasize to other organs. Early diagnosis is imperative for a favourable outcome and imaging studies are required for proper oncological staging. In veterinary medicine, there is great divergency in the terminology used in sonographic studies to describe mammary tumours, thus limiting the quality of reporting and risk assessment of bitches with mammary neoplasia. Accurate oncological staging necessitates reliable imaging, but current sonographic reporting lacks standardization, hindering risk assessment. While a modified BI-RADS™ lexicon improves sonographic reporting, a comparable standardized protocol for CT assessment is absent. This study aimed to establish a standardized CT reporting protocol for canine mammary tumours, correlating qualitative and quantitative features with histological classifications to improve diagnostic accuracy and clinical management.

Medical records (January 2021–October 2024) of bitches undergoing total body CT scans (pre- and post-contrast) for oncological staging at two veterinary institutions were retrospectively reviewed. For qualitative description and standardization of

the nomenclature, the terminology was based on the Modified BI-RADS™ Lexicon (1) and adapted to CT description (2). Following qualitative description, lesions were assessed quantitatively regarding the density based on the Hounsfield Unit (HU) values both on pre and post contrast evaluation. All CT studies were anonymized and blindly evaluated. Tumours were divided into malignant and benign. All quantitative data was assessed for normality (Shapiro-Wilk). Comparisons between groups were performed with Mann-Whitney Test. Qualitative data were compared between groups with the Fisher's Exact Test and if significancy was found, the sensitivity and specificity was calculated.

Twelve CT studies were included. Fifty-three mammary tumours with confirmed histological type were evaluated. Forty-five tumours were malignant (84.9%) and eight were benign (15.1%). Only the enhancement pattern and the presence of calcification were different between groups. The presence of heterogenous enhancement pattern and rim enhancement were strongly associated with malignancy (43% sensitivity, 96% specificity; 43% sensitivity; 100% specificity, respectively). As for calcification, it presented 37.8% sensitivity, 100% specificity for malignancy. Increased tumour volume was significantly associated with an increased likelihood of malignancy (p value < 0.001). No difference was observed of the density between the groups in the pre- and post-contrast studies.

Conclusions. This study demonstrates the feasibility and utility of a modified BI-RADS™ lexicon adapted for CT in characterizing canine mammary tumours. Specific qualitative CT features (heterogeneous enhancement, rim enhancement, calcification) exhibited high specificity for malignancy, potentially improving diagnostic accuracy and aiding clinical decision-making in conjunction with tumour size assessment. Further prospective studies are warranted to validate these findings and refine this standardized CT reporting protocol for broader clinical implementation.

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7068 | Proteomic approach of formalin fixed paraffin embedded tissue of canine breast inflammatory carcinoma

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Breast tumors are common in bitches and women, and dogs are considered an experimental model for human. The most aggressive of mammary tumors is inflammatory carcinoma, which can develop from any type of carcinoma. In this type of tumor, surgical excision is not indicated, with diagnosis often accompanied by metastases, and low survival. Then, the aim of this study was to investigate diagnostic targets that can identify the transition from carcinomas to inflammatory carcinomas, to avoid an unfavorable prognosis, using proteomics tool as a histological bank of formalin-fixed and paraffin-embedded samples.

Five 20 µm fragments of paraffin blocks were used to form 3 groups ($n = 5$/group): non-inflammatory carcinoma (NIFC), invasive non-inflammatory (NIFINC) and inflammatory (IFC). The samples were treated to remove the paraffin and in-gel tryptic digestion was performed followed by proteomics with a shotgun approach. The data were submitted to multivariate (principal component analysis [PCA] and heatmap) and univariate analysis (ANOVA followed by Fisher LSD test) in MetaboAnalyst 6.0 (http://www.metaboana). Gene ontology pathway enrichment was conducted in ShinyGO 0.82 (https://bioinformatics.sdstate.edu/go/).

Results. A total of 364 proteins were found and 77 were common in all groups. In the PCA, there was a separation of the NIFC, NIFINC and IFC groups, evidenced by the heatmap. Eight proteins exhibiting differential abundance were identified. Trafficking protein particle complex subunit 6B (TRAPPC6B) was found to be more abundant in NIFC, while caspase 14 (CASP14) and histone H4 were higher in NIFINC. Additionally, albumin (ALB), LMBR1 domain containing 2 (LMBRD2), and lysozyme C, spleen isozyme (1,4-beta-N-acetylmuramidase C) were found to be more abundant in IFC. In contrast, desmoglein-1 (desmosomal glycoprotein 1, DG1, DGI) (DSG1) and thioredoxin-dependent peroxiredoxin were decreased in IFC group.

Thus, the identified proteins are biomarkers of aggressiveness and participate in the oncogenesis process of breast carcinoma, as well as being part of the tumor microenvironment. We highlight the desmoglein-1 (desmosomal glycoprotein 1, DG1, DGI) (DSG1) and thioredoxin-dependent peroxiredoxin that are common proteins in differentiated tumors (1) and regulate the cell proliferation and apoptosis (2). Although IFC is not considered an undifferentiated tumor, it is an aggressive form of breast cancer, and the downregulated proteins can be considered biomarkers for this tumor type.

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6521/7694 | Anterior colpotomy for the excision of a vaginal mass in a dog: A case report

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An intact, 12-year-old, German Shepherd, 30 kg, bitch was admitted due to chronic constipation and irregular vaginal hemorrhagic discharge. Regarding the reproductive history, the dog presented irregular estrous cycles for the last 2 years. The last proestrus occurred 1.5 years before, followed by occasional vaginal hemorrhagic discharge till admission. The dog was clinically healthy. A large, painless mass in the anterior part of the vagina was found during digital vaginal palpation, while during digital rectal

palpation it was observed that the vaginal mass was compressing the rectum. The blood test showed leukocytosis and anemia, indicative of chronic disease. The vaginal cytologic examination was compatible with normal proestrus phase. No metastases were found in the x-rays and the abdominal ultrasound. The ultrasound revealed ovarian cysts and masses and a vaginal mass of mixed echogenicity at the anterior part of the pelvic cavity. The surgical excision of the mass was planned. Median laparotomy was performed and ovariohysterectomy was started. Before the removal of the uterus, laparotomy was extended caudally to pull uterus and expose the anterior vagina. A longitudinal anterior colpotomy was then performed and a single intraluminal pedunculated mass was easily removed. The vagina was sutured with simple continuous suture and colpopexy was performed. The ovariohysterectomy was completed. Histopathology revealed vaginal leiomyoma and ovarian granulosa cell tumor and cysts. Estradiol 17-beta was found increased in the ovarian cyst fluid, while in the blood serum it was in basal levels. The dog was discharged one day after the operation without urination and defecation problems. It has remained healthy for 5 years.

Vaginal and vulvar tumors are the second most common reproductive neoplasms in the bitch next to mammary tumors. Leiomyoma is the most frequent benign vaginal mass of dogs. Most of the times, it is intraluminal and pedunculated and hormone-dependent. Therefore, the concurrence of ovarian cysts or masses that excrete estrogens and exaggerate vaginal masses is probable. The treatment of these masses is surgical excision with either colpotomy, when the mass is intraluminal, or colpectomy, when it invades the vaginal wall. Surgical approach may be caudal - via episiotomy, cranial - via posterior laparotomy or with a combination of these. Additional ovariohysterectomy is advisable to avoid recurrence of the vaginal mass. When the pelvic canal remains intensely dilated after mass removal, colpopexy is suggested to avoid a possible prolapse. In our case, posterior laparotomy combined with anterior colpotomy allowed the removal of a large, single, intraluminal, pedunculated vaginal mass and was easily performed in combination with ovariohysterectomy, without complications.

6520/7853 | Therapeutic management of canine transmissible venereal tumour: A case series

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Clinical cases. a) A young, male, intact, stray, dog was admitted with three small, red, soft, non-painful, pedunculated masses observed on the ventral surface of the glans penis. The dog was otherwise clinically healthy. Hematocrit, neutrophil and platelet count were slightly decreased. No metastases were obvious in thoracic and abdominal radiographs. Cytology revealed round cells, with round nuclei and cytoplasmic vacuoles, compatible with transmissible venereal tumor (TVT). The masses were surgically excised, and vincristine was intravenously administered at a dose of 0.6 mg/m^2 weekly, for a total of three sessions. The surgical wounds healed without complications. For the last 18 months post-surgery, the dog has been clinically healthy without any recurrence.

b) A 2-year-old, female, intact Caniche dog was admitted with intermittent vaginal hemorrhagic discharge. The dog was systemically healthy apart from a small, red, soft, non-painful, pedunculated vestibular mass that was detected during vaginal examination. The mass projected through the vulva and was bleeding after handling. Blood test results were unremarkable. No metastases were seen in survey radiographs. The mass was surgically removed, followed by cytologic examination. TVT was confirmed and vincristine was administered, at a dose of 0.6 mg/m^2 weekly for three sessions. The surgical wound healed well and the dog has remained healthy and recurrence-free for the last 5 years.

c) A 3-year-old, female, intact, cross-breed dog was admitted with vaginal hemorrhage and a mass projecting from the vulva. The mass was small, solitary, red, soft, non-painful, friable and pedunculated with a bleeding surface, originating from the clitoris fold. The dog was otherwise healthy. Blood test results were within normal limits. No metastases were found in survey radiographs. A TVT diagnosis was made based on cytology. Vincristine was initiated, but the mass size did not decrease after 7 doses. Episiotomy was performed and the mass, including the clitoris, were surgically excised, followed by 3 more vincristine doses weekly, at a dose of 0.6 mg/m^2. The surgical wound healed without complications. The dog has been healthy and recurrence-free for the last 4 years.

Canine TVT may be treated with several modalities: chemotherapy, surgery, cryosurgery, electrosurgery, irradiation, immunotherapy, and a combination of the above. Chemotherapy is most commonly elected because of its high efficiency and practicality, but it is also associated with adverse effects. Surgical excision as single therapy often results in recurrence. According to Takariyanti et al. (2021), in small, single, localized, easily accessible, non-invasive masses, surgical excision combined with chemotherapy is recommended to reduce chemotherapy sessions. The first two cases fitted exactly the above criteria and surgical excision was performed. Additionally in the second case, TVT was diagnosed soon after excision, so chemotherapy was added. The third case did not respond to chemotherapy, possibly due to difficulty of vincristine in penetrating into the clitoris fold, necessitating surgical excision. Chemotherapy combined with surgical excision in these cases accelerated clinical remission and reduced the total chemotherapy dose and possible morbidity related to adverse effects.

A2 | Incidence of progesterone-mediated conditions after deslorelin treatment in female cats: A multicentric study

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Deslorelin, a GnRH superagonist, is growingly being used off-label in female cats for reproductive control, including ovarian

suppression, estrus induction, puberty delay, and management of post-spaying urinary incontinence. Its mechanism of action involves an initial surge in LH and FSH, causing a transient "flare-up effect" leading to increased estradiol levels and ovulation, followed by prolonged ovarian suppression (6–37 months), with fertility restoration upon implant removal or degradation.

A key concern associated with deslorelin treatment in queens is the progesterone (P4) rise following ovulation, which can lead to several pathologies, including insulin resistance (diabetes mellitus), pyometra, xanthomatosis, acromegaly, and feline mammary fibroadenomatosis (FMF). Rare cases of blindness due to lipid deposits in the eyes have also been reported. This study aimed to identify P4-mediated conditions in queens after potential ovulation induction triggered by the deslorelin flare-up effect.

A retrospective analysis was conducted on 206 queens treated with deslorelin implants (4.7 and 9.4 mg) at five European veterinary hospitals. Implants were administered in both periumbilical and interscapular regions. The collected clinical data included breed, age at implantation, prior deslorelin treatments, estrous cycle stage at implantation, implant dosage, and the presence of P4-mediated conditions.

P4-mediated conditions mediated were documented in 21/206 (10.2%) of queens. FMF was observed in 3.9% of queens, mammary hyperplasia was noted in 2% of adult females, viable pregnancies concluding in parturition occurred in 2%, pyometra was diagnosed in 1%, and aggressiveness along with diabetes mellitus was reported in 0.5%. The study reported several P4-mediated conditions in treated queens. FMF was noted exclusively affecting cats under one year of age while mammary hyperplasia and pyometra were observed in older queens. These findings are consistent with the well-known role of P4 in mammary tissue proliferation and uterine conditions. Viable pregnancies were detected at five weeks of gestation only three weeks after implantation, suggesting conception shortly before treatment in queens incorrectly classified as being in anestrus at the time of implantation. In other cases, outdoor access during flare-up allowed potential matings resulting in pregnancy. Both these situations. highlight the need for thorough pregnancy screening or P4 measurements before deslorelin administration. Aggressiveness might be explained by the estradiol rise during the flare-up period while diabetes association with deslorelin treatment remains inconclusive, as it was diagnosed five months post-implantation. Although P4 is known to influence sensitivity to insulin in dogs, further investigation is needed to establish a definitive link between deslorelin and diabetes in queens.

The study confirms the efficacy of deslorelin in ovarian suppression while emphasizing the associated risks, particularly regarding P4-mediated conditions. Though incidence rates remain relatively low, the clinical significance of these complications warrants careful patient selection and monitoring when using deslorelin off-label. Future research should focus on evaluating individual variability and implementing preventive strategies to minimize adverse effects.

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6474 | Factors associated to survival at 24 h in German Shepherd and Dobermann newborn puppies: Not only birthweight and Apgar score

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Given the high percentages of perinatal mortality in dogs, newborn survival is a topic of interest for veterinary clinicians. Although the important role of Apgar score (AS) and birthweight (BW) was previously reported (1,2), the possible influence of other parameters is still under investigation. Moreover, a breed-oriented approach in newborns studies was recently proposed for a better understanding of data (2). The present study aimed to assess the possible association between some maternal (age, parity and litter size) and neonatal factors (AS and BW) and survival at 24 h of age in German Shepherd and Dobermann puppies.

Newborn puppies born by elective cesarean sections were enrolled. Data about maternal age, parity, litter size, Apgar score and birthweight were recorded.

On a total of 138 newborn puppies enrolled (59 German Shepherd from 10 litters and 79 Dobermann from 7 litters), 9 German Shepherd and 3 Dobermann puppies died within the first 24 h after birth, while 50 German Shepherd and 76 Dobermann were alive. The statistical analysis showed a negative effect of lower litter size ($p < 0.05$), lower AS ($p < 0.05$) and lower BW ($p < 0.001$) on newborn puppy survival in German Shepherd, while in Dobermann newborn puppies a negative effect of lower maternal age ($p < 0.001$), lower parity ($p < 0.001$), and lower AS ($p < 0.01$) was detected. Significant correlations among factors within each breed were found. In German Shepherd, significant correlations were found for maternal age and parity ($R = 0.78$, $p < 0.001$), AS and litter size ($R = -0.46$, $p < 0.001$), AS and BW ($R = 0.43$, $p < 0.01$), and BW and litter size ($R = -0.60$, $p < 0.001$). In Dobermann, maternal age was correlated with parity ($R = 0.79$, $p < 0.001$), litter size ($R = -0.71$, $p < 0.001$), AS ($R = 0.46$, $p < 0.001$), and parity was correlated with BW ($R = 0.37$, $p < 0.001$), and litter size ($R = -0.62$, $p < 0.001$). When the effect of the breed on the studied parameters was assessed, the statistical analysis showed that breed influenced parity, litter size and BW, but not AS.

In conclusion, data from the present study confirmed the recognized role of AS and BW as factors associated to short-term puppy survival, but maternal factors can have also a role, especially in specific breeds. Therefore, when short-term survival of puppies is assessed, a more comprehensive evaluation of maternal and neonatal parameters should be performed.

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6508/7808 | Effects of passaging and cryopreservation on canine uterine myocytes in adherent cell culture

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Dystocia is a common complication during canine pregnancy (1), frequently caused by uterine inertia. Conservative medical treatments for uterine inertia are limited. To better understand its etiopathology and to evaluate the contractility of uterine myocytes in vitro, we established a protocol for culturing canine uterine myocytes in adherent cell culture. This model offers a standardized and reproducible tool in line with the 3R principle. While widely used in reproductive medicine research for humans and other species, it could also help explore new therapeutic strategies for dystocia in dogs. A particular focus of this study was the effect of passaging and cryopreservation on proliferation and expression of contraction-associated markers, as prior research has shown it impacts mesenchymal cell viability (2). Uterine interplacental tissue was collected from three dogs undergoing caesarean sections for medical reasons. The tissue was prepared stereomicroscopically and digested enzymatically. Cells from the circular and longitudinal myometrial layers were transferred into separate T25 culture flasks. The cells were either cultured in DMEM/F12 medium containing 2% penicillin G/streptomycin and 10% heat-inactivated fetal bovine serum at 37°C with 5% CO2 or cryopreserved with 10% DMSO by slow cooling to −80°C and storing at −196°C. Fresh and cryopreserved cells from the same dog were analyzed at passages one and three. Growth rates were measured every 24 h over 96 h, Live Cell Imaging documented cell morphology, while immunofluorescence was used to analyze the expression of alpha smooth muscle actin (SMAα) and the localization of the oxytocin receptor (OXTR) in both muscle layers.

Cells from both myometrial layers expressed the contraction-associated markers SMAα and OXTR up to passage three, even after cryopreservation. Morphologically, the cells showed no significant differences. However, growth rate analysis over 96 h revealed a marked decrease in proliferation in two of the three dogs after cryopreservation. The third dog's cells were less affected by cryopreservation, suggesting individual variability. Despite this, a general decline in proliferation was observed from passage one to passage three in all cell types, irrespective of prior treatment. Interestingly, similar reduced, but stable proliferation rates were observed in spontaneously immortalized canine uterine myocytes up to passage 28, with the expression of SMAα and OXTR remaining largely unaffected.

As the expression of contraction-associated proteins and characteristic cell morphology in circular and longitudinal myocytes were largely unaffected by cryopreservation or cell ageing, cells up to passage three appear a suitable and vital model to study hormonal influence on canine uterine myocytes at cellular level. Declining proliferation rates especially after passage three, as well as individual susceptibility to cryogenic damage suggest the use of young and freshly harvested cells or characterized immortalized cell lines.

This study was funded by the Akademie für Tiergesundheit e.V.

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7087/7794 | Treatment with a deslorelin-slow-release implant alters protein expression of androgen receptor and prostaglandins in the canine epididymis

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Prostaglandins are involved in the physiological function of the canine epididymis and have been postulated to be under the control of androgens. Treatment with a deslorelin slow-release implant (DSRI) and its effects on the testis are well investigated (1, 2), but they are poorly characterized in the epididymis. We hypothesize that protein expressions of the androgen receptor (AR) and selected parameters of the prostaglandin pathway are reversibly affected by downregulation due to treatment with a DRSI. Our study reveals new insights into protein expression patterns in the canine epididymis.

Five healthy male beagle dogs with normospermia were treated with a 4.7 mg DSRI. Five months later, hemicastration and implant removal were performed ("downregulation"). After another five months and regaining of semen quality within the reference range, the remaining testis and epididymis were removed ("recovery"). Three healthy, normospermic untreated beagle dogs served as controls. The epididymides were separated into three segments, head, body and tail, fixed in formalin and embedded in paraffin for further investigations. Immunhistochemistry was performed for AR, PTGS2, HPGD, PTGES, EP2 and EP4. Slides were descriptively evaluated and compared between the groups (downregulation/recovery/control) and the segments (head/body/tail).

During downregulation, immunopositive staining for AR, HPGD, EP2 was less intense, whereas PTGS2 and PTGES showed stronger immunopositive signals compared to the controls. Whereas AR staining was nuclear, staining against the other targets was cytoplasmatic. Principal and basal cells stained immunopositive for all targets in all samples and segments, with additional staining in smooth muscle cells (AR, PTGS2, EP2) and in blood vessels (all). Additionally, EP4-positive "halo-like-cells" were identified for the first time in the canine epididymis, but only during downregulation and recovery, not in untreated controls. Comparing downregulation to recovery samples, AR, HPGD, EP2 stained stronger and PTGES weaker in the recovery group. The comparison of recovery and untreated control samples revealed a stronger HPGD and EP4 staining during recovery. Subjectively, only for EP2 a segment-specific staining intensity was identified, with the head showing a stronger immunopositive signal than the tail.

This study indicates an altered expression of the AR and selected parameters of the prostaglandin pathway as a consequence of DSRI treatment, indicating that epididymal function partly depends on androgens (3) and that different functions of the individual segments likely require different mediators. The results are in good agreement with our recent gene expression

studies (4). Subjectively, protein expression of AR, PTGS2, PTGES and EP2 was fully restored after recovery, matching recovery of testicular endocrine and germinative function (2) and indicating reversibility of DSRI-effects. Whether stronger HPGD and EP4 staining during recovery compared to the controls indicates still ongoing epididymal recrudescence, requires further investigation. Despite providing significant new insights, future studies should specifically focus on the time-line of recovery and restoration of epididymal functionality.

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7104 | Description of a Golden Retriever Cohort: An open science study to better characterize the first days of life and their short, medium and long-term consequences in dogs

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In many mammalian species, events occurring during the perinatal period induce physiological changes that can persist throughout an individual's life, with consequences for long-term health (developmental origins of health and disease concept). In dogs, four major canine longitudinal studies are in progress ("Dogslife", "Golden Retriever Lifetime Study", "Generation Pup", "Dog Ageing Project"). The objectives of these cohorts are to identify environmental, genetic, and lifestyle factors that influence aging in dogs and the risk of common disorders. However, none of these cohorts have carried out longitudinal follow-up from birth to adulthood to evaluate the influence of gestation and early life on health. This abstract describes the design of a longitudinal cohort of Golden Retriever dogs (TGC) that has been set up to collect data prospectively from the end of gestation until adulthood. The TGC focuses on three main objectives: (1) to develop minimally invasive clinical studies in order to investigate the effects of the fetal and neonatal periods on the health of dogs in the short, medium and long term, (2) to breed and select puppies to become future assistance dogs, (3) to support the training of students in reproduction, neonatology and preventive medicine.

Launched in 2022, the project initially constituted an "early cohort (EC)" of 113 Golden Retriever dogs, followed prospectively from the end of gestation to 18 months of life. Pregnant females (18 bitches in the EC) were admitted to a dedicated maternity room about two weeks before parturition where they remained with their puppies until adoption at 2 months of age. Once weaned, dams returned to their owners, whereas puppies were adopted to become assistant dogs, breeding females (for TGC purposes), or companion dogs. Puppies and their dams underwent close clinical follow-up, first in the maternity room and after 2 months of life during dedicated check-up veterinary visits (at 2, 3, 4, 6, 12 and 18 months of age). A wide range of clinical parameters (e.g., temperature, weight, clinical scores, morphometric measurements, fecal scores, coproscopy, behavioral information, skin pH and trans-epidermal water loss) and biological samples (blood, swabs (buccal, cutaneous, rectal, ear, genital), urine, hair, feces, skin surface tape stripping) were collected. All samples were stored in a dedicated biorepository. This cohort is now being perpetuated using the same protocol, with additional follow-up at 24 months and then annually. In total, 587 variables are being recorded.

Preliminary results confirmed the feasibility of collecting extensive, high-quality data and biological samples. The minimally invasive procedures allowed for continuous, detailed monitoring of the early life stages and their effects on health status. Our goal is to maintain this cohort indefinitely, with the infrastructure that has been created serving as a foundation on which other studies can be built. The TGC makes available to the research community not only some of the data it collects but also its research platform, acting as a facilitator to explore new hypotheses and develop new technologies in connection with developmental biology.

6440/7312 | Effect of two artificial photoperiods on testicular proliferation and apoptosis and reproductive related hormones

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Natural manipulation of the reproductive activity of the domestic cat depends on a thorough understanding of the effect of exogenous light (1). The aims of this study were to test the effect of a long (LP) and a short artificial photoperiod (SP) on: (1) Testicular cytoskeleton and germ cell proliferation and apoptosis (2) to determine serum melatonin, testosterone (T), and antimullerian hormone (AMH).

Eight intact, 3.5–5 kg, 2–5 years male cats that belonged to our colony were housed free in windowless rooms. Four males were exposed to a short (SP, 6:18 L/D) and the other four to a long photoperiod (LP, 16:8 L/D) for 3 months each. Both photoperiods were preceded by an adaptation of 3 months of 10/14 L/D. At the end of the experiments the males were castrated and placed for responsible adoption (IACUC 21/001). Blood samples were drawn during the dark phase, between 4 and 5 h am under a red monochromatic light of <5 luxes (5 W, Proex, Argentina) at the end of the third month of each photoperiod for melatonin (USBiological, Tucker, GA, USA), T (Elecsys Testo II, Roche Diagnostics, Mannheim, Germany), and AMH (Elecsys®, Cobas, Roche Diagnostics International Ltd., Switzerland) evaluations. Histological testicular sections were processed for the immunohistochemical detection of proliferating germ cells (PCNA), apoptotic cells (Caspase-3) and a cytoskeletal protein (vimentin). Data were compared between photoperiods by the Wilcoxon's

test. Relationships between hormone concentrations were calculated with Sperman's correlation test. Data were expressed as mean ± SEM and the level of significance set at $p < 0.05$ (SPSS 24.0; IBM SPSS, Inc., Armonk, NY, USA).
Serum melatonin was higher in the SP than in LP (989.03 ± 35.29 vs. 800.91 ± 56.90 pg/mL; $p < 0.05$). Neither AMH (5.81 ± 0.90 vs. 6.14 ± 0.45 ng/mL; $p > 0.05$) nor T (5.41 ± 1.72 vs. 3.74 ± 1.05 ng/ml; $p > 0.1$) differed between the two photoperiods. There was no correlation between melatonin and other hormones while T and AMH were strongly and negatively correlated ($r = -0.94$; $p < 0.01$). There was a tendency to higher PCNA-positive germ cells percentage in the LP than in the SP (59.69 ± 2.73 vs. 52.54 ± 3.10; $p < 0.1$). Conversely, germ cell apoptosis was higher in the SP than in the LP (13.99 ± 1.46 vs. 11.18 ± 1.65; $p < 0.05$). No differences in vimentin immunoreactivity were found between SP and LP photoperiods (4.83 ± 0.58 vs. 4.71 ± 0.63; $p > 0.1$).
These results show that cats under an artificial LP are reproductively characterized by an increased testicular proliferation, decreased apoptosis and diminished serum melatonin. This knowledge can be useful for breeding and contraceptive lightning programs. Further research is warranted to maximize the use of light in the reproductive management of this species.

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7127/7867 | Studies on the ovarian function in female Bernese mountain dogs

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In the Bernese mountain dog (BMD), the decreased fertility calls for strategic measures. The whelping rate 2009–2019 was low, 63%, as was the litter size: in a third of litters born there were only 1–4 pups (1). A small litter size is considered to depend predominantly on maternal factors, mainly related to the ovarian function. A poor ovarian function may also lead to a low pregnancy rate, for instance in cases of anovulation. The aim of the present study was to investigate the early ovarian function as a potential cause for the reproductive problems in the BMD.
Twenty-two BMD bitches and 36 control bitches of 22 different breeds were included. The serum progesterone concentrations were analyzed in estrus and at the time of pregnancy diagnosis, approximately 4 weeks after mating. The concentration of anti-Müllerian hormone, AMH, correlated with the number of ovarian follicles, was analyzed in estrus. Heterophilic antibodies have been described to be more prevalent in the BMD than in most other breeds and may interfere with immunoassays (2). Their presence was also analyzed in estrus. The concentration of C-reactive protein, CRP, an acute phase protein that is elevated during pregnancy, was measured in estrus and at pregnancy diagnosis. Ratios were compared using the Fisher's exact test, and continuous variables were compared between BMD and controls using t-test.
The pregnancy rate in BMD was 54.5% compared to 75% in control bitches. Progesterone concentrations at the time of pregnancy diagnosis did not differ between BMD and other breeds ($p = 0.15$). Anovulation (diagnosed as no rise in progesterone concentrations) was seen in three BMD bitches but in no control bitch. This difference was not statistically significant ($p = 0.08$). Presence of heterophilic antibodies did not differ between BMD and other breeds ($p = 0.36$). The AMH concentration during estrus was lower in BMD bitches than in other breeds ($p < 0.001$) and the concentration of CRP at pregnancy diagnosis was also lower in BMD bitches than in other breeds ($p < 0.001$).
The pregnancy rate of BMD was low, as previously described. The semen quality of BMD has previously been shown to often be of inferior quality (3). The present study suggests an etiology to a decreased fertility involving the females. Giant breeds are known to have lower AMH concentrations than small and medium sized breeds (4). However, the presence of anovulatory cycles, which is uncommon in the general dog population, and the low AMH concentration during estrus may indicate an impaired folliculogenesis and warrants further investigations. More studies are also needed to evaluate the role of CRP during canine pregnancy and the importance of the lower CRP concentrations at pregnancy diagnosis in the BMDs.

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7141/7956 | A case of pseudoplacentation endometrial hyperplasia in a dog: Morphological features and sex steroid receptor distribution

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Pseudoplacentation endometrial hyperplasia (PEH) is an uncommon condition in dogs. Characterized by tissue organization resembling the endometrial layers at placentation sites, some authors mistakenly refer to it as placental-like structures. This case report details the early sonographic presentation and sex hormone receptor expression (PGR, ERα, and ERβ) at PEH sites and unaffected uterine areas.
A 21-month-old female Bernese Mountain Dog was presented for a routine uterine health check approximately 21 days after the end of her 2nd heat. No mating history was registered. The dog was in good health, with vaginal cytology indicating a luteal phase. The only notable finding was increased mucous accumulation in the caudal vagina, with bacteriological analysis revealing a low-grade physiological mixed flora. Ultrasound revealed two fluid-filled uterine cavities (one in each horn), suggesting either early pregnancy or cystic uterine lesions.
Weekly follow-ups over four weeks showed the structures developing a nodular appearance, a hyperechoic center and striations radiating from the periphery to the center. Initially no fluid was detected within the structures or the uterine lumen. Blood tests remained unremarkable, with a serum progesterone concentration of 5.98 ng/mL. Finally, however, the lesions increased in size, developing a villous appearance, and being characterized by echogenic endoluminal fluid within both structures and the uterine lumen, raising concerns about potential uterine infection. Thus, ovariohysterectomy was recommended

and performed. Tissue samples (uterine sites with and without lesions and ovaries) were fixed in formalin for histological analyses.

Macroscopically the affected endometrium appeared thickened with greyish tissue covered by a mucus-cloudy fluid. Histology confirmed the pseudoplacental appearance of the lesions, with the endometrium exhibiting notable epithelial proliferation arranged in layers resembling uterine placentation sites. The uterine surface had a papilliform appearance, and the superficial glands resembled glandular chambers. Deep uterine glands were partially separated from the superficial uterine compartment by a connective tissue layer characteristic of placentation sites. No decidualization was observed, consistent with the process being embryo-driven. Immune system infiltration was moderate without necrosis. Unaffected uterine sites exhibited typical morphology for late luteal phase, with some areas of superficial proliferation. Corpora lutea showed vacuolized lutein cells, consistent with the late luteal phase. Likewise, the uterine surface epithelium displayed characteristics typical of this stage. Immunohistochemistry showed weaker PGR and ERα staining at PEH sites, whereas ERβ staining was enhanced, particularly in the epithelial compartments. The cellular localization of each receptor was similar at both uterine sites. Thus, adding to strong stromal signals, PGR was primarily detected in the superficial glands, with no signals in the surface epithelium, and weak or no staining in deep uterine glands. ERα was localized in the glandular epithelium, with no (or weak) signals in the surface epithelium. ERβ was evenly distributed throughout the endometrial compartments, presenting strong epithelial signals, particularly in the surface epithelium at PEH sites.

In summary, this case report describes the sonographic course of PSE, starting with its onset, and provides the first insights into hormone receptor staining patterns, indicating differences between affected and unaffected uterine sites in PEH-affected uteri.

7110/7866 | Canine serum N-glycosylation changes during infection and pregnancy

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It is well established that levels of acute phase proteins (APPs) change both during canine pregnancy and during infection. Many of the APPs are glycoproteins. Significant changes of the N-glycosylation pattern during healthy canine pregnancy have recently been demonstrated (1), similar to changes observed in pregnant women. Diagnosing inflammatory disorders during pregnancy is complicated by the fact that pregnancy in itself induces inflammatory changes. The aim of the present study was to evaluate whether the overall canine N-glycan pattern is affected in bitches with a bacteriological infection, pyometra, and a primary viral infection, kennel cough, and to evaluate whether these changes are similar to the ones observed in pregnant bitches.

Serum samples from ten dogs diagnosed with pyometra, ten dogs diagnosed with kennel cough, ten pregnant dogs day 24–30 of pregnancy and nine healthy control dogs were analyzed. A method previously optimized for N-glycan quantification in canine serum (1), based on RapiFluor-labelling (2) and subsequent glycan mapping by HILIC-UPLC-FLR-MS analysis was used for analysis. A one way ANOVA was applied to compare the glycan/glycan group levels from pyometra, kennel cough, pregnant and control dogs. Post hoc analysis was performed using Dunnett's correction for multiple comparisons.

The two dominating glycans were glycans A2G2S2 and FA2. The levels of A2G2S2 were significantly higher in pregnant dogs and dogs with pyometra, compared to control dogs. The corresponding levels were on average 7.5 and 4.7 percentage points higher, respectively. The levels of the glycan group 'terminally galactosylated glycans' were significantly lower in pregnant dogs (9.96%, SD 1.61) and in dogs with pyometra (11.0%, SD 1.67) compared to healthy dogs (13.5%, SD 1.82). Furthermore, levels of high mannose glycans were significantly higher in pregnant dogs (4.26% SD 0.50) and in dogs with pyometra (4.28% SD 0.38) compared to control dogs (4.92% SD 0.53). No significant differences were observed for the FA2 glycan, or the glycan groups 'sialylated', 'galactosylated' and 'agalactosylated complex glycans'. Significant differences for both individual N-glycans and N-glycan groups were observed for pregnant dogs as compared to controls. The N-glycan changes associated with pregnancy were rather small, i.e. a few percentage points. Differences found to be statistically significant showed the same pattern for pregnant dogs and dogs with pyometra, and similar changes were seen in dogs with kennel cough. The overall canine N-glycan pattern does not seem suitable for differing inflammatory changes present in pregnant dogs from changes in dogs with pyometra or kennel cough. N-glycosylation can regulate function, stability and quality control of glycoproteins, such as APPs, and further studies of N-glycosylation in inflammatory processes are warranted.

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6955/7765 | Too young to be sweet: A rare case of type 1 diabetes mellitus in a kitten

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Type 1 Diabetes Mellitus (DM), resulting from immune-mediated destruction of pancreatic β-cells leading to absolute insulin deficiency, is exceptionally rare in cats. In contrast, Type 2 DM—characterized by insulin resistance and β-cell dysfunction—is prevalent among overweight adult cats.(1) To date, Type 1 DM in felines has been poorly documented. This case report details an unexpected diagnosis of diabetic ketoacidosis (DKA) in a kitten, underscoring the importance of considering diabetes even in pediatric patients.

A 5-week-old male Burmese kitten presented with severe dyspnea, dehydration, anorexia, vomiting, and apathy. Clinical signs commenced two days prior and deteriorated following a dexamethasone injection (0.2 mg/kg SC) administered by a private veterinarian the day before presentation. Anamnesis revealed that from as early as three weeks of age, the kitten had

been voraciously consuming its mother's dry food—an atypical feeding behavior at that developmental stage.

Clinical examination showed tachypnoea (40/min) with costo-abdominal effort, a heart rate of 240 bpm, a core temperature of 38.8°C, and a body weight of 450 g (BCS 3/9). Initial diagnostics revealed severe hyperglycemia (22.2 mmol/L). Abdominal ultrasound indicated severe gastroenteritis with gastric overdistension, reduced peristalsis, mesenteric lymphadenopathy, and bladder sediment. Thoracic radiographs displayed mild, diffuse increased lung opacity, suggestive of early aspiration pneumonia. Echocardiography excluded cardiogenic dyspnea.

Severe dyspnea and the kitten's fractious behavior complicated handling, while dehydration and hemoconcentration limited blood sampling to a complete blood count. Results showed moderate leukocytosis ($28.8 \times 10^3/\mu L$) with neutrophilia, eosinophilia, lymphocytosis, and pronounced lipemia. The initial working diagnosis included gastric overload due to excessive dry food intake, contributing to respiratory distress. The kitten received oxygen therapy, intravenous fluids, butorphanol, amoxicillin-clavulanic acid, and a continuous rate infusion of metoclopramide. Although initial stabilization was achieved by early evening, blood glucose levels rose again overnight, eventually peaking at 31 mmol/L. The following morning, persistent hyperglycemia and marked ketonuria (4.6 mmol/L) confirmed DKA.

Given the poor prognosis and in agreement with the owner, euthanasia was performed. Histopathology of the pancreas revealed severe atrophy of the islets, with the remaining islet cells showing swollen, vacuolated cytoplasm and small peripherally located nuclei, interstitial fibrosis, and multifocal lymphocytic inflammation. Additionally, hepatic lipidosis and acute alveolar oedema were diagnosed. Insulin immunostaining of the pancreas showed only sparse β-cells, mainly arranged in small clusters or as isolated cells. These findings support profound β-cell loss, consistent with type 1 DM.

This case highlights the necessity of considering DM in pediatric patients, despite its extreme rarity. Given the predisposition of Burmese cats to pancreatic dysfunction (2), diabetes should be an early differential diagnosis in this breed, even in atypical presentations. The kitten's early polyphagia, severe hyperglycemia, and metabolic decompensation following corticosteroid administration suggest unrecognized congenital diabetes. Awareness of type 1 DM in young cats is crucial to prevent diagnostic delays, particularly in cases with unexplained hyperglycemia and gastrointestinal symptoms.

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A10 | Minimally invasive repair of a congenital inguinoscrotal hernia in a Cavalier King Charles Spaniel using the percutaneous internal ring suturing (PIRS) technique: Case report

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Clinical case: A five-month-old male, Cavalier King Charles Spaniel, was presented with a soft, non-painful, left-sided scrotal enlargement. The scrotal swelling was manually reducible and exhibited normal temperature. Ultrasonography confirmed fat tissue/omentum within the inguinal canal/scrotum, suggesting a unilateral left inguinoscrotal hernia. The hernia was diagnosed as involving adipose tissue, with no signs of complications, and testicular viability was to be preserved.

A minimally invasive technique, Percutaneous Internal Ring Suturing (PIRS) was chosen to minimize surgical trauma, postoperative discomfort, and recovery time —especially important for a young, active animal. Under general anesthesia, the dog was positioned in dorsal recumbency. Preoperatively, the hernia was manually reduced. A 5 mm laparoscopic port was inserted in the midline post-umbilically, and a minilap trocar was placed cranio-lateral to midline. A 2/0 non-absorbable monofilament suture (Ethilon®) was introduced percutaneously using a Tuohy needle and passed around the internal inguinal ring under laparoscopic visualization, applying a suture-loop technique with two skin entry points. The suture was tied externally, effectively closing the ring without open dissection and while maintaining patency for the spermatic cord and associated vessels, preserving testicular blood supply.

Postoperative analgesia consisted solely of meloxicam for five days. The patient showed no signs of discomfort, requiring no additional analgesia. Exercise was restricted for 7–10 days, and follow-up evaluations at two weeks and one month confirmed normal blood flow and testicular viability via Doppler ultrasonography. No recurrence or complications were observed up to four months post-surgery. The puppy resumed normal activity within days, demonstrating the advantages of the minimally invasive approach.

Congenital inguinoscrotal hernias are rare in dogs, often requiring surgical intervention to prevent complications such as strangulation or organ entrapment. Traditional open herniorrhaphy remains the standard, yet minimally invasive methods like PIRS are emerging as promising alternatives in veterinary medicine.

This case demonstrates the feasibility of adapting the PIRS technique, previously described in ovine models, for canine congenital inguinal hernia repair. This minimally invasive approach resulted in minimal tissue trauma, reduced postoperative pain, and a quicker return to normal activity. Further studies are needed to evaluate its broader application in small animal surgery. Long-term follow-up will be essential to confirm testicular function and reproductive outcomes.

In the author's knowledge, this is the first reported case of a congenital inguinoscrotal hernia repair in a dog using the PIRS technique. The successful adaptation of this technique suggests a viable alternative to traditional open surgery, possibly allowing

not to castrate the individual and potentially setting a precedent for its wider implementation in veterinary practice.

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A11 | Atypical glandular proliferation suggestive of endometrial/vaginal adenocarcinoma in a Labrador Retriever

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Clinical case. A 5 year-old female Labrador Retriever with chronic kidney disease was referred for evaluation of a persistent vaginal-uterine remnant distended with fluid, approximately 2 years after ovariohysterectomy. Ovarian remnant tissue was discarded, by measuring AMH, and a computed tomography (CT) was performed to exclude metastases. Despite two prior surgeries to reduce the uterine/vaginal stump, the diverticulum continued to accumulate fluid, leading to periodic strangury due to compression of the urethra. Multiple ultrasound-guided drainages were performed for symptom relief.

Laparoscopic exploration revealed a mass-like structure at the vaginal remnant, closely adhered to the bladder, impairing ureteral visualization. To avoid ureteral injury, extensive dissection was not attempted. A laparoscopic-assisted approach, combined with vaginal endoscopy—which identified a membrane obstructing the vaginal lumen cranial to the cingulum—was utilized to create a controlled vaginal fistula, allowing drainage by linking the diverticulum to the vaginal lumen. Biopsies were obtained and a Folley catheter was placed connecting the diverticulum with the vulva. Postoperatively, fluid accumulation decreased and spontaneous urination was restored.

Histopathology suggested an atypical glandular proliferation consistent with endometrial adenocarcinoma. The cytological characteristics indicated malignancy, though the exact origin and biological behavior required further investigation. The CT scan identified a heterogeneous soft tissue structure involving the left ureter at the L4 region and a mass-like lesion within the vaginal remnant extending towards the bladder trigone. No pulmonary or abdominal metastases were detected, except a calcified pulmonary nodule suggestive of a benign granuloma.

Given the histopathological findings and imaging results, the patient was referred to an oncology specialist for evaluation of treatment options.

Uterine neoplasms are rare in dogs, accounting for 0.3–0.4% of all canine tumors, with endometrial adenocarcinoma being an uncommon but potentially malignant entity (3, 4). Most uterine tumors are incidental findings during ovariohysterectomy, rarely cause clinical signs unless they reach significant size or invade adjacent structures. However, in cases of hormonally active or malignant lesions, persistent clinical signs may develop even after surgical removal of the uterus (1, 2).

Cystic or proliferative structures in the vaginal remnant post-ovariohysterectomy are unusual and raise concerns regarding residual reproductive tissue, neoplastic transformation, or chronic inflammation (5). This report describes a complex case of a suspected endometrial/vaginal adenocarcinoma within a vaginal diverticulum, in a dog without history of hormonal treatments or ovarian remnant syndrome, leading to recurrent fluid accumulation, urinary dysfunction, and requiring surgical management.

This case highlights the complexity of diagnosing and managing suspected endometrial adenocarcinoma in dogs, particularly when arising within a vaginal remnant post-ovariohysterectomy. While uterine neoplasia is rare, they should be considered in cases of persistent reproductive tract abnormalities. A laparoscopic-assisted approach offers precise exploration, biopsy collection and minimally invasive management of structures, such as diverticula, while preserving ureteral integrity (1, 2).

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7098/7762 | Effect of the ABCA1 inhibitor probucol on post thaw characteristics of dog spermatozoa

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During freezing and thawing, capacitation-like changes occur within the sperm plasma membrane, impairing fertilizing capacity. In extended fresh canine semen, addition of probucol (PRO), a cholesterol transporter antagonist specific for ABCA1 reduced capacitation-like changes. The aim of this study was to investigate effects of probucol (PRO) on post-thaw dog semen characteristics.

Ejaculates from 17 stud dogs were collected. Sperm-rich fractions from dogs collected on the same day ($n = 2$–3) were pooled (13 pooled samples) and divided into 5 aliquots that were frozen according to the Uppsala protocol with treatment T1: untreated control, T2 addition of 250 µM PRO and T3 of 500 µM PRO before dilution with cooling medium and equilibration for 1 h (+4°C), T4 addition of 250 µM PRO and T5 of 500 µM PRO before dilution with freezing extender. Straws were stored in liquid nitrogen at −196°C until analyses. Kinematic data (IVOS II, IMV Technologies), sperm concentration (NucleoCounter SP-100, ChemoMetec) and sperm morphology (Hancock's solution) were assessed.

For tyrosine phosphorylation, a semen smear was prepared on a glass slide, fixed with 4% paraformaldehyde, permeabilized with Triton-X and incubated with mouse anti-phosphotyrosine antibody (clone 4G10®; Merck Millipore, Darmstadt, Germany). Then, slides were incubated with Alexa Fluor 488-conjugated goat anti-mouse IgG (Thermo Fisher Scientific, Breda, the Netherlands) and stained with Hoechst 33342 (Sigma,St. Louis, MO, USA) and microscopically evaluated as described by Petrunkina (3). Because not all data were normally distributed, comparisons among treatments were done by Friedman test followed by Wilcoxon test.

Motility, progressive motility (P), viability, total number of morphologically abnormal sperms as well as ALH, BCF, DAP, DCL, DSL, VAP, VCL, and VSL were reduced after thawing, irrespective of treatment ($p < 0.01$). Motility, P, linearity (LIN), straightness (STR) and total capacitation were decreased by addition of PRO in comparison to controls ($p < 0.01$); but not the other kinematic characteristics and pathomorphology. Tyrosine phosphorylation demonstrated a decrease in non-capacitated sperm after freezing-thawing ($p < 0.001$) that was less pronounced after addition of 500 µM PRO (T3 and T5).

Although addition of the cholesterol transporter antagonist probucol at 500µM decreased capacitation like changes after freezing-thawing of dog sperm, the treatment did not counteract the decrease of all other sperm characteristics investigated.

7148/7761 | Hormonal evolution and changes in different serum markers after gonadectomy and/or deslorelin implant administration in adult male dogs during a 6-month period

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Surgical and medical sterilization are widely used techniques for fertility control and behavioral management in male dogs. However, their effects on endocrine regulation remain incompletely understood. This prospective clinical study aimed to evaluate and compare the hormonal changes induced by orchidectomy (ORX) and the use of adeslorelin (DES) subcutaneous implant (Suprelorin®, Virbac) in healthy male dogs over a 6-month period.

Thirty-four healthy, sexually mature male dogs, aged between 9 and 72 months old (28 ± 17.2 months) and weighing 26.07 ± 8.59 kg, were enrolled. All dogs were privately owned animals presented for castration at consultations in two Veterinary schools. Dogs were randomly assigned to three groups at inclusion date: (G1) ORX + placebo ($n = 6$); (G2) ORX + DES ($n = 8$); (G3) DES only ($n = 8$). A fourth group of twelve intact owned dogs was included as control (G4). Blood samples were collected at Day 0 (D0), Month 1 (M1), Month 2 (M2), and Month 6 (M6) to assess serum levels of LH, FSH, AMH, CPSE with ELISA canine specific kits and steroid hormones by a GC-MS method. Statistical analyses included two-way ANOVA and Mann–Whitney U tests; Significant differences were set when $p < 0.05$.

At baseline (D0), LH concentrations were variable but comparable between groups, with no statistically significant differences. However, from D0 to M6, distinct patterns emerged among treated groups. LH levels remained elevated and stable in G1 but significantly decreased in both DES-treated groups (G2 and G3, $p < 0.0001$). No statistical difference was observed between G2 and G3. FSH showed no significant variation across groups at any time point and compared to D0. Testosterone and DHT dropped below the detection limit (<0.016 ng/mL) after treatment in G1, G2 and G3. At M6, dogs belonging to G3 had significantly higher residual testosterone levels than G1 and G2 groups ($p < 0.05$), though still within the suppressed range (<0.4 ng/mL). Estradiol also declined significantly from D0 to M1 in G1, G2 and G3, and remained low up to M6. AMH decreased to undetectable levels in G1 and G2 but increased significantly in G3 ($p < 0.0001$), suggesting Sertoli cell stimulation when testicular tissue remains (1). CPSE levels were comparable between groups at D0 but declined progressively in G1, G2 and G3, reaching low concentrations at M6, indicating suppression of prostatic secretory activity.

Testosterone and CPSE dropped in all dogs from G1, G2 and G3 between M1 and M2, and remained basal for at least 6 months.

In G2, the concomitant use of orchidectomy with deslorelin implants dramatically lowered the LH level, which is different from what was observed in G1, where dogs were just gonadectomized. Interestingly, FSH level remained stable in all groups. Compared to D0, AMH level increased in all G3 dogs, demonstrating that it is a good marker of the efficacy of the deslorelin implant in male dogs (1).

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7128/7818 | Antimicrobials resistance profile of *Escherichia coli* and *Staphylococcus pseudintermedius* isolated from healthy breeding bitches in Northern Italy

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Antibiotics are frequently and unjustifiably administered in dog breeding facilities, to increase fertility and reduce neonatal mortality. The aim of this study was to investigate the antimicrobial resistance profile of *Escherichia coli* and *Staphylococcus pseudintermedius*, the first a commensal bacterium of the intestinal tract, the second a colonizer of skin and mucous membranes, in healthy breeding bitches. The prevalence of multidrug-resistant (MDR) strains, Extended Spectrum Beta Lactamase (ESBL)-producing *E. coli* and methicillin-resistant *S. pseudintermedius* carrying the mecA gene was evaluated.

The study included 74 healthy bitches, 5 from each of 15 breeding kennels. Two bacteriological samples were collected from each animal, one from the perivulvar skin and one from the

rectum, using sterile wet swabs (ESwab, 480CE, Copan Italia Spa, Brescia), and processed by the IZS Venezie within 72 h. Dog breeders were given a structured questionnaire covering antibiotic use. Questions required categorical response options ranging from "always" to "never". For bacteriological isolation, classical microbiology procedures were applied and mass spectrometry was used for species identification. Minimum inhibitory concentration test was performed for antimicrobial susceptibility assessment (1). ESBL-producing *E. coli* strains were phenotypically confirmed (2), and the *mecA* gene was checked in oxacillin-resistant *S. pseudintermedius* colonies. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (3). The answers to the questionnaire were scored and the breeding kennels were categorised as MU (minimum antimicrobials use), SU (seldom use) and RU (regular use).

The association between the percentage of resistance to each antimicrobial agent of *S. pseudintermedius and E. coli* and the breeding kennel category, percentages of ESBL *E. coli*, MRSP and MDR strains were assessed by means of Fisher's exact test. Significance was set at $p < 0.05$.

A total of 116 *Escherichia coli* and 36 *Staphylococcus pseudintermedius* strains were isolated. The resistance profile of *E. coli* showed complete resistance to Beta-lactam/Beta-lactamase inhibitor combinations (ampicillin and amoxicillin-clavulanic acid) and to first-generation cephalosporin. Resistance to the third-generation cephalosporins, cefpodoxime and cefovecin, was 32.39% and 30.99%, respectively. A similar resistance percentage was found for tetracyclines (29.58% for tetracycline and 28.17% for doxycycline). Lower percentages of resistance were found for folate pathway inhibitors (trimethoprim-sulfamethoxazole, 21.13%) and aminoglycosides (gentamicin 7.04%, kanamycin 12.68%, amikacin 1.41%), while it was 7.04% to fluoroquinolones (enrofloxacin, pradofloxacin). Additionally, 9 ESBL isolates (7.76%) were detected.

S. pseudintermedius isolates showed high resistance to Beta-lactam/Beta-lactamase inhibitor combinations (amoxicillin-clavulanic acid and oxacillin 29.41%, ampicillin 82.35%, penicillin 88.24%) and tetracyclines (tetracycline and doxycycline, 44.12%). Lower resistance towards other antimicrobial agents was detected (aminoglycosides from 17.65% to 32.35%, first- and second-generation cephalosporins 26.47%–20.59% and fluoroquinolones 5.88%) . Of the isolated *S. pseudintermedius*, 16.67% were mecA-positive. 45 MDR (42.86%) were detected, 29 (40.85%) *E. coli* and 16 (47.06%) *S. pseudintermedius*. ESBL, MRSP and AMR prevalence did not result associated with the breeding kennel category.

These results confirm the need to prioritize antimicrobial stewardship and to sensitise to the possible misuse of antimicrobials in dog breeding.

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6459/7768 | What makes the difference – Ultrastructure of in vivo and in vitro matured feline oocyte

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The oocyte quality and competence are crucial for successful application of in vitro techniques in any species. In cats, the ART advances are sought mainly in hope that developed methods and techniques will become useful for wild Felidae gene conservation. Unfortunately, despite long time ongoing research the results of in vitro maturation, fertilization and embryo culture in cats are still lower than expected.

The study aimed to compare the ultrastructural organelle distribution of in vivo matured oocytes with oocytes collected from donors of different ages (prepubertal and mature females), both before and after in vitro maturation.

In total 25 oocytes were collected and divided into following experimental groups: in vivo matured oocytes collected from oviducts (I. $n = 5$, in vivo-M) and GV oocytes collected through ovarian cortex slicing of ovaries obtained from donors before puberty and from adult queens that were evaluated before (II. $n = 5$, Pp-noIVM. III. $n = 5$, A-noIVM) and after in vitro maturation (IV. $n = 5$, Pp-IVM. V. $n = 5$, A-IVM). All oocytes were fixed in 2.5% glutaraldehyde and processed for observations in transmission electron microscope (TEM). Analysis included mitochondrial morphometric traits, % of visible ooplasm covered by lipid droplets (LD), number of cytoplasmic vacuoles (CVs) per μm^2 and number of contact sides (CS). Statistical inference was based on ANOVA with LSD post hoc test.

The key distinguishing feature of in vivo matured oocytes compared to all other groups was the lowest number of cytoplasmic vacuoles (CVs). In vitro matured oocytes from prepubertal donors closely resembled in vivo matured oocytes, except for CVs count. In contrast, oocytes from adult queens after IVM exhibited significantly smaller mitochondrial diameters and fewer mitochondrial cristae when compared to in vivo ones. Additionally, oocytes from adult donors had larger mitochondria, and less CS and lower % of surface occupied by LDs before IVM than after IVM, whereas young non-matured oocytes exhibited the opposite trend, with larger % LDs and increased mitochondrial cristae in comparison to Pp-IVM. Significant differences were also observed in oocytes from prepubertal and adult queen subjected to IVM, with Pp-IVM having a larger mitochondrial diameter and more cristae than A-IVM. However, before maturation, mitochondrial diameter was greater in A-noIVM oocytes.

The primary goal of in vitro procedures is to obtain oocytes competent for fertilization and subsequent embryonic development. Cytoplasmic organelles, their spatial distribution, and functional interactions play a crucial role in proper maturation and developmental potential. The results presented above highlight the impact of maturation conditions (in vivo vs. in vitro) on oocyte cytoplasmic architecture. These findings may contribute

to optimizing culture conditions that better preserve cytoplasmic integrity during in vitro procedures.

6270/7934 | Bluetongue-induced fatal pregnancy loss in a Rafeiro do Alentejo dog

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Pregnancy loss in dogs is a complex clinical challenge, often linked to bacterial, viral, parasitic, hormonal, or environmental factors. While infectious causes such as *Brucella canis*, Canine herpesvirus (CHV-1), and *Toxoplasma gondii* are well-documented, less common agents are rarely suspected during clinical workup. This case report describes the diagnostic process and clinical evolution of a pregnant Rafeiro do Alentejo bitch that presented with a severe reproductive and systemic disorder caused by Bluetongue virus (BTV) infection. Although BTV is known to cause reproductive failure in ruminants, its impact on canine reproduction remains poorly documented.

A 2-year-old pregnant Rafeiro do Alentejo bitch, living in a rural environment with no direct contact with livestock, was presented to the clinic with brown-orange, malodorous vulvar discharge lasting two days. The bitch was vaccinated and regularly dewormed, with no known history of previous reproductive issues. Upon admission, the animal showed mild apathy, pink mucous membranes, and vital parameters within acceptable ranges (T = 38.7°C; HR = 92 bpm; RR = 24 rpm). An estimated 5% dehydration was noted, with mild abdominal discomfort on palpation. Abdominal ultrasound revealed an enlarged uterus containing heterogenous fluid and thickened walls. Initial bloodwork (complete blood count, biochemistry, and electrolytes) showed normal values except for mild hypoalbuminemia (2.5 g/dL) and low creatinine (0.2 mg/dL). Panel test screening for *Dirofilaria* spp., *Leishmania* spp., *Ehrlichia canis*, and *Anaplasma* spp. was negative. Supportive care included intravenous fluid therapy (Ringer's lactate), broad-spectrum antibiotics (ampicillin and metronidazole), antiemetics (maropitant), gastric protection (omeprazole), and analgesia (methadone).

Over the next 48 h, the bitch's condition deteriorated, having developed respiratory distress while urine output ceased despite a small bladder volume. Hematological reassessment revealed anemia, leukocytosis, mild neutrophilia, thrombocytopenia, and decreased hematocrit. Biochemistry indicated worsening hypoalbuminemia (2.3 g/dL), severe azotemia (creatinine = 9.86 mg/dL; BUN > 140 mg/dL), and mild increases in ALP and GGT (106 U/L and 18 U/L, respectively). Thoracic imaging confirmed pulmonary edema, and abdominal ultrasonography showed persistent uterine changes and hypoechoic renal cortices. Serology for Neospora caninum and *Leptospira* spp. was negative, while *Toxoplasma gondii* IgG was positive with negative IgM. Testing and serotyping evidenced BTV-3 infection (Ct 25).

Despite oxygen therapy, diuretics, and continued supportive care, the clinical condition worsened, with severe respiratory distress, vomiting, abdominal pain, progressive hypothermia (37.2°C), and minimal urine output (71 mL/24h). Euthanasia was performed on day 7 due to poor prognosis. Postmortem findings confirmed severe uterine alterations, acute renal failure, pericardial and pleural effusion, and pulmonary edema.

This case underscores the diagnostic challenges posed by atypical infectious causes of abortion in dogs. BTV, primarily known for affecting ruminants and transmitted by *Culicoides* spp. biting midges, is rarely considered in canine reproduction. The absence of direct contact with livestock and aborted material highlights the potential impact of vector-borne transmission and BTV's role on canine fertility. Enhanced surveillance in dog populations in rural areas, especially in regions with high abundance of midges, may warrant a better understanding of the overall BTV's impact in dogs health.

6530 | A case of foreign body-induced vaginitis in a spayed bitch

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A five-year-old mixed-breed spayed female dog started displaying lethargy, inappetence and vulvar licking. Seven days into the symptoms, the bitch was visited by the referring veterinarian. Clinical examination revealed elevated body temperature and a painful reaction to vulvar palpation. Blood tests showed high C-reactive protein, as well as neutropenia and a slight anemia. Abdominal ultrasound did not reveal any abnormalities. A 10-day antibiotic treatment course with enrofloxacin and metronidazole (dosages not reported) was prescribed. Clinical signs improved during antibiotic treatment but vulvar licking persisted, therefore the bitch was presented to the Veterinary Hospital for a second opinion. A thorough physical and reproductive examination revealed marked perineal and vulvar swelling and a significant pain reaction from the bitch upon any attempt at manipulation. Sedation was performed by subcutaneous administration of dexmedetomidine (8 µg/kg), methadone (0.1 mg/kg) and ketamine (1 mg/kg). A cranial vaginal culture sample was collected using a swab protected from vulvar and cranial vaginal contamination. Vaginal cytology was performed, revealing predominantly non-keratinized parabasal cells and a small number of neutrophils. Vaginal examination using a vaginal speculum revealed a hyperemic vaginal mucosa with small abrasions and an awn grass foreign body located in the vertical portion of the vagina. The foreign body was removed successfully and bupivacaine was administered intravaginally. Meloxicam (0.2 mg/kg) was administered intravenously and prescribed for the following 4 days at 0.1 mg/kg once a day orally. Anesthesia was reversed with atipamezole. Vaginal culture identified *Streptococcus* spp. however, no antibiotic treatment was initiated. Following the removal of the foreign body, clinical signs improved within 4 days, and the bitch fully recovered. Discussion: The differential diagnoses for vulvar and vaginal swelling in spayed female dogs includes urine pooling, exposure to chemical irritants, neoplasia, vaginal trauma, or vaginitis (1). Vaginitis is often secondary to non-infectious causes such as congenital anomalies of the genital tract, foreign bodies, trauma, vaginal tumors, urinary tract disorders or consequent to pre-pubertal gonadectomy. Although congenital

abnormalities commonly present at a younger age and in intact females, vaginal anomalies such as clitoral hypertrophy, vestibulovaginal stenosis, vaginal septa and fibrous bands should also be considered in a case of chronic or recurrent vaginitis. These conditions may predispose to secondary inflammation, retention of secretions, or foreign body entrapment, and may not be easily detectable without vaginoscopy. Despite bacterial growth on vaginal culture, antibiotic treatment was not prescribed, as *Streptococcus* spp. is a normal component of the vaginal flora (2). This case highlights the importance of a thorough reproductive examination, including vaginoscopy and vaginal cytology, and emphasizes that empirical antibiotic treatment may mask symptoms, but not always treat the cause of inflammation.

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6325/7783 | Partially and non-responsive deslorelin treatments in female and male cats

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Lack of efficacy after deslorelin is occasionally observed but its incidence has not been formally reported. This paper describes a series of cases of cats in which deslorelin failed to provide an adequate response.

Medical records of both female and male cats implanted with deslorelin were assessed retrospectively. Cases with absent or incomplete responses to treatment were selected and their anamnestic, clinical, and laboratory data were analyzed. Before implantation (with doses of 4.7 or 9.4 mg of deslorelin), all cats underwent thorough general and reproductive examinations, including vaginal cytology, reproductive ultrasound and hematology, biochemistry. For research individuals, progesterone and testosterone were assayed, the latter 1 h after GnRH stimulation (50 µg IM gonadorelin).

Among the 147 cats (63 males and 84 females) treated with deslorelin during the study period, 7 cats (4 males and 3 females) did not respond adequately to treatment. The age range of this subset of cats was from 8 months to 7 years. Five cats were implanted in the periumbilical area and 2 males intrascapularlly. Tomcat 1 mated with two females, at 14 and 85 days post-implantation, which resulted in the delivery of 4 and 6 kittens, respectively. Nonetheless, he gained weight and became more affectionate toward his owners. Tomcat 2 never exhibited basal testosterone concentrations following GnRH stimulation (16 ng/mL 4 months post-implantation), and penile spikes never disappeared, although testicles decreased in size. Tomcat 3 continued to mate with queens successfully, and semen motility and morphology remained excellent under deslorelin treatment. The cat was reimplanted 15 months after the first treatment, responding fully. Tomcat 4 never exhibited testicular atrophy, penile spike regression, or basal testosterone concentrations (12.4 ng/mL 1 year post-implantation). Queens 5, 6 (sisters), and 7 continued cycling regularly after treatment (estrus confirmed by cytology), and the latter became pregnant 1.5 months after implantation and queened, displaying normal maternal behavior. Queens 5 and 6 were ovariohysterectomized and the implant was recovered during the surgery. In queen 7 the implant was searched for through palpation periumbilically without success.

Inadequate responses to deslorelin were identified in 6% of tomcats and 4% of queens. Four/7 cats (cats 4, 5, 6 and 7) were classified as fully non-respondent. In the remaining 3, partial (behavioral and reproductive) changes were noticed. Although persistent estruses have been observed (1), the maintenance of a regular estrus cycle after deslorelin in queens is reported for the first time in this study. Longer time of downregulation onset (28 weeks until complete effect have been reported (2)), defects of the implant or of its storage and loss of implant after administration cannot be ruled out as causes of atypical response. However individual factors such as genetic mutations in the case of the sister queens, should be considered as possible determinants of non-responsiveness to deslorelin. Further research is needed to study individual factors that lead to absence of response to deslorelin.

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7136/7804 | Toward optimal cat oocyte transport: Impact of retrieval and storage conditions on in vitro maturation

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Due to the critical conservation status of many felid species, assisted reproductive technologies (ARTs) have been refined for genetic recovery programs. Following an animal's death or a medically required ovariohysterectomy it is crucial to optimize oocyte transport conditions to the laboratory to minimize the impact on oocyte quality and ensure the success of ARTs. With the development of ovum pick up (OPU) in felines (1), this issue becomes even more relevant. The domestic cat serves as a valuable model to wild felid species. Transport conditions are critical factors influencing subsequent maturation potential (2). Previous studies have shown that exposing ovaries to low temperatures during transport can cause cellular autolysis, leading to cytoplasmic vacuoles, damaging the microtubular cytoskeleton, or directly affecting nuclear structure (3,4). Nevertheless, feline oocytes exhibited greater cryotolerance than other species, allowing storage at 4°C for up to 24 h without compromising meiotic competence or in vitro blastocyst development (5). No studies have yet investigated the effects of transporting isolated feline oocytes in a holding medium. This research assesses the

impact of storing isolated oocytes at room temperature for 18 h in a commercial holding medium on their nuclear maturation.
For this study, 22 ovaries from domestic cats were processed following elective ovariectomy. One ovary from each pair was subjected to either slicing or aspiration for oocyte retrieval, resulting in the recovery of 514 oocytes, of which 270 were selected for in vitro maturation (grade I and II). Each pool of oocytes obtained with each method was divided into two groups. The first one ($n = 73$ slicing-0 h and $n = 51$ OPU-0h) underwent direct in vitro maturation (IVM) for 28 h (supplemented TCM-199) at 38.5°C and 5% CO_2. The second group ($n = 96$ slicing-18h and $n = 50$ OPU-18h) was stored at room temperature (18-20°C) for 18h in an equine commercial holding medium. After 18 h of storage, oocytes were washed in IVM medium and subsequently in vitro matured. After 28 h, 254 oocytes were denuded with 0.1% hyaluronidase, fixed in 4% paraformaldehyde (PFA) and washed in phosphate-buffered saline (PBS). Chromatin was stained with 1 µg/mL Hoechst 33342. Oocytes were then washed, placed in mounting medium (50%PBS, 50% glycerol, 0.0025 µg/mL Hoechst) between a coverslip and slide. Oocytes ($n = 254$) were observed under fluorescence microscopy to assess nuclear maturation. Germinal vesicles, metaphase I, and metaphase II were recorded. A one-way ANOVA test was performed, considering $p < 0.05$ as statistically significant.
Maturation rates were 54.13 ± 3.28% and 45.01 ± 8.47% for slicing-0 h and OPU-0 h groups respectively and 33.30 ± 1.65% and 38.19 ± 5.46% for the slicing-18 h and OPU-18 h groups respectively. Significant differences were observed between 18 h groups ($p < 0.01$) compared to 0h groups, indicating that storage for 18 h may significantly affect oocyte maturation capacity, regardless of the retrieval method used. However, no significant differences were found between slicing 0h and OPU 0h ($p > 0.05$), suggesting that retrieval method does not negatively impact oocyte maturation.

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6545 | Diagnostic and clinical insights into ovarian remnant Syndrome in cats: A focus on AMH and AFC

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Ovarian Remnant Syndrome (ORS) is a complex condition that can present with various clinical signs include vocalization, lordosis, attraction of male animals, and even allowing mating. The use of Anti-Müllerian Hormone (AMH), widely regarded as the most reliable marker of human ovarian reserve (1), is a current area of interest as a diagnostic biomarker for ORS in pets. However, previous studies with limited sample sizes have not reached a consensus on its diagnostic accuracy (2,3) or established a definitive threshold value (4). Additionally, the relationship with AMH and Antral Follicle Count (AFC) is limited to a single study (5) consisting of intact cats. Thus, it is anticipated that performing AFC alongside AMH measurement will provide valuable insights into the diagnosis and clinical course of ORS in cats.
This study involved 25 female cats diagnosed with ORS and the methodology included examination of the reproductive tract, blood sampling for serum AMH measurement (ECLIA method) (6), surgical intervention to remove OR, histopathological examination, and AFC determination of OR. The statistical analysis employed descriptive measures such as mean and percentage, along with the Pearson correlation test to assess relationships between variables.
The mean serum AMH level was found to be 0.975 ± 0.22 ng/mL (range 0.02–4.71) in 25 cats in which ORS was confirmed postoperatively. The previous surgical site selections resulting in ORS were found to be similar, with 52% being lateral and 48% median laparotomy. The clinical findings of ORS appeared on average 128.5 ± 25.9 days (range 7–390) after the surgery. In all cases, lordosis and rolling were consistently observed as the common signs of estrus behavior. One particular case #10, with an ovarian remnant volume of 0.1 ml and a serum AMH level of 0.13 ng/mL, was found to have even allowed mating. The mean volume of the OR was 0.19 ±0.02 ml (ranged 0.02-0.50). It was noticed that a majority of the OR's (78%) contained follicular cysts. While no correlation between AMH and AFC was detected, a negative correlation ($p < 0.017$) of AMH with follicular cysts was identified.
This study is the first to assess AFC with remnant volume, and their relationship with AMH in cats with ORS, using the largest sample size in feline ORS literature. While no direct association was found between AMH levels and ovarian remnant volume or the severity of clinical signs, follicular cysts were linked to decreased AMH concentrations. In addition, it was found that the mating behavior was unrelated to the remaining ovarian tissue or serum AMH levels.
This study was a PhD Thesis Project supported by the Bursa Uludag University, Scientific Research Projects Unit.

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7151/7951 | Ultrasonographic fetal gender determination in cats

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Ultrasonographic fetal gender identification is often requested by owners and breeders during monitoring of pregnancy both for economic and emotional reasons. In domestic animals, fetal sex determination is performed with ultrasound by visualization of the fetal genital area or genital tubercle[2]. In cats, fetal gender determination can be obtained between the 38th and 43rd day of pregnancy by visualizing the external genitalia in a midsagittal scan, just below the tail. The prepuce and scrotum can be identified as two distinct protrusions, while the vulva appears as a single triangular bulge located ventral to the tail[1]. The aim of this study is to describe an alternative approach for fetal sex identification in cats, using different scanning planes to identify the genitalia.

Ten pregnant queens were presented for pregnancy monitoring. They were monitored with ultrasonographic scans between the 38th and the 55th days of pregnancy. Ultrasonography was performed with a Samsung RS85 ultrasound machine equipped with a linear transducer (2–14 MHz). The fetal external genitalia were visualized using both a midsagittal and longitudinal ventrodorsal and transverse scans.

Out of 41 fetuses, 18 were recognized as males (18/27) and 7 as females (7/14). In male fetuses, using a transverse scan of the perineal area the scrotum appeared as an ovoid area, with a medium hyperechoic line representing the scrotal septum, localized ventral to the tail and caudal to the perineum; the prepuce appeared as a bulging ventral to the scrotum. Using a longitudinal ventrodorsal scan, the scrotum appeared as an oval-rounded protuberance with a medium hyperechoic line at the end of fetal body. In female fetuses, both transverse and ventrodorsal scans revealed the vulva as a triangular or ovoid structure defined by at least two hyperechoic lines converging ventrally; in some cases two small rounded hypoechoic areas could be visualized just dorsal to the vulvar labia, representing the dorsal skin folds of the vulva.

As far as the authors know, the only one described method to assess the fetal gender in cats is published by Zambelli and coworkers[1]. Unlike previous descriptions, the authors found that transverse or longitudinal ventrodorsal scans facilitate visualization of the genital area even beyond day 43 of pregnancy making gender identification possible at later stages, whereas after day 43, midsagittal scanning is no longer useful for sex determination because of the closer position of the fetal tail to the perineum. In conclusion a correct visualization of the genitalia requires experience of the operator but it can be obtained even in late pregnancy with longitudinal ventrodorsal and transverse scans of the fetus. As previously described in cats and dogs[1],[2], larger litter sizes make fetal sex determination more challenging because of the reduced intra-abdominal space, increased fetal movements and lack of fetal fluids.

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7107 | The pellet method as an alternative for canine sperm cryopreservation

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Sperm cryopreservation is an important reproductive biotechnology alternative in breeding dog development, that allows sperm to be preserved for an undetermined period and transported over large distances. The development of suitable protocols that control cell damage from this process is still the highest challenge found. With the improvement in microbiological control, and sample storage, the pellet (PL) cryopreservation becomes another method to the conventional straws (ST) method since it can freeze the sperm in a lower volume avoiding the sample waste when evaluated after thawing, and when applied techniques such as intracytoplasmic sperm injection (ICSI) and artificial insemination (AI). Thus, this study aimed to compare the PL and ST methods in kinetics, morphology, and membrane integrity of dog sperm after frozen/thawed process.

Eight small breed stud dogs ($n=8$) from a commercial kennel ranging in age from two to five years old with a weighted mean of 3.2 ± 0.57 kg were selected. Sperm collection was carried out by digital manipulation. The fresh sample was evaluated in kinetics by CASA system, concentration, morphology, and membrane integrity. Then, centrifuged and resuspended in a TRIS-Yolk extender (citric acid, 20% egg yolk, OEP, fructose, and amikacin added 7 % of glycerol as a cryoprotectant) in a final concentration of 60×10^6 spermatozoa/mL. The final volume was divided into the treatment: straws of 0.5 mL (ST) and pellet of 50 µL (PL). Both samples were cooled at 4°C for 2 h. Then, ST was placed on a surface at 6 cm liquid nitrogen for 20 min and PL on the surface of a preformed crushed dry ice slab for 5 min. After that the treatments were immersed in liquid nitrogen and kept in a cryogenic bottle until analysis. Both treatments were thawed in a water bath (37°C/30 ss) and analyzed using the same parameters applied to fresh samples. The variables were analyzed in comparison of straws and pellet method by paired t-test considering $p < 0.05$.

PL showed higher kinetic parameters of total motility (72.0 ± 4.0 vs 59.5 ± 8.65 %), progressive motility (51.12 ± 6.83 vs 33.75 ± 4.02 %), average path velocity (92.92 ± 3.95 vs 77.93 ± 5.92 µm/s), straight-line velocity (77.81 ± 5.7 vs 65.87 ± 6.33 µm/s), and curvilinear velocity (115.48 ± 4.78 vs 96.17 ± 7.24 µm/s). Also, PL presented a higher percentage of rapid cells (65.0 ± 5.29 vs 42.37 ± 6.50 %), and a lower percentage of cells with medium (7.12 ± 2.35 vs 17.37 ± 4.68 %) and slow velocity (22.75 ± 2.18 vs 33.12 ± 6.6 %). Higher membrane integrity was found in the PL treatment (87.0 ± 2.36 vs 76.93 ± 7.36 %). No differences were found in sperm morphology.

The pellet method is a viable alternative to dog sperm cryopreservation, yielding better results compared to a conventional straw method.

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6168 | Inguinal hernia during pregnancy in a bitch – Successful surgery and neonatal survival

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Inguinal hernia in dogs is an uncommon but recognized pathology, but cases involving reproductive structures present a significant diagnostic and therapeutic challenge. Veterinary literature

describes various approaches to surgical hernia treatment, but cases where the hernia contains a gravid uterus are rare and require special attention (1). In advanced pregnancy, the presence of fetuses in the hernial sac may lead to circulatory disorders, fetal death, and septic complications. In the described case, surgical intervention enabled a successful outcome and neonatal survival (2).

Case Report. A 7-year-old, 16 kg mixed-breed bitch was brought to a veterinary clinic with a suspected neoplastic mass in the caudal abdomen. The mass measured approximately 20 cm in diameter, reached the ground, and occupied the entire space between the pelvic limbs. The bitch was found on the street by caretakers who decided to help her. The owners reported that she maintained a normal appetite and exhibited normal urination and defecation.

Clinical examination revealed a normal response to stimuli and no signs of pain. An inguinal hernia was diagnosed, and ultrasonographic examination of the hernial contents confirmed the presence of a uterus containing two viable fetuses. Blood tests, radiography, and fetal biometry measurements were performed. Laboratory results showed no abnormalities, while radiographic imaging confirmed the presence of two fetuses based on the detection of fetal skeletons. Biometric measurements indicated a biparietal diameter (BPD) of 23.5 mm on average. A cesarean section was planned for two days later.

The surgery involved opening the hernial sac at the site of the visible enlargement in the inguinal region. Two live neonates were extracted from the uterus, with an APGAR score of 10 after brief resuscitation (3). In addition to the uterus, intestinal loops and the spleen were also present in the hernial sac. An ovariohysterectomy was performed, and the abdominal organs were repositioned to their anatomical locations before closing the hernia.

Postoperatively, the neonates displayed an active suckling reflex and were placed with their mothers. Unfortunately, 14 days after surgery, one of the puppies died due to suspected cardiorespiratory failure, while the other survived and was successfully reared. The bitch's healing process proceeded without complications.

This case illustrates a successful surgical management of a gravid inguinal hernia diagnosed at term, resulting in the survival of one neonate. Although similar cases are often associated with poor outcomes due to fetal ischemia and uterine entrapment, timely intervention in this case prevented such complications. However, the clinical decisions were facilitated by the advanced stage of pregnancy, which allowed for immediate cesarean section and ovariohysterectomy. Therefore, while the case highlights important practical aspects of late-gestation hernia management, it provides limited guidance for earlier diagnosis or intervention during pregnancy. Future studies and case reports focusing on earlier stages of gestation would be valuable in improving treatment strategies in such complex scenarios.

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6368/7881 | Development of a scoring system for the ultrasonographic assessment of cystic endometrial hyperplasia in dogs: A pilot study

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Cystic endometrial hyperplasia (CEH) is a uterine disorder characterized by cystic dilation of endometrial glands, commonly affecting older female dogs. It is linked to reduced fertility, poor conception rates, and an increased risk of life-threatening infections such as pyometra. CEH also contributes to impaired sperm attachment and endometrial inflammation. Although ultrasound is the primary diagnostic tool, documentation and interpretation of findings have not been standardized yet. Therefore, the aim of this project was the development and validation of a scoring system for CEH assessed using B-mode ultrasound which may be used in clinical and research settings. For this study, ultrasound video clips from 32 dogs diagnosed with CEH were selected, excluding pyometra cases. Initially, the three authors independently assigned a severity grade (0 = normal, 1 = mild, 2 = moderate, 3 = severe) to each case without any support of a grading system, anamnesis or clinical background. Interobserver agreement was calculated using Fleiss' kappa. In a second step, the images were analyzed for uterine morphology, number and size of visible cysts, uterine diameter, outline, and echotexture. Measurements were taken from areas with and without cysts and compared using a Student's t-test to identify differences. The data from this step were used to define and select parameters for a novel scoring system. To validate the scoring system, the same specialists re-evaluated the images. Interobserver agreement was again calculated using Fleiss' kappa and the intraclass correlation coefficient (ICC). In the initial evaluation without using the scoring system, interobserver agreement was $\kappa = 0.441$, with greater consistency in normal and severe cases. The average cyst diameter was 0.25 cm (range: 0.19–0.4 cm). The t-test showed no significant difference in uterine diameter between cystic and non-cystic areas , except in severe cases where larger cysts distorted the uterine outline. Cysts as small as 1 mm were measurable, while smaller ones contributed to a more heterogeneous echotexture. Cysts exceeding 4 mm consistently altered the uterine contour. Cyst distribution varied, though complete examination of both uterine horns and the body was not always possible due to the retrospective study design. Based on these findings, the scoring system considered the following parameters such as: presence of cysts < 1 mm, number of cysts between 1 and 4 mm, number of cysts >4 mm, heterogeneous echotexture of the endometrium and uterine outline. Following the development of the scoring system, specialists re-evaluated the images. Interobserver agreement improved significantly ($\kappa = 0.616$), with an ICC of 0.910, demonstrating high reliability.

In this project we could show that a scoring system for CEH can increase inter-observer reliability, addressing the limitations of subjective evaluation. This scoring system will be validated by more experts in the field. It may improve diagnostic consistency among different examiners and standardize documentation in clinical practice and in research projects.

7092 | Testicular FNA in male dogs with infertility

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Fine-needle aspiration (FNA) of the testes is a minimally invasive diagnostic tool used to evaluate spermatogenesis and identify underlying pathological processes (3). This study aims to assess the cytological findings in testicular FNA samples from infertile male dogs and correlate them with potential causes of infertility.

Testicular fine-needle aspiration (TFNA) was performed on two healthy mature pedigree dogs (1 and 4 years old) with azoospermia. After detailed collection of history and a range of basic examination sessions, the option of FNA was chosen to check spermatogenesis disorders.

After the dogs were sedated with medetomidine (10 mcg/kg) and propofol (3-8 mg/kg), a 21- or 23-gauge butterfly needle with 10 ml syringe were used. Suction was stopped as soon as some blood-tinged fluid appeared into the butterfly tube, the needle withdrawn and the aspirate squirted onto a glass slide and smeared out. Air-dried smears are stained in three steps and take about 10–20 s each. The smear is first fixed in methyl alcohol solution, followed by staining with solution I containing L-xanthene (an eosin variant), and then solution II containing methylene blue and azure A. The slide is then rinsed with water and let them dry. Thereafter, smears were examined under light microscopy (Leica DM500) at 1000x magnifications under oil immersion.

A quantitative and qualitative analysis was made on each smear. Nuclear and cytoplasmatic characteristics were considered in order to identify the following cell populations: spermatogonia, primary spermatocytes, secondary spermatocytes, early spermatids, late spermatids, spermatozoa and Sertoli cells (1).

The investigation has shown that the correlation of indexes was characteristic of Sertoli cell-only syndrome - SEI (the ratio between the number of Sertoli cells per 100 spermatogenic cells was used to determine the Sertoli cell index) of dogs were 278 and 410 each, partial obstruction - SI (spermatic index was the relative percentage of each cell population and the percentage of spermatozoa with respect to the total number of germ cells) was 76.2 in 1-st dog and hypospermatogenesis - SI was 16.9 in 2-nd dog.

Finally, the relationship between SI and SEI indexes (SSEI) was used to explore the ratio between mature spermatozoa and nursing cells, representing the ability of the testis to produce sperm cells (2). 1-st dog - SSEI were 346 in left testis (LT) and 4,9 in right testis (RT). 2-nd dog - SSEI were - 23.8 in LT and 1.18 in RT. No adverse effects, such as pain, swelling, infection or hematoma were observed during or after TFNA in any dog.

Testicular FNA is a valuable tool for diagnosing infertility in male dogs. It provides rapid, minimally invasive insights into testicular health, allowing for targeted treatment or breeding management decisions. When combined with clinical history, hormone assays, and semen analysis, FNA enhances the accuracy of infertility diagnosis and helps determine the appropriate therapeutic approach.

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6847 | Association between circulating adipokines concentrations in canine pregnancy and neonatal birth weight and litter size

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The maternal metabolic environment has a significant impact on the growing fetus and is hence a primary determinant of neonatal birth weight. In women, maternal obesity and insulin resistance are associated with higher neonatal birth weight. The adipose tissue is considered an active endocrine organ that produces cytokines, termed adipokines, which affect metabolic homeostasis, satiety, and reproduction.

The goal of this study was to determine associations between circulating adipokine concentrations and litter weight, litter size, and neonatal survival in pregnant dogs. Predicting these litter characteristics before whelping can help assure safe delivery and a healthy litter, especially in breeding programs aiming to optimize maternal and neonatal outcomes.

We hypothesized that circulating maternal concentrations of insulin, leptin, adiponectin, visfatin, and betatrophin during pregnancy are predictive of litter size, neonatal birth weight, and short-term survival. These adipokines are involved in glucose regulation, lipid metabolism, and inflammatory responses, all of which may influence fetal development and viability.

This study measured adipokines throughout pregnancy in 28 pregnant bitches of five breeds (Maltese, Shih Tzu, Border Collie, Jack Russell Terrier, and Australian Shepherd) using serum collected at five time points: estrus (prior to pregnancy), 25–30 days post-ovulation, 40–50 days post-ovulation, 1–4 days pre-parturition, and 5–7 weeks post-partum. Bitch and sire age and body weight were recorded, body condition score and percent body fat were calculated, and fasting serum samples were analyzed for insulin, leptin, adiponectin, visfatin, and betatrophin using validated ELISA kits. Pregnancy was confirmed and monitored by serial ultrasonography. Litter outcomes were documented immediately following parturition and during postpartum follow-up.

Normalized litter weight and average puppy weight were calculated based on dam and sire body weights. Litter characteristics, including size, average puppy weight, total litter weight, and puppy survival to 5–7 weeks, were recorded and analyzed using general linear models, controlling for confounders such as breed size, age, and body condition. In addition to the hormonal measurements, HOMA-%B and HOMA-IR were calculated to assess beta-cell function and insulin resistance, respectively, providing further insight into maternal metabolic status.

Key findings included a negative association between HOMA-%B values at 25–30 days post-ovulation and survival rate (1% increase in HOMA-%B = 0.23% decrease in survival, $p = 0.015$), and a positive association between HOMA-%B at 40–50 days and normalized litter weight (1% increase = 0.46 g/kg increase, $p = 0.037$). Adiponectin concentrations at multiple time points were negatively associated with normalized average puppy weight and total litter weight. A consistent negative association was also found between betatrophin concentrations and normalized litter weight at all time points, indicating its potential role as a limiting factor in fetal growth.

Leptin concentrations at the time of whelping were negatively associated with litter size (1% increase = 0.01 decrease in puppies, $p = 0.022$). Post-partum concentrations of leptin and adiponectin were positively associated with survival rate (1% increase = 0.12% and 0.42% increase in survival, respectively), suggesting that these adipokines may play a role in maternal recovery and lactation efficiency.

These findings suggest that adipokines—particularly adiponectin and betatrophin—along with insulin sensitivity markers such as HOMA-%B, play significant roles in determining fetal growth and survival.

6518/7693 | Determination of the efficacy of various markers in cat spermatogonial stem cells

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This study investigated the expression patterns of Fibroblast Growth Factor Receptor 3 (FGFR3), Integrin β1 (ITGB1), and Chemokine Receptor Type 4 (CXCR4) in domestic cat spermatogonial stem cells (SSCs). The classification between prepubertal and pubertal cats was based on the identification of spermatogenic cells in testicular tissue through histological analysis. Additionally, body weight, penile spine development, testicular weight, and spermatozoa presence were evaluated in pubertal and prepubertal groups.

The study was conducted on five prepubertal and five pubertal cats. A total of 32 testicular sections were obtained using a cryostat and examined through an immunofluorescence staining protocol following fixation. Three seminiferous tubules were analyzed per section. Microscopic analyses were performed using a Leica DM2500 fluorescence microscope, and images were analyzed with ImageJ software. Statistical analysis was conducted using SPSS 30 software.

In immunofluorescence analyses, the mean intensity of ITGB1 protein was $1.75 \pm 0.30 \times 10^5$ in the prepubertal group and $1.70 \pm 0.21 \times 10^5$ in the pubertal group, with no statistically significant difference ($p = 1$). For CXCR4 protein, the mean intensity in the prepubertal group was $2.15 \pm 0.19 \times 10^5$, whereas in the pubertal group, it was $1.32 \pm 0.21 \times 10^5$, a statistically significant difference ($p = 0.018$). FGFR3 protein expression showed no significant difference between groups, with mean intensities of $1.19 \pm 0.11 \times 10^5$ and $1.21 \pm 0.11 \times 10^5$, respectively ($p = 1$).

The findings indicate that ITGB1 protein is strongly expressed in all seminiferous tubules during both prepubertal and pubertal periods and plays an active role in basal membrane cell interactions. CXCR4 protein was highly expressed in the seminiferous tubules during the prepubertal period but localized to fewer cells in the basal compartment in the pubertal stage. FGFR3 protein was expressed in gonocytes during the prepubertal period and exhibited a specific staining pattern on the basal membrane and spermatozoon tail regions in pubertal cats. This study provides valuable insights into the biological identity and dynamic roles of SSCs in domestic cats, contributing to the understanding of feline spermatogenesis and potential conservation efforts for endangered species.

5987 | An uncommon sex-cord stromal tumor in a spayed bitch – A case report

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An 8-year-old Keeshond bitch which had undergone an ovariohysterectomy at three years of age, was presented because male dogs had been trying to mate her for the past 6 months. The owner was not being treated with any topical estrogen. On clinical examination the vulva appeared enlarged and slightly edematous. Vaginal cytology showed 90% anuclear and 10% nuclear superficial cells and a few neutrophilic granulocytes. Vaginoscopy revealed edema and hyperemia in the vaginal mucosa, progesterone was 0.23 ng/mL. Ultrasonographic imaging of the ovarian areas revealed a small nodular area on the right, measuring 1x1cm. Anti-Müllerian hormone (AMH) was < 0.3pmol/l, luteinizing hormone (LH) < 1ng/ml, thyroxin (T4) was within the normal reference. The patient was seen again after 21 days showing the same clinical picture, vaginal cytology and vaginoscopy. Progesterone was < 0.2 ng/ml, LH < 1 ng/mL and AMH < 0.3 pmol/L. Ultrasound revealed both adrenal glands to be of normal shape and size (width 0.7 cm/length 1.3 cm). An adrenocorticotropic hormone stimulation test did not show elevated estradiol, 17-hydroxyprogesterone or cortisol concentrations. During exploratory laparotomy under general anesthesia, a nodular tissue area of 0.5 × 0.6 × 0.7 cm caudal to the right kidney was identified and removed. The nodular tissue was diagnosed by histopathological examination as a sex-cord stromal tumor compatible with an interstitial cell tumor (luteoma). Ovarian tumors are uncommon in all domestic species. All sex-cord stromal tumors including granulosa cell tumors, thecoma and interstitial tumors (luteoma, lipid cell tumor, steroid cell tumor) have the potential to be hormonally active, producing signs of masculinization, persistent anestrus, or hyperestrogenism as in the present case. This is a rare case of an ovarian tumor probably arising from the remnant of ovarian tissue after 5 years of ovariohysterectomy. The bitch was exhibiting classical signs of estrus not due to remnant granulosa cells from the ovarian follicles, but rather a remnant of interstitial tissue that had developed into an estradiol-producing luteoma making diagnosis even more challenging.

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7018 | Relationship between hygiene practices in breeding catteries and kennels on gastrointestinal parasite infestation

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Gastrointestinal parasitism in canine and feline facilities is common and may be responsible for clinical signs such as

digestive disorders, retarded growth or mortality. However, the precise effect of hygiene practices on preventing parasite infestation remains poorly studied in cat and dog breeding facilities. This study aims to assess the relationship between breeding facility sanitary management and parasite infestation.

A total of 37 French breeding facilities were included in this study between 2019 and 2023, comprising 19 catteries and 18 kennels. For each facility, a questionnaire was completed regarding facility management, animal housing conditions and housed animals. Additionally, pooled fecal samples (1–7 samples) were collected by breeders separately from each run. These samples were then analyzed using flotation coproscopy with saturated sodium chloride. Data analysis was conducted using JAMOVI 2.6 software. The association between the presence of parasites and seven variables at the run level (age of animals, floor and wall covering, animal density, species, disinfection, use of detergent) was tested using χ^2 tests, with a p-value <0.05 considered statistically significant. "Run sanitary score" was then determined based on these variables. Similar analyses were then used to test the association between six variables at the facility level (species, presence of diarrhea, quarantine area, infirmary, anthelmintic administration, mean sanitary score of the run) and the presence of parasites (eggs, oocysts excreted in feces).

Among the 37 breeding facilities studied, data on 149 runs were collected, with 77 from cats and 73 from dogs (mean of 4 runs per facility). Eggs of ascarids, strongyles and trichurids, as well as oocysts of coccidia, were identified in 38% (14/37) of facilities and 17.2% (25/149) of runs. The prevalence of gastrointestinal parasites was significantly higher in canine runs (23%, 17/73) than in felines (10%, 8/77, $p=0.031$). Multi-infestations were observed in 8% (12/149) of runs, only in kennels (6/18), with 2 to 3 different parasites identified. The number of positive runs was significantly lower when a detergent was routinely used (12%) compared with those with no detergent usage (27%, $p=0.034$). Similarly, the number of positive runs was lower in systematically disinfected runs (11%) compared with the non-disinfected ones (44%; $p<0.001$). Age of animals, floor and wall coverings, animal density, and the sanitary score of the runs were not associated with the presence of parasites. At the facility level, the prevalence of parasites was significantly higher when the average sanitary score of the runs was low compared to when it was considered good (respectively 22%, 8/37 and 5%, 2/37; $p=0.040$). A history of diarrhea, quarantine area, infirmary, or the administration of anthelmintic was not found to be associated with parasite infestation.

This study highlights the importance of adapted sanitary practices on parasite infestation in canine and feline breeding facilities. The systematic use of detergents and disinfectants significantly reduces parasite prevalence. Veterinary visits and breeder training remain essential for improving sanitary and medical prophylaxis in facilities, thereby minimizing health risks and enhancing animal welfare.

5375/7763 | Ultrasonographic appearance of the vagina in 17 healthy dogs according to the estrous cycle: Prepubic and perineal approaches

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The ultrasonographic appearance of the vagina in dogs has not been fully described (1).Vaginoscopy, vagino-urethrography, and CT can detect vaginal lesions, but they require sedation or exposure to radiation. Transcutaneous ultrasound is a safe, non-invasive technique that can be performed on awake patients.The ultrasonographic appearance of the vagina in pathological conditions has been evaluated, but only limited portions of the organ were described (2).The aim of this study is to describe the normal B-mode ultrasonographic appearance of the vagina in female dogs during the stages of the estrous cycle: proestrus (P), estrus (E), diestrus (D), and anestrus (A), using both prepubic (Pr) and perineal (Pe) approaches.

Seventeen female dogs with no history of previous matings, pregnancies, illnesses or surgeries, were included in the study. All the bitches included in the study had unremarkable physical examination. Clinical and ultrasonographic evaluations were repeated for each phase, in all bitches, based on vaginal cytology and progesterone results. All exams were performed without sedation using an Esaote MyLab X8 ultrasound machine (Esaote, Genoa, Italy) with a multifrequency linear probe (4–15 MHz). For each dog, two scans were performed: one transcutaneous Pr (on a dorsal, sagittal, and transverse plane along the midline, cranially to the os pubis) and one transcutaneous Pe (on a sagittal and dorsal plane, ventral to the anal sphincter).The B-mode appearance of the vaginal wall and its contents was described and the vaginal wall thickness in transverse section was measured. Thickness variation across the estrous cycle phases was compared using a Student t-test and a one-way ANOVA for pairwise comparisons ($p<0.05$ was considered as statistically significant). The perineal scan was also used to visualize the urethral orifice and the distal segment of the urethra. The authors hypothesized that the B-mode appearance of the vaginal wall changes between the follicular and luteal phase.

The study included 17 dogs of different breeds (1.8 ± 0.3 years and 15.8 ± 16.7 kg).In all dogs, the vagina (Pr and Pe scans) showed collapsed walls and absence of content in all estrous phases. The vaginal wall was thicker and hypoechoic during P and E, whilst the echogenicity increased and the diameter decreased during D and A. These variations were attributed to the presence of tissue oedema during P and E. The mean vaginal wall thickness in the Pr scan varied significantly across estrous phases ($p<0.05$), except for the PE comparison ($p=0.9$). For the Pe scan, thickness varied significantly across phases ($p<0.05$), except for the PE ($p=0.7$) and DA ($p=0.06$) comparisons. The external urethral orifice was visible in all dogs during all phases.

The B-mode ultrasonographic appearance of the vagina in female dogs during the estrous cycle can be described using Pr and Pe approaches. Significant increases in thickness were observed during AP and PE, and significant decreases during ED and DA.

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7055 | Comparative values of serum progesterone in elective cesarean section in bitches: A retrospective study

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In some breeds, accurate determination of the bitch's parturition date is essential to avoid complications such as dystocia and to determine on the need for an elective caesarean section (ECS). The breeds most affected include brachycephalic breeds, but also those with very large or small litter. In fact, ECS can be performed before or during the first stage (FI) of the parturition (ECSFI). Several methods are used to determine FI, such as LH/ovulation control, decrease in rectal temperature, cervical dilation, fetal heart rate (HR) and/or the measurement of the prepartum decline in progesterone levels (P4). However, with regard to the latter parameter, some studies suggest that it may be higher in certain breeds (1). However, an exact cut-off value that is applicable to all bitches for the performance of an ECSFI has not yet been established due to the high individual variability (2). Therefore, the aim of this retrospective study was to evaluate the P4 values obtained in bitches on the day before and on the day of the ECSFI and to compare whether the progesterone values obtained varied between bitches of different breeds.
The data were obtained from the ECSFI performed in our hospital during the last year. In all the cases, the bitches underwent routine ovulation monitoring (by serial vaginal cytology and serum P4 values) and ECSFI under the same conditions. In all cases, serum P4 levels were determined by QLIA (IMMULITE 1000®). The ECSFI was scheduled 65 ± 1 days after the LH peak in all the bitches. Cesarean section was performed if HR was <200 bpm and/or P4 ≤2 ng/mL. In all cases, ECSFI was performed without any complications and viable puppies with a good APGAR score were obtained. The P4 values on the day of the surgery (time 0) or one day before (time −1) were recorded and compared (mean ± SD). All statistical analyses were performed with GraphPad PRISMA, using normality tests and mean comparisons (Mann-Whitney and one-way ANOVA). A total of 27 healthy bitches from 9 different breeds were included. The mean age of the bitches was 3 years, with a mean litter size of 4.74 ± 2.9 pups. The mean HR at time 0 was 183.88 ± 20.67 bpm. Of the 27 ECSFI, 7 were performed on day 64 after the LH peak, 15 on day 65 and the remaining 5 on day 66. Globally, serum P4 levels were significantly higher ($p < 0.05$) at time-1 (3.44 ± 2.1 ng/mL) compared to time 0 (1.46 ± 0.85 ng/mL). However, no significant differences were observed between the three breeds compared at the two times (Great Dane *n*:11; American Bully *n*:6 and French Bulldog *n*:4).
In conclusion, our study suggests that under our conditions we observed significant differences in P4 values obtained on the day before or on the day of ECSFI surgery, with no apparent breed effect. However, future studies are needed to confirm these data.
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6519/7814 | Survival and integrity of domestic cat testicular tissue after short-term in vitro culture

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Testicular tissue culture is a promising strategy to safeguard male fertility, especially in prepubertal individuals at risk of losing reproductive potential. While recent advances in organotypic culture systems have demonstrated the feasibility of in vitro spermatogenesis (IVS) in rodent models (1,2), the application of this technology to other species, particularly felids, remains underexplored (3). With the ultimate goal of developing a system to promote feline male germ cell proliferation and differentiation, this study aimed to evaluate the effects of a medium developed for rodent IVS (2) and of its supplementation with retinol and taurine, known for their roles in germ cell differentiation and their antioxidant activity, on the survival and integrity of domestic cat cultured testicular tissue.
Testicular tissue fragments (1 mm^3) from immature and early pubertal cats (testes weight <1 g; $n = 7$) were placed on agarose gel blocks, nearly submerged in rodent-IVS base medium (BM) [2] or the same medium supplemented with 1 µM retinol (RET) or 60 µM taurine (TAU), and cultured at 38.5°C with 5% CO_2 for 48 h. Samples were then fixed and processed for histomorphometric analysis (area of tubular, necrotic and fibrotic interstitial tissue) and immunohistochemical detection of apoptosis (activated caspase-3), proliferation (MCM-7) and spermatogonial (PGP9.5) markers. Fresh fragments were collected and processed as non-cultured controls (FC). Data, expressed as mean ± SD, were analyzed by Kruskal-Wallis test, followed by Dwass-Steel-Critchlow-Fligner post-hoc test ($p < 0.05$).
The tubular area did not change during culture in BM (0.737±0.139 mm^2 vs. 0.854 ± 0.083 mm^2 in FC; $p = 0.134$), while it significantly decreased in samples cultured in RET or TAU (0.541 ± 0.156 and 0.651 ± 0.130 mm^2; $p = 0.003$). Although an increase in necrotic area was observed (from 0 mm^2 in FC to an average of 0.110 ± 0.110 mm^2 in cultured groups; $p = 0.002$), no increase in fibrotic area was observed in BM and TAU compared to FC (0.174 ± 0.125 and 0.228 ± 0.118 mm^2 vs. 0.146 ± 0.083 mm^2; $p = 0.277$). Immunohistochemical analysis revealed a significant increase in caspase-3 positive cells in all cultured groups compared to FC (71 ± 39 (BM), 65 ± 44 (TAU) and 127 ± 101 (RET) vs. 8 ± 8 cells/fragment (FC); $p < 0.001$), but the number of spermatogonial and proliferating cells remained stable across all conditions (ranging from 1328 to 1810 cells/fragment in cultured groups vs. 2006 cells/fragment in FC for PGP9.5, $p = 0.286$; from 1204 to 1445 cells/fragment in cultured groups vs. 1291 cells/fragment in FC for MCM-7, $p = 0.815$).
Short-term culture in BM preserved cat testicular tissue structure and tubular area, while all conditions maintained

spermatogonial and proliferating cells. Taking all parameters into account, BM alone emerged as the most suitable medium for short-term culture of cat testicular tissue. Although RET and TAU are promising molecules for supporting germ cell development, their benefits were not evident within 48 h, warranting further investigation under long-term conditions to fully assess their potential in promoting feline IVS.

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Reproduction in Domestic Animals

ABSTRACT

Posters

6817 | Can the use of frozen canine semen that is stored outside of my semen bank influence the fertility data obtained at my centre? A retrospective study

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Retrospective studies indicate that the fertility achieved after artificial insemination (AI) with canine frozen semen varies between 50 and 75%. Many factors have been suggested to influence this fertility, such as the method of semen cryopreservation, number of AI, bitch age, breed, ovulation control or type of AI used, among others. In this regard, recent studies have confirmed the satisfactory pregnancy rates achieved by endoscopic-assisted transcervical insemination (TCI) for frozen canine semen (1). However, the influence of many other factors that may affect fertility outcomes in the clinical practice has not yet been fully evaluated retrospectively (2). For this reason, the aim of this retrospective study was to compare the whelping rate and litter size obtained after TCI of canine semen collected, frozen and stored in our semen bank (indoor group) with semen collected, frozen, stored and imported to our centre from an external canine semen bank (outdoor group).

Data were obtained from the AI performed in our Hospital from 2019 to 2023. The inclusion criteria were as follows. All ovulation timing controls were performed in the same way (serial vaginal cytology and serum progesterone levels by IMMULITE 1000). In both groups, the TCI of one dose of frozen semen (straws, not pellets) (150–250 million motile sperm post-thawing) was performed 3 or 4 days post-ovulation. Pregnancy was diagnosed by ultrasound 25–27 days after LH peak. In the indoor group, the Uppsala cryopreservation method was used. In the outdoor group, the methods of cryopreservation were multiple, the most frequent being CLONE, Uppsala, Biladyl, Caniplus, MOFA, among others. Whelping rates (total number of bitches producing at least one live pup divided by the total number of bitches inseminated with the same group of semen $\times 100$) and litter sizes (mean $\pm$ SD) were compared between indoor and outdoor groups. A chi-square statistic with Yates correction was performed (significant differences at $p < 0.05$).

A total of 100 TCI (37 different breeds) were included. The overall whelping rate was 75% with 5.47 ± 2.70 pups per litter. The whelping rate differed between the groups ($p < 0.05$), being higher in the indoor group (82.35%; $n = 51$) compared to the outdoor group (67.34%; $n = 49$). However, no significant differences in litter size were observed between the two groups (5.11 ± 2.44 pups per litter and 5.92 ± 2.98 pups per litter, indoor and outdoor groups, respectively).

In conclusion, our data suggest that, from a practical point of view, the use of imported canine frozen semen significantly reduces the whelping rate in the bitch after TCI. Therefore, future studies are needed to evaluate the putative influence of the number of shipments, the type of dry shipper containers, the age of the animal at the time of the cryopreservation and/or the influence of the time of storage in the bank semen before its use, among other factors.

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5422/7759 | Diagnostic value of endoscopically guided transcervical catheterisation for cytological and bacteriological sampling in canine endometritis

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In the bitch, the diagnosis of subclinical uterine diseases is usually challenging. Endometritis is frequently present in asymptomatic animals and occasionally overlooked by routine ultrasound scan. Previous studies have suggested that cytologic and microbiologic sample collection through transcervical catheterisation (TCC) is valuable for the diagnosis of uterine disorders (1, 2). No study has simultaneously evaluated microbiological samples obtained by TCC uterine lavage, direct uterine sampling post-ovariohysterectomy and deep vaginal sampling in bitches with and without endometritis. The aims of this study

were: 1) To evaluate the diagnostic value of endometrial cytological samples obtained by endoscopically guided TCC. 2) To determine the sensitivity and specificity of TCC for the identification of uterine bacterial growth. 3) To compare vaginal and uterine bacteriological findings.
Ten post-pubertal bitches were submitted for ovariohysterectomy. Before the surgical procedure, vaginal bacteriological and cytological sampling and TCC were performed. Uterine microbiological and cytological samples were also obtained by TCC. After ovariohysterectomy, direct uterine bacteriological sampling and full-thickness biopsy were carried out. The agreement between endometrial cytology and full-thickness biopsy and between bacteriological results were evaluated using Cohen's kappa (κ) coefficient. Sensitive and specificity were also calculated for bacteriology cultures and cytology, using direct uterine sampling and endometrial full-thickness biopsy as the gold standard. The level of significance was set at $p < 0.05$.
The agreement in the diagnosis of endometritis between the TCC cytology and histopathology was substantial ($k = 0.62$; $p < 0.05$). Sensitivity of TCC cytology was acceptable (66%) and specificity was excellent (100%, $p < 0.01$). The agreement between TCC and direct uterine bacteriological sampling was moderate ($k = 0.60$; $p < 0.01$). Sensitivity of TCC bacteriological samples was excellent (100%) and specificity was acceptable (75%, $p < 0.05$). Conversely, the agreement between vaginal and uterine bacterial analysis obtained via TCC ($k = 0.05$; $p > 0.1$) or by direct uterine sampling ($k = 0.1$; $p > 0.1$) was poor.
This study highlights the diagnostic value of endoscopically guided TCC for cytological and bacteriological sampling in canine endometritis. The substantial agreement between TCC cytology and full-thickness biopsy, along with the moderate agreement in the bacteriological analysis, is evidence that the use of both techniques together is reliable for the diagnosis of endometritis. The poor agreement between vaginal and uterine bacteriological results further confirms the presence of different microbiota between these cavities and, therefore, the limitation of the use of vaginal samples for the diagnosis of uterine infections.

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7124/7817 | Evaluation of the AndroDog simulator by students—preliminary results

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The teaching of practical skills in the veterinary curriculum is increasingly restricted by animal welfare regulations. At the same time, better teaching simulators are becoming available, which are designed to mimic real patients and allow students to practice their skills without the need to use live animals (1). The recently developed AndroDog Andy (vetiqo.com/produkte/andy/) aims to facilitate training in andrological examination and semen collection. It features interchangeable prostate and scrotum models, simulating a variety of clinical conditions, including healthy and pathological variations.
The examination using the AndroDog was taught to and practised by 3rd- and 5th-year students under direct supervision. Demonstrations were followed by hands-on practice, with students permitted unlimited time to refine their examination techniques.
After training, participants provided feedback on the use of the simulator as a learning tool via a structured evaluation form with five-point Likert scales. Currently, responses have been collected from 5 fifth-year and 14 third-year students ($n = 19$). Throughout the upcoming weeks, we will conduct additional courses, proceed with the evaluation, and consequently gather further data.
Most students agreed that the simulator improved their knowledge (12 strongly agreed, 7 agreed) and practical skills (13 strongly agreed, 6 agreed). The training also enhanced their motivation (14 strongly agreed, 5 agreed) and self-confidence (8 strongly agreed, 7 agreed, 4 neutral). Students perceived that engagement with the simulator did not hinder the learning process (15 strongly agreed, 3 agreed and 1 no response). Notably, students did not feel inadequate while using it (14 strongly agreed, 4 agreed and 1 neutral), nor did they find the use of the simulator stressful (15 strongly agreed, 3 agreed and 1 neutral). However, most students indicated that they did not feel confident in the andrological examination before training (14 disagreed with the statement 'I already know the exam well').
The majority found the testicular examination (2 students strongly agreed, 12 agreed, 5 were neutral), prostate assessment (4 students strongly agreed, 11 agreed, 4 were neutral) and semen collection simulation (2 students strongly agreed, 10 agreed, 3 were neutral, 2 disagreed and 2 chose 'I cannot judge') to be realistic. Some students noted that their limited prior experience with real canine andrological exams made assessment difficult.
The simulator was provided free of charge by Vetiqo. Preliminary findings suggest that the Androdog simulator is an effective tool for improving student knowledge, practical skills, and confidence in andrological examinations. However, it remains unclear how well simulator-based training translates to real-world competency, as most students lack experience with live patient exams. Additionally, the strong positive response could partially reflect the novelty effect, a typical phenomenon seen when new simulators are introduced. Further educational research is needed to better understand the long-term skill retention and comparative effectiveness against traditional learning methods, as well as the extent to which the simulator actually prepares students to examine real dogs and interpret the findings.

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6404 | Intrauterine bacteria in bitches undergoing emergency caesarean section

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Previously, it was believed that a healthy uterus was sterile except during proestrus and estrus. However, recent studies have

challenged this assumption. Bacteria have been detected in the uterus, amniotic fluid, placenta, and even meconium of healthy puppies1-3;4;5. The colonisation of a healthy bacterial flora in the uterus during fetal development is likely to play a crucial role in neonatal health. Infectious diseases, often bacterial, are one of the most common causes of puppy mortality, second only to hypoxia caused by difficult births. The aim of the present study was to evaluate bacterial microbiota in the uterus of bitches without any sign of infection. Materials and methods. Samples were collected from the uteri of 13 privately owned bitches undergoing caesarean section (CS). Included breeds were; Chihuahua, Staffordshire Bullterrier, Borderterrier, Mixed Breed, Golden Retriever, Miniature Schnauzer, Vostotjnoevropejskaja Ovtjarka, Dachshund, Bearded Collie, American Bully, Bracco Italiano, Flatcoated Retriever, and Russkaja Tsvetnaya Bolonka. All bitches were treated with emergency CS because of dystocia. Permission to collect samples was obtained from each owner. Samples were collected with swabs from the uterus between implantation sites. The swab samples were cultured for bacterial analysis by direct inoculation onto two blood agar plates which were incubated at aerobic and anaerobic conditions, as well as on lactose purple agar, MacConkey agar, and Colistin-Oxolinic Acid-Blood Agar. All plates were incubated at 37°C and examined after 24 and 48 h. Additionally, the swabs were analysed for fungi by culturing on Sabouraud agar, which was incubated at 30°C for 5 days. All isolated bacteria were identified to the species level using MALDI-TOF. Results. In one litter, two live puppies were born before admission. The other 12 bitches had not given birth to a puppy before CS. In total, 70 puppies were born, of which 5 were stillborn (7.1%). None of the bitches had more than one stillborn puppy. Bacterial growth was found in samples from five bitches (38.5%). *Clostridium ulcerans*, *Staphylococcus pseudintermedius, StaphylococcusT capitis, Mycoplasmopsis canis* and *Streptococcus canis* were grown in mixed culture, whereas *Staphylococcus aureus* was found in almost pure culture. Three of five bitches with bacterial growth had stillbirth in the litter, while 2/8 without bacterial growth had stillborn puppies.

Bacteria were isolated from the uterus in 38.5% of the bitches included in our study. As all the bitches underwent emergency CS because of signs of dystocia, it cannot be excluded that bacteria may have entered through an open cervix. Patency of the cervix was not assessed. Stillbirth was not more common in litters in which intrauterine bacteria were isolated than in litters in which the culture was negative.

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7153 | Severe metritis caused by retained placentas in a Queen—Case report

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Retained placenta is a postpartum complication characterised by the failure to expel placental tissue due to incomplete separation of the chorionic villi from the endometrial crypts. This condition is uncommon in feline species and may lead to uterine infections due to tissue putrefaction. In bovines, in which incidence can reach up to 40%, the retained placenta is often associated with complications during birth, inadequate assistance, or immune incompetence affecting the detachment from the endometrium (1). This report describes a case of metritis secondary to retained placenta in a domestic cat.

An 18-month-old primiparous female cat presented with a three-day history of delivery. The owner reported that the cat had delivered four kittens, one of which died shortly after birth. Over the 3 days following delivery, the cat exhibited intermittent reddish-brown putrid vaginal discharge. Clinical examination revealed lethargy, mild dehydration, and pale mucous membranes. Transabdominal ultrasonography identified a tubular structure measuring 2.7 cm in diameter within the uterus, containing hypoechoic material alongside two amorphous intraluminal structures with well-defined echogenic borders. These findings suggested an inflammatory/infectious process (metritis) and the presence of retained placentas. Laboratory tests indicated anaemia (HCT 22%), leukopenia ($3.7 \times 10^3/\mu L$) due to neutropenia ($1.9 \times 10^3/\mu L$) and lymphopenia ($1.3 \times 10^3/\mu L$). Surgical treatment via ovariohysterectomy was performed. During the procedure, the uterus was incised, revealing two necrotic structures adhered to the endometrial wall, which were confirmed histopathologically to be necrotic placental tissue. Microbiological culture and antibiogram of the uterine contents detected the presence of beta-hemolytic *Streptococcus* and *Pasteurella multocida*, both of which were sensitive to the prescribed treatment of amoxicillin with potassium clavulanate. Following the procedure, the animal was discharged and showed significant improvement, with a successful follow-up 10 days later when sutures were removed.

Accurate diagnosis and effective therapy were crucial for the patient's recovery, highlighting the risk of systemic complications from delayed diagnosis. The underlying factors predisposing cats to retained placenta remain unclear. It has been reported as a case secondary to experimental gestational interruption with aglepristone, treated with prostaglandins (2). Abnormal trophoblastic infiltration also led to the retained placenta and uterine perforation in a bitch (3), however, such a finding was not present in this case's histological analysis. This queen's unaccompanied parturition might have presented predispositions to its occurrence. An immune system failure in the uteroplacental

junction breakage as described for bovine (4) could be the case, considering the present leucopenia, however, testing for feline retroviruses was declined by the owners. The topic presents an interesting area for future studies to further elucidate this issue.

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7149 | Preputial neoplasms in dogs: A retrospective study

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Preputial neoplasms in dogs are considered rare (1). However, incidence in tropical countries, such as Brazil, may suggest otherwise. This study aimed to characterise the clinical and oncological aspects of preputial tumours in dogs and assess their prevalence in the Brazilian context, where their occurrence is regarded as rare in the international literature.

A retrospective study was conducted at the veterinary hospital of UNESP Botucatu (São Paulo, Brazil) where relevant keywords were searched in the system to evaluate all cases of canine preputial tumours from January 2018 to April 2024. Clinical data, including breed, age, weight, size, tumour characteristics, treatments and outcomes, were collected and analysed. The statistical analysis compared the variables of coat colour classification, sun exposure, tumour's largest lateral diameter, surgical procedure, histological classification and outcome to assess associations.

The 52 identified cases represented 5.4% of male consultations (including those for elective orchiectomy) during the same period. The mean age was 10.0 ± 4.7 years. Mixed-breed dogs accounted for 53.8%, with an average weight of 17.3 ± 9.2 kg. Among the defined breeds, Pitbulls predominated (36.5%). Additionally, 13.5% of the patients had white or partially white coats. Sunbathing was reported in 46.1% of the patients, and it was present in all but one (13) white or partially white dogs. Histopathological analysis revealed 25% carcinomas, 36.5% hemangiomas/hemangiosarcomas, and 9.6% carcinoma associated with hemangioma/hemangiosarcoma, and 11.5% as mast cell tumours (MCTs). Of the 46 surgical procedures performed, 19 (36.5%) were nodulectomies, one of which involved a reconstructive technique. Mass resection with penectomy and urethrostomy were performed in 26 (56.5%) patients, four of whom required reconstructive techniques. The rate of major complications was primarily associated with penectomies. Among follow-up responders (20), eight dogs were still alive. Euthanised animals had an average survival time of 12.6 ± 13.1 months, while those that died naturally lived 6.9 ± 11.6 months from diagnosis. The overall survival time for deceased animals was 8.9 ± 11.85 months. Statistically, a predominantly or totally white coat, larger tumour diameter, and histopathological classification (carcinoma and hemangioma/hemangiosarcoma) were associated with penectomy ($p < 0.05$). Although MCTs are usually the most common skin neoplasms (2), the prepuce showed a distinct pattern, likely due to sparse fur. Squamous cell carcinomas and hemangiosarcomas, which are associated with lighter skin and sun exposure (3), are the predominant factors in this study. The higher incidence observed in tropical countries may be related to increased UV exposure and outdoor activities (2). Additionally, the prevalence of Pitbulls, a breed commonly found in these regions, may indicate both environmental influences and genetic predisposition.

This study indicates that the prepuce may have a different neoplasia incidence compared to other skin locations. The protective anatomical role of the penis makes tumour resection in the prepuce particularly challenging. While penectomy may seem like the safest procedure to remove all neoplastic tissue, there are numerous potential short- and long-term impacts.

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6999/7913 | Influence of glucocorticoid therapy on the reproductive cycle of bitches—Preliminary results

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From human medicine, it is already known that Glucocorticoids can influence the hypothalamic–pituitary–gonadal axis by inhibiting the pulsatile secretion of GnRH from the hypothalamus (1–3). The aim of this study was to investigate the effects of exogenous glucocorticoids on the reproductive cycle of bitches, as there are no studies focused on this yet. Twelve female dogs of different breeds, aged between 16 and 131 months, were presented to our clinic due to neuropathies ($n = 5$), chronic enteropathies ($n = 3$) or other reasons ($n = 4$). All dogs received a starting dose of cortisone (Prednisolon) between 1 and 2 mg/kg once a day, which was gradually reduced when the symptoms resolved. In these dogs a gynaecological examination was performed, and the owners answered a questionnaire about their reproductive performance.

At the start of cortisone treatment, one 15-month-old dog was prepubertal. One dog was after its first and four dogs were after their second heat. Five already had three or more heats with regular inter estrous intervals. One had irregular interestrous intervals between 6 and 12 months. No oestrogen influence could be seen during the gynaecological examination of any of the bitches. The prepubertal dog still had no signs of its first heat, nine dogs showed a prolonged anestrus, and two dogs had no alterations in their estrous cycle. The prepubertal bitch showed no signs of heat till the age of 35 months. Eight of the nine dogs with prolonged anestrus were treated from 6 to 24 months with dosages between 0.1 and 0.85 mg/kg. These dogs had no signs of heat from 12 to 28 months. The remaining dog with prolonged anestrus received cortisone for a 12-month interval. She came into heat during cortisone treatment, but 51 months after the end of treatment, no further estrus has been observed. The two dogs without cycle alterations received 0.1 to 0.18 mg/kg cortisone for

4 months and 6 years, respectively, and showed regular inter estrous intervals.

These preliminary results suggest that cortisone treatment may suppress the estrous cycle in bitches even at reduced dosages and in prepubertal dogs. This tends to apply especially when treatment extends over several months, although individual exceptions may occur. To emphasise if shorter treatment periods don't result in cycle alterations and if estrous suppression remains after the end of treatment further research with more dogs is needed.

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6466/7931 | Early dynamics of the gut microbiota in golden retriever puppies during the first 12 h of life: A preliminary study

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The early hours of life represent a critical period for the dynamic development of the gut microbiota (GM). At birth, the newborn's intestinal tract contains oxygen, creating an environment that favours the initial colonisation by aerobic bacteria. This early colonisation is essential, as these bacteria consume the available oxygen, facilitating the establishment of facultative and strict anaerobic bacteria later on (1). Despite the fundamental importance of this process, its detailed progression remains poorly understood and understudied in dogs and other mammals.

In this preliminary study, we aimed to assess the evolution of the gut microbiota in Golden Retriever puppies during the first 12 h of life. The study included 14 healthy puppies born vaginally from 3 dams. Rectal swabs were collected at birth (H0) and at 4 (H4), 8 (H8) and 12 h (H12). Bacterial DNA was extracted, amplified (V3-V4 regions of 16S rRNA), and sequenced (Illumina MiSeq). Taxonomic affiliation used the 16S SILVA database (version 138.1 PINTAIL 100). Statistical analysis (ANCOM) evaluated changes in taxa at H4, H8 and H12 relative to H0, with p-values adjusted using the Holm method.

Four phyla were identified in the 56 collected faecal samples: Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria. Altogether, 33 taxa were present at the family level, and 45 at the genus level. Between H0 and H12, a significant increase was observed in the abundances in 6 of those taxa ($p < 0.05$ for all comparisons; Figure 1). Among them, Pasteurellaceae (0% at H0, 16 ± 29% at H12), and specifically Haemophilus (1% at H0, 2 ± 3% at H12) and Frederiksenia (0% at H0, 3.31 ± 8% at H12) were facultative anaerobes. Whereas, Clostridiaceae (0% at H0; 16 ± 29% at H12) and mainly Clostridium sensu stricto 1 (0% at H0, 13 ± 29% at H12), and Peptostreptococcaceae (1 ± 2% at H0, 12 ± 25% at H12) and mainly Terrisporobacter (0% at H0, 3 ± 7% at H12) and Romboutsia, (0% at H0, 8 ± 18% at H12) were strict anaerobes.

The results confirm the rapid shift in GM during the first hours after birth, from an oxygen-rich to oxygen-poor environment, favouring colonisation by strict anaerobes and facultative anaerobes. These findings emphasise the importance of these early dynamics in facilitating the transition to a more mature microbiota. Further studies with larger sample sizes and longer follow-up periods are needed to fully understand the functional implications of these microbial changes in the development of gastrointestinal dysbiosis, being most probably responsible for numerous chronic conditions in adulthood.

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7085 | In utero parvovirus infection of an entire dog litter

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Canine parvovirus (CPV) is still a leading infectious cause of canine mortality and weaning puppies are particularly at risk as maternally derived antibodies (ab) can block active immunisation (1). Early neonatal mortality due to CPV is rare (2), as is in utero infection, that has been reported to cause cerebellar hypoplasia (3). Aim of this case-report is to describe the clinical presentation of CPV prenatal infection in a litter.

Postmortem examination was performed on 5 puppies of an English Staffordshire Bull Terrier bitch, aged 3.5 years, at her 2nd parturition. After a healthy pregnancy, a c-section was performed at the Veterinary Teaching Hospital of Turin (Italy) because of uterine inertia (the previous parturition had been natural). At presentation, blood parameters were normal and foetal heart rate was > 210 bpm. Surgery was done under inhalatory anaesthesia (sevoflurane in O2), without complications. Five term puppies (3 females, 2 males) were extracted, but none could be resuscitated, none started to breathe after clearing of the airways. O2 was administered with a tight-fitting mask. Heart rate was around 200 bpm at birth but progressively decreased until cardiac arrest, despite chest compressions and epinephrine administration (0.2 mg/kg IO) (4). No drug had been administered to the dam that could be reversed in the newborns.

At necropsy, puppies weighed 240–265 g, had abundant hemorrhagic fluid in the abdomen and the Docimasic test was negative in all of them. No macroscopic lesions were observed in the organs. The intestine showed moderate mucosal lymphocyte infiltration, while pancreas, liver, kidneys, and lungs were free of lesions. The spleen had signs of lymphocyte depletion. Microbiological exams led to unremarkable results and search for *Salmonella* spp. and *Neospora caninum* was negative. Virologic investigations using PCR on a pool of intestinal tissue detected CPV2 DNA and sequencing of the VP2 gene identified the antigenic variant CPV-2a.

This report shows how CPV is still a risk in dog breeding and how it can act in unusual presentations. The bitch had been yearly vaccinated with modified live virus vaccines: unfortunately, we have not the CPV ab titre at parturition, but only at about 90 days following it and it was very high (> 1:640, IMB-VacciCheck-Canine, Agrolabo, Italy). The high titre may have followed infection. We can hypothesise that the bitch had not responded properly to the CPV vaccinations and was infected during late pregnancy: her ab titre may have protected her from

developing clinical signs, but the virus could cross the placenta and infect the foetuses. Foetal heart rates suggest the absence of cardiac lesions, but we have no histological proofs of this: macroscopically, the organs were normal, and the unexpected diagnosis did not suggest proceeding with histology.
Further studies on maternal immunity and placental transmission of CPV would be valuable in enhancing preventive strategies to protect against such infections in the future.
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7039 | Neutrophil and erythrocyte dynamics in canine vaginal cytology: A predictive marker for estrus-diestrus transition

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Canine vaginal cytology is widely used for estrous cycle staging, but its predictive value for the estrus-diestrus transition and its correlation with serum progesterone levels require further validation. This study aimed to evaluate whether neutrophils can serve as reliable cytological markers of diestrus and whether their fluctuations correspond with serum progesterone levels.
Materials and Methods: Vaginal cytology samples were collected from 13 bitches ($n=13$) at different estrous cycle stages, including proestrus, estrus, and early diestrus. Serial samples were obtained to confirm intra-day progesterone consistency, with a focus on evaluating the percentage of parabasal, intermediate, superficial, anucleated, neutrophil, and erythrocyte cells. Cytological samples were prepared using Diff-Quik staining and analysed under light microscopy (1). Serum progesterone concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) (2). Statistical analysis was performed using Pearson's correlation and one-way ANOVA, with a significance threshold of $p<0.05$.
Neutrophils were highly correlated with diestrus ($r=0.81$, $p=0.03$) and showed a significant increase in this stage (80.67% in early diestrus, 19.33% in late diestrus, and 0% in estrus), confirming their role as a predictive marker for the estrus-diestrus transition. Superficial cells were the dominant cell type in estrus, comprising 23.19% and 29.07% across different subsets, while anucleated cells accounted for 55.19% and 24.63%. Intermediate cells were most frequent in late diestrus (43.29%) and proestrus (26.36%), while parabasal cells were largely confined to proestrus (82.4%). Diestrus samples had significantly higher progesterone levels compared to estrus ($p=0.02$). No significant correlation was found between erythrocyte percentage and any estrous stage ($p>0.05$).
This study confirms that neutrophil influx is an important predictive marker for the estrus-diestrus transition, with a strong correlation to serum progesterone levels. Superficial cells are the dominant cytological cell type in estrus, further supporting their role in estrous cycle monitoring. The use of ELISA for progesterone determination provides reliable hormonal validation of cytological changes. These findings validate vaginal cytology as a cost-effective diagnostic tool, particularly in settings where hormonal assays are unavailable. Future research should refine cytological progesterone estimation models to improve breeding and reproductive management strategies.
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6532/7856 | Serum CPSE as a monitoring tool for finasteride therapy in canine benign prostatic hyperplasia

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With advancements in veterinary care contributing to an increasing population of aging dogs, age-related conditions such as benign prostatic hyperplasia (BPH) are being diagnosed more frequently in practice. Clinical signs and transabdominal ultrasonography are commonly used for diagnosis, while histopathology remains the gold standard (1). Serum CPSE has emerged as a valuable biomarker, with elevated levels distinguishing BPH-affected dogs from healthy ones (2). Medical management typically involves dihydrotestosterone (DHT) inhibitors, with finasteride being the most widely used 5-alpha reductase inhibitor. Finasteride effectively reduces prostatic volume (3) by suppressing DHT synthesis, yet its impact on CPSE levels during treatment remains underexplored. This study aimed to assess the utility of CPSE as a monitoring tool for BPH treatment by evaluating changes in serum CPSE levels alongside prostatic volume (PV) in dogs receiving finasteride therapy. Seven Beagle dogs diagnosed with BPH received 5 mg of finasteride daily for 16 weeks. Prostatic volume and serum samples were collected over a 32-week period, with samples obtained every 2 weeks during the 16-week treatment phase, followed by sample collection every 4 weeks during the 16-week post-treatment monitoring phase. Serum CPSE levels were measured using ELISA kit (Odelis CPSE, Virbac, France). The results indicated that BPH-affected dogs had an average body weight of 13.0 ± 2.0 kg, an initial prostatic volume of 22.9 ± 5.1 cm^3, and serum CPSE levels of 193.5 ng/mL. These pre-treatment values (week 0) were set as the baseline, representing 100%, to facilitate comparison with post-treatment measurements. Significant reductions in PV were observed from week 4 ($70.1\pm4.3\%$, $p<0.001$) through week 16 ($42.1\pm5.1\%$, $p<0.001$). After treatment cessation, PV increased by week 20 ($63.1\pm6.8\%$). By weeks 24, 28, and 32, PV returned to baseline levels with no significant difference ($p>0.05$). Similarly, serum CPSE levels significantly decreased from week 2 ($84.9\pm4.8\%$, $p=0.43$) to week 16 ($43.5\pm11.6\%$, $p=0.018$), reaching the lowest level at week 16. Following treatment cessation, CPSE levels gradually increased, with a significant rise at week 20 ($74.4\pm4.6\%$, $p=0.018$). By weeks 28 and 32, CPSE levels returned to baseline, with no significant difference observed ($p>0.05$). This study demonstrated that finasteride reduced PV within the first 4 weeks and lowered serum CPSE levels within the first 2 weeks, with these reductions maintained throughout

the treatment period. The parallel decrease in CPSE levels and PV suggested that CPSE could serve as a useful biomarker for monitoring treatment response in BPH dogs. However, after treatment cessation, serum CPSE levels increased earlier than PV. By week 20, CPSE levels rebounded to 74.4% of baseline, while prostatic volume remained lower at 63.1%. This suggested that CPSE may act as an early marker of prostatic cell proliferation recurrence, preceding detectable changes in PV. Due to the delay between CPSE elevation and prostate regrowth, ultrasonography may serve as a complementary tool to enhance the accuracy of BPH monitoring.

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6515/7855 | Investigating the ratio of actual to estimated normal prostatic volume in dogs with subclinical and clinical benign prostatic hyperplasia

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Research on the relationship between prostatic size and clinical signs in canine BPH remains limited. Understanding this association in dogs is crucial for improving diagnostic accuracy and treatment strategies. Clinical signs include urethral discharge, hematuria, rectal tenesmus and urinary dysfunction. This study aimed to evaluate the ratio of actual to estimated normal prostatic volume (RAE) as an indicator of prostatic enlargement and its association with clinical symptoms in dogs with subclinical and clinical BPH.

A retrospective study was conducted at the Small Animal Teaching Hospital, Chulalongkorn University, from 2015 to 2023. Clinical data from dogs diagnosed with BPH via ultrasonography and clinical signs were analysed. The actual prostatic volume (aPV) was measured using Kamolpatana et al.'s formula (1): PV (cm^3) = [(L × W × D) / 2.6] + 1.8, where L (length), W (width), and D (dorsoventral diameter) were obtained via transabdominal ultrasonography. The estimated normal prostatic volume (ePV) was calculated using Sannamwong et al.'s formula (2): ePV (cm^3) = (0.33 × body weight [kg]) + 3.28. Dogs with aPV exceeding ePV were diagnosed with BPH, and RAE (aPV/ePV) was used to assess the degree of enlargement.

Of the 411 dogs, 279 (67.9%) had subclinical BPH, while 132 (32.1%) had clinical BPH. Clinical BPH dogs were significantly older (median: 10 vs. 9 years, $p = 0.015$), though body weight did not differ ($p = 0.126$). The median RAE was significantly higher in clinical BPH (2.5 [1.9–3.5]) than in subclinical BPH (1.9 [1.5–2.3], $p < 0.001$). ROC curve analysis identified an RAE cut-off of 1.87, with sensitivity = 82.6%, specificity = 51.3%, and AUC = 73% (95% CI: 68%–78%). Logistic regression analysis showed that RAE > 1.87 increased the risk of clinical symptoms 4.98 times (95% CI: 3.00–8.28, $p < 0.001$). Among clinical cases, dogs with urinary symptoms had the highest RAE (2.9, IQR: 2.0–3.4), followed by those with gastrointestinal and reproductive symptoms: however, the differences between symptom groups were not statistically significant ($p > 0.05$).

This study underscores the ratio of actual to estimated prostatic volume (RAE) as one of the key markers for evaluating prostatic enlargement in canine BPH. The findings confirm that clinical BPH cases exhibit significantly higher RAE than subclinical cases, highlighting the association between greater prostatic enlargement and increased severity of canine BPH. With an RAE cut-off of 1.87, this parameter effectively differentiates clinical from subclinical BPH. Due to its moderate specificity, ultrasonography and other clinical assessments are still essential for a thorough evaluation. As BPH often progresses silently and leads to other prostatic disorders, early identification of at-risk cases using RAE could improve disease monitoring, clinical decision-making and patient management.

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7161/7840 | Trends and comparison of progesterone concentrations throughout pregnancy in two groups of rough collie bitches along with a comparative analysis of two distinct fluorescence immunoassay instruments for canine progesterone assessment

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The evaluation of serum progesterone (P4) assay in the bitch is essential for the management of the reproductive cycle, as well for the diagnosis and monitoring of pathologies in a breeding female. This interest has led to the release of several diagnostic tools on the market, characterised by ease of use and rapid availability of results (1,3). Furthermore, several studies have sought to determine the normal ranges of progesterone throughout pregnancy in order to provide a model on which therapeutic decisions can be based (2). The aims of this study are: a) to evaluate whether there is a significant difference in progesterone concentration between bitches with an uneventful pregnancy and those with suspected hypoluteinism (P4 < 10 ng/mL prior to day 50 of pregnancy) and b) to compare P4 results obtained using two different instruments for canine progesterone assay.

The population of this preliminary study consists of 5 female Rough Collies, aged 2 to 5 years, from the same kennel, whose P4 concentration was monitored from ovulation (T0) until the 58th day of gestation (T4). Serum samples were collected every 3–7 days based on clinical evaluation, following pregnancy diagnosis, by cephalic vein puncture, allowed to clot at room temperature prior to centrifugation at 4000 rpm for 10 min and processed using the Vcheck Bionote V200 analyser (Bionote, Minnesota, USA). The sample were kept frozen at −20°C until a second measurement with the Tosoh AIA 360 (Tosoh corp., Minato-ku, Tokyo, Japan). Data were classified based on the type of pregnancy (*N* = normal, SH = suspected hypoluteinism), the assay used (Vcheck or AIA360) and the sampling periods: T0 = ovulation, T1 = 20–30 days post ovulation (dpo), T2 = 30–40

dpo, T3 = 40–50 dpo and T4 = 50–58 dpo. The correlation between the two diagnostic methods was performed. Statistical analysis was conducted using ANOVA for repeated measures, considering treatment with natural progesterone (N vs. SH), time and their interactions as fixed effects. The effect of the individual bitch was considered as a repeated and random effect. The two analytical P4 methods were compared using Pearson's correlation at the different time points and overall. Significance was set at $p < 0.05$.

No differences were detected for P4 between N and SH. The effect of time intervals was significant for both analytical methods ($p < 0.05$). Vcheck showed a P4 concentrations with a 30% difference at T2 and T3 between group N and H. This gap, however, is not observed with AIA360 at T2 and was lower at 21.7% at T3. Significant correlations between the two methods were RT4 = 0.85 and the overall was $R = 0.82$, $p < 0.05$.

Present results suggest that there is a low correlation between the two methods during the oestrus monitoring and at T = 3. This difference could lead to misinterpretation. It is advisable to interpret the results carefully and correlate them with clinical findings. However, a more representative sample and further investigations are needed to draw remarkable conclusion.

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7074 | Relationship of placental proteome and viability of canine neonates

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The placenta is the functional pregnancy unit responsible for maintaining fetal life. During dystocia, placental detachment can compromise neonatal survival. Then, this study aimed to investigate the molecular mechanisms of the placental function that support neonatal viability in bitches undergoing caesarean section by placental proteome.

Placentas from 31 puppies were collected from three regions: marginal hematoma (MARGINAL), far from the umbilical cord insertion (MIDDLE), and near to the insertion of the umbilical cord (CORD). Neonates were assessed using the APGAR score and classified into NORMAL, MODERATE, and WEAK groups. The samples were prepared for tryptic digestion and mass spectrometry was conducted by ESI-Q-TOF MS/MS. After mass spectrometry, data normalisation and analysis were performed with MetaboAnalyst, using univariate (ANOVA) and multivariate (non-hierarchical cluster analysis) analyses based on principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and VIP score.

A total of 411 proteins were identified in the 3 placental regions. The MARGINAL region exhibited a broader diversity of proteins, and the MIDDLE region played a more significant role in metabolic activity, particularly in oxidative stress response, maternal-foetal exchange, and cellular proliferation. The neonates were grouped, and a distinct proteome emerged. The WEAK group showed a higher concentration of proteins associated with hypoxia and tissue degeneration. Conclusion. The MIDDLE region of the canine placenta is the most metabolically active and sensitive to foetal distress, with distinct molecular alterations in the WEAK APGAR group, emphasising the placenta's role as an indicator of neonatal viability.

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6273 | Morphological and morphometric changes in the uterine and ovaries tissue of queens treated with an anti-GnRH vaccine

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The anti-GnRH vaccine could be used for control cat reproduction. However, few studies have examined its histopathological impacts on uterine tissues and ovarian function. The aim was to evaluate the morphological and morphometric changes of uterine and ovarian tissue in adult queens receiving two doses of Improvac vaccine.

Adult queens ($n = 12$), mixed short hair breed, aged from 2 to 4 years, were maintained under artificial illumination. The experiment had the approval of the IACUC (101–9-19 T). Queens were divided into two groups (G): control received saline solution (CON, 0.5 mL, sc; $n = 3$), and treated received anti-GnRH vaccine (Improvac, Zoetis, TRT, 0.5 mL, sc; $n = 9$) during interestrus. Queens received two doses 30 days (D) apart. TRT queens were divided into 3 G and ovariohysterectomized at 120D (G1, $n = 3$), 270D (G2, $n = 3$), and 330D (G3, $n = 3$). CON queens were ovariohysterectomized at 90D. The uterine and ovaries was histological processed and stained with H&E. The morphometric study was performed on microphotographs using ImageJ 1.53 t. The uterus was evaluated for endometrial thickness (ET, μm), internal and external glandular diameter (IGD and EGD, μm), and the height of glandular cells (HGC, μm). In the ovaries, the maximum and minimum diameters of the follicles and oocytes were measured to calculate the mean diameter. Five primordial follicles, five unilaminar primary, five multilaminar primary, and five secondary follicles were examined in each ovary. Data were analysed with GLIMMIX of SAS. The TRT group developed soft tissue masses at the vaccination site, which later disappeared.

All CON showed normal endometrium (NE). In the TRT, five queens had endometrial atrophy (EA), one queen had NE, and

three had endometrial hyperplasia (EH). TRT had an increase in ET compared with CON (715.76 ± 35.75 [EA: 565.31 ± 0.01, NE: 669.65 ± 0.03, EH: 975.75 ± 0.01, $p = 0.0001$; respectively)] vs. 340.67 ± 21.86, $p = 0.0001$). We observed an increase in the IGD in the TRT compared to CON (20.59 ± 0.01 [EA: 19.87 ± 0.01, NE: 20.49 ± 0.04, EH: 21.8 ± 0.01, $p = 0.0001$; respectively] vs. 13.21 ± 0.02, $p = 0.0001$). Similarly, the EGD was higher in TRT with EH compared than CON (39.18 ± 0.00 vs. 35.55 ± 0.01, $p = 0.0001$). The HCG in CON were higher compared to TRT (9.76 ± 0.01 vs.7.16 ± 0.01 [EA: 6.42 ± 0.01, NE: 6.36 ± 0.03, EH: 8.67 ± 0.01, $p = 0.0001$; respectively], $p = 0.0001$). Histological evaluation of the ovaries revealed that five TRT had follicular cysts (G1 $n = 2$, G2 $n = 1$, G3 $n = 2$). TRT with EA showed higher diameters of primordial follicle oocytes than CON ($p = 0.03$). Likewise, the oocytes of unilaminar primary and multilaminar primary follicles from the TRT with EA and EH significantly increased compared to CON ($p = 0.005$, $p = 0.0001$, $p = 0.02$ and $p = 0.0001$; respectively). The TRT with EA and EH showed higher primordial, unilaminar primary, and multilaminar primary follicles diameters ($p = 0.03$, $p = 0.04$, $p = 0.0001$, $p = 0.005$, $p = 0.0001$, $p = 0.0001$; respectively). However, no differences were observed in the diameter of oocytes and secondary follicles ($p = 0.40$, $p = 0.07$; respectively).

The use of the Improvac vaccine in queens causes changes in endometrial histological structure and alterations in the development of oocytes and ovarian follicles.

6416 | When labour never starts: Exploring prolonged gestation and aseptic fetal demise in the dog

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Clinical case. A 4-year-old female Border Collie was referred for a second opinion due to prolonged pregnancy, now at day 73 from the last mating, without signs of labour onset. The bitch had been mated over a 5-day period, and no medical treatment had been administered during gestation. The bitch was fed a commercial diet formulated specifically for pregnant and lactating bitches. Ultrasound performed at an external clinic on day 56 confirmed viable foetuses with heart activity, while a subsequent scan 7 days later showed no detectable foetal heartbeats. At the time of referral, the bitch was clinically stable, without vaginal discharge, and by vaginal examination foetal structures were not palpable. A POCUS ultrasound confirmed the absence of foetal heart activity in all visible foetuses. No free gas was noted, but there was increased echogenicity in the foetal fluids and developed foetal structures with corticomedullary differentiation of the kidneys. Mild abdominal effusion was observed. Preoperative blood work revealed only mildly elevated ALT and total protein levels, with no signs of infection or systemic inflammation. Caesarean section was recommended and performed. Intraoperatively, a markedly hypotonic and dilated uterus was observed, containing approximately 2 L of brown, gelatinous, malodorous fluid. Five deceased foetuses were removed, with some lacking associated placental tissue suggesting that the placentas had likely disintegrated. The cervix remained fully closed. Samples for bacterial culture (aerobic and anaerobic) were collected with swabs from the uterus between implantation sites and progesterone analysis were ordered during surgery. Supportive therapy included intravenous fluids, opioid analgesia, and antibiotic treatment with amoxicillin (20 mg/kg q8h) and enrofloxacin (5 mg/kg q24h), pending culture results. Progesterone levels were 16.4 nmol/L, and treatment with aglepristone (10 mg/kg, two injections 24 h apart) was initiated. Cabergoline (5 µg/kg for 5 days) was prescribed to suppress lactation. Bacterial cultures remained negative after 48 h; enrofloxacin was discontinued, but amoxicillin therapy was continued. Follow-up ultrasound examinations were conducted at 7-day intervals, showing gradual uterine involution with complete resolution of intrauterine fluid. The foetuses were submitted for necropsy and microbiota research, although results were pending at the time of writing.

This case highlights the challenges in managing prolonged pregnancy in bitches and raises important considerations regarding the causes of intrauterine foetal death in the absence of infection and with serum concentration of progesterone above 6 nmol/L at day 73 after last day of mating. Possible etiologies may include endocrine dysfunction, uterine inertia, or aseptic foetal death secondary to placental insufficiency or foetal stress (1). In the absence of bacterial growth on culture and no systemic inflammatory markers, a diagnosis of aseptic intrauterine foetal death is most likely. This phenomenon has been reviewed and is thought to be related to placental insufficiency, hypoxic foetal death, or subclinical infection not detectable by standard culture techniques (2). Further investigation into the role of maternal-foetal microbiota and hormonal dynamics is indicated.

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6538/7952 | Unilateral ovariectomy to maintain fertility in a 2-year-old Boxer with granulosa cell tumour

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Tumours of the ovaries in small animals are rare findings, this is due to the high number of ovariectomized animals and the fact that only in cases where the tumour has clinical symptoms a pathohistological examination is performed. As a rule, older animals are affected, an exception is germ cell tumours (GCT), which are also detected in younger animals. Due to their hormonal activity, GCTs can negatively affect the reproductive cycle, showing prolonged estrus symptoms or irregular estrus intervals, which can result in metropathies. Usually in patients presenting with an ovarian mass, an ovariohysterectomy is performed, however if the owner wishes to preserve fertility a unilateral ovariectomy might be a viable treatment option.

In October 2023 a 2-year-old female intact Boxer was brought to the clinic with owners reporting a prolonged estrus cycle (> 3 months). A gynaecological exam showed serosanguineous vulvar discharge, with the vulva being mildly enlarged. Vaginal cytology showed small and large intermediate cells, as well as some superficial cells. Endoscopically, the vaginal mucosal folds appeared edematous, pink and round. A progesterone serum concentration was measured < 2 ng/mL. An ultrasound of the ovaries was performed, finding multiple follicles of varying sizes. Ovulation induction was attempted with Choriongonadotropin,

this however had no effect and so a 4.7 mg deslorelin (Suprelorin) implant was implanted. This successfully suppressed heat symptoms for 3 months. Repeat gynaecological exams showed normal symptoms of heat, however the ultrasound of the ovaries had showed abnormal changes, large cavernous structures of irregular echogenicity. It was decided to remove the deslorelin implant and AMH levels in the blood were tested revealing an elevation of 22 ng/mL.

Through a unilateral ovariectomy the affected ovary was removed. Macroscopic findings showed a multinodular, partly cystic increase in circumference. Histological findings indicate a cystically altered granulosa cell tumour.

After one failed attempt to mate the dog, she was presented in our clinic with fluid filled pregnancy-associated uterine vesicles and a minimum of six foetal masses within the vesicles were detected. However, a week later the dog showed signs of vaginal discharge and presented again for a re-check. Progesterone levels were still adequality high at 49.42 ng/mL, however there was only one viable foetal heart beating detected in ultrasound. Changes in embryo diameter, crown rump length, biparietal head diameter, and other parameters progressed normally. A c-section was scheduled for day 63 of pregnancy and a healthy female puppy was delivered.

The cause for resorption of the foetuses cannot be said with certainty. It is to be believed that the lost function of the excised ovary is compensated for by the remaining ovary. Other causes of subfertility could be due to genetics or because of damages to the endometrial lining. Other short communications have shown, that a unilateral ovariectomy resulted in normal pregnancy and litter sizes and that it can be speculated that it is the capacity of the uterus and not the number of ovaries that is the limiting factor for litter size.

6177/7832 | Pre- and post-natal gastrointestinal parasitological evaluation in a dog breeding kennel

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Kennels are environments of high population density and intense interaction between animals, which favours the spread of parasites. Breeders play a fundamental role in preventing parasites, in order not only to protect the health of their dogs, but also to avoid possible repercussions for public health. This study aimed to evaluate the degree of parasitism in different groups of dogs within a multi-breed breeding kennel. Specifically, it focused on assessing parasitic infection rates among adult males, adult females (including pregnant females), neonates and puppies. By identifying parasitological burdens across these groups, the study sought to provide insights into parasite distribution and contribute to improved management and control strategies in breeding facilities. A total of 56 stool samples were analysed, 42 of which came from adult and young dogs, and 14 from newborn dogs aged 2 months or less (from 6 litters). All 6 litter's dams were tested during that lactation or pregnancy. These samples were gathered mostly in the spring and preserved at 4°C (maximum 2 weeks) until they were submitted to various coprological techniques, including the Baermann Method, the Faecal Smear with modified Ziehl-Neelsen stain, the Flotation Method (Willis) with and without immersion oil, the Mini-FLOTAC and the Direct Immunofluorescence. In this kennel, adult dogs were dewormed two times a year, while puppies were dewormed from the age of 1 month and every 15 days until they were 2 months old, using febantel and pyrantel. Lactating bitches were not dewormed, unless puppies were found with parasites. Sanitation was carried out once or twice a day with the help of a neutral detergent, with the exception of unpaved parks, where faeces were collected every 2 days.

In adult and young dogs, the prevalence rates observed were 95.5% (37/40) for hookworms, 32.5% (13/40) for *Toxocara canis* and 15.0% (6/40) for *Cystoisospora* spp., resulting in an overall prevalence of 95.23% (40/42). It was found that 67.5% (27/40) of dogs had single infections, 17.5% (7/40) had double infections and 12.5% (5/40) had triple infections. In the neonates and puppies, 3 types of parasites were identified: *T. canis*, *Cystoisospora* spp., with varying prevalence (40 to 100% for *T. canis* and 60% to 100% for *Cystoisospora* spp.), as well as Giardia spp. present in 33.33% (2/6) of litters, resulting in an overall prevalence of 80 to 100%, depending on the age of the puppies. All dams were tested positive during pregnancy and/or during lactation for hookworms and *T. canis/Cystoisospora* spp.

Internal parasites continue to be a challenge, and to ensure the sustainability and the salubrity of kennels, it is essential to educate breeders about parasitosis and encourage them to adopt preventative measures, avoiding high treatment costs. Given that many of these parasites have zoonotic potential, it is crucial to continue with studies that assess the sanitary conditions of kennels, identify the prevalence of parasites and the risk factors associated with their emergence, promoting the education of both owners and veterinarians, in line with the One Health concept.

6550 | Evaluating miRNA inheritance and gene expression in canine oocytes and zygotes

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Oocyte maturation and the maternal-zygotic transition are essential processes in early embryonic development. These processes are regulated by intricate post-transcriptional interactions, with microRNAs (miRNAs) playing a pivotal role in modulating gene expression. In this study, we aimed to evaluate the expression of maternal genes, C×37, BMP-15, and GDF-9, critical for oocyte development, along with the miRNAs predicted for these genes through bioinformatics analysis. based on the binding site-predicted targets available in the databases.

Furthermore, we assessed the potential inheritance of these miRNAs to zygotes generated by in vitro fertilisation (IVF). Oocytes were collected from ovaries of ovariohysterectomized adult bitches, and a total of 317 immature oocytes, 388 in vitro matured oocytes (IVM) (cultured for 72 h, at 38°C, 5% CO_2 in TCM199–Hepes, supplemented with 10% foetal calf serum, 0.25 mM pyruvate, 10 IU/mL hCG, 2 µg/mL ß-estradiol, 50 µg/mL progesterone, 100 IU/mL penicillin, and 20 µg/mL streptomycin), and 108 presumptive zygotes (obtained by co-culturing in vitro matured oocytes with 1×10^6 sperm/mL in Fert-TALP medium for 24 h under identical incubation conditions), were used for the study. miRNAs miR-8901, miR-1301, and miR-196a were selected as predicted regulators for C×37, BMP-15, and GDF-9, respectively, based on PCR amplification cycles and dissociation curve analysis. mRNA and miRNA expression levels were quantified at three developmental stages (immature oocytes, IVM oocytes, and zygotes) using quantitative PCR (qPCR). Statistical analyses, including ANOVA followed by Duncan's test, were conducted to compare expression levels, and Pearson correlation analysis was performed to evaluate the relationship between mRNAs and their corresponding miRNAs. Our results demonstrated that all three selected miRNAs were expressed in oocytes and detected in zygotes, suggesting maternal inheritance. The relative expression of GDF-9 decreased ($p<0.05$) from immature oocytes to zygotes, while BMP-15 and C×37 increased significantly ($p<0.05$) in IVM oocytes. At the miRNA level, miR-196a showed a significant increase from immature oocytes to zygotes, whereas miR-1301 and miR-8901 increased ($p<0.05$) in IVM oocytes. Additionally, a significant negative correlation ($r=-0.790$) was found between miR-196a and GDF-9, suggesting a post-transcriptional regulatory mechanism during the maternal-zygotic transition. This study provides the first evidence of miRNA inheritance in canine zygotes, highlighting the potential role of these miRNAs in regulating gene expression during early development. These findings contribute to a better understanding of the molecular mechanisms underlying oocyte maturation and fertilisation in canines. Furthermore, the results may inform advancements in assisted reproductive technologies for dogs, including in vitro maturation and fertilisation protocols, improving fertility rates in canine breeding and genetic conservation.

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6425 | Effect of freezing rate on canine sperm quality: A comparative study of two cryopreservation protocols

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During cryopreservation, spermatozoa endure considerable physical and thermal stress, and their intrinsic properties further influence post-thaw quality. This variability underscores the importance of optimising each step of the process to enhance sperm survival and improve freezing consistency (1). Adjusting the freezing rate has shown promising results in stallions and boars (1,2), but its effects in dogs remain underexplored.

Semen from 11 healthy adult dogs of nine different breeds (weight range: 9–40 kg) was collected and split into two aliquots per dog, ensuring each served as its own control. The sperm-rich fraction was centrifuged at 720×g for 5 min; the supernatant was removed, and the pellet was resuspended in a two-step TRIS-egg yolk extender containing glycerol and Equex paste. Samples were then cooled from 37°C to 4°C over 2 h in a bain-marie placed in a refrigerator. Freezing was performed using a controlled-rate freezer (IceCube 1810; SyLab, Austria). The control freezing curve cooled samples at −17°C/min from 4°C to −80°C, then −7°C/min to −130°C. The alternative curve, derived from equine protocols (3), cooled at −10°C/min from 4°C to −25°C, then −17°C/min to −130°C. Post-thaw sperm was evaluated using a CASA system (ISASv1, Proiser, Spain) for motility and kinematic parameters—including total motility (TM), progressive motility (PM), curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP)—as well as amplitude of lateral head displacement (ALH), beat cross frequency (BCF), wobble (WOB), straightness (STR), and linearity (LIN). Sperm morphology was evaluated on eosin–nigrosin stained smears. Plasma membrane integrity, acrosome integrity, and mitochondrial membrane potential were assessed using SYBR14/PI, PNA-FITC and JC-1 fluorochromes under fluorescence microscopy. Paired samples t-tests or Wilcoxon signed-rank tests were used depending on data distribution (IBM SPSS 19.0).

The median age of the dogs was 4.9 years (IQR = 3.8). After thawing, sperm quality was significantly reduced in the alternative freezing curve compared to control ($p<0.05$), except for WOB, normal morphology, major morphological anomalies and mitochondrial membrane potential, which did not differ significantly. Progressive motility decreased by 9.37% ($p<0.001$), total motility by 5.91% ($p<0.05$). VCL, VSL and VAP were also impaired (−11.60 µm/s, −9.74 µm/s, and −7.58 µm/s respectively; $p<0.01$). Minor morphological anomalies increased ($p<0.05$), and acrosome integrity decreased by 5.3% ($p=0.05$). No significant differences were observed for major defects, teratozoospermia index or abnormal morphology.

Under the tested conditions, the alternative freezing curve negatively affected post-thaw semen quality in dogs. Specifically, slower cooling between −5°C and −10°C may have increased osmotic stress, leading to decreased motility and acrosome integrity. These findings highlight the importance of species-specific optimisation of freezing curves in canine semen cryopreservation.

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5443/7793 | Subclinical hypothyroidism in female dogs and its link to fertility problems

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Three bitches were presented for the first time at our Clinic due to fertility problems: a 3-year-old German shepherd, a 4-year-old

Cavalier King Charles Spaniel, a 4-year-old Border collie. All bitches had already been mated twice in previous heats. The optimal time of mating was determined by progesterone measurements and cytological examination of vaginal smears, but the bitches did not become pregnant. None of the bitches had any previous basic blood test for morphology, biochemistry (liver function, kidney function and thyroid hormones). Animals were regularly dewormed and vaccinated against infectious diseases. Blood was taken from all three bitches for haematological, biochemical and hormonal [estradiol 17 B, progesterone, TT4, TSH, TgAA (Vetlab Laboratory, Poland)] determinations during the anestrus period. Ultrasonography of urogenital tract was performed. Systemic diseases and ovarian cysts were excluded. The results obtained directed the suspicion of subclinical hypothyroidism (no clinical signs of hypothyroidism). In all three bitches, TT4 and TSH levels were within normal limits (although in the lower ones). In contrast, TgAA in the German Shepherd were above normal range and in the other bitches within the highest norms. As the bitches were approximately 1.5 months away from their next heat, it was decided to include thyroid supplementation with Thyroforce (Vetfood, 1 capsule per 10 kg b.w.). Further tests for TT4 and TgAA were performed after 1 month of supplementation and all were within physiological limits. Supplementation was continued. Optimum time of mating was determined based on progesterone blood levels and cytology of vaginal smears. The optimal time of mating was determined, and the bitches were mated twice, 2 days apart. Twenty-three days after the second mating, an ultrasound was performed and, in all bitches, foetal ampules were found. The German Shepherd gave birth to six puppies, the Cavalier King Charles Spaniel five puppies, and the Border collie seven puppies.

Clinical hypothyroidism in dogs is fairly well understood—based on clinical signs and blood results, long-term hormonal treatment is implemented. Subclinical hypothyroidism is much more difficult to diagnose due to the lack of clinical symptoms. Its interpretation based on thyroid hormone tests is not always clear. The relationship between hypothyroidism and infertility in the bitch is controversial and subclinical hypothyroidism is underestimated in canine reproduction. Sometimes supplementation is enough, without including hormonal treatment. Thyroforce contains key ingredients such as tyrosine, iodine, iron, zinc, vitamins D3, A, E, B vitamin complex and ashwagandha. Each of these elements plays an important role in the production and regulation of thyroid hormones, supporting health and hormonal balance in animals. This also gives room to sustain their reproduction. At the time of writing this report, our Clinic is caring for a further 4 cases with a similar substrate of infertility.

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6282/7802 | Effect of zinc and manganese nanoparticles on the motility and viability of canine sperm stored in a liquid state at 5°C

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Previous studies have shown that the use of zinc nanoparticles (ZnNPs) in the preservation of bull, boar, and turkey semen had a positive effect on improving the biological properties of sperm (1). Zinc provides antioxidant activity by preventing the production of reactive oxygen species (ROS) by abnormal sperm and leukocytes, thus inhibiting lipid peroxidation (2). In turn, manganese nanoparticles (MnNPs) can improve the antioxidant capacity of preserved turkey sperm (3). Divalent manganese ions increase sperm motility, viability, capacitation, and acrosome reaction, and minimise oxidative stress (4). The aim of this study was to evaluate the effect of adding ZnNPs and MnNPs to the Tris-fructose-citric acid extender on the motility and viability of canine sperm stored at 5°C.

Semen was collected from four mixed-breed dogs using digital manipulation. The ejaculate was diluted with different variants of Tris-fructose-citric acid extender without (control) and with the addition of different concentrations of ZnNPs and MnNPs: 200 µg/mL; 100 µg/mL; 50 µg/mL and 25 µg/mL) to a concentration of 30 ×106 spz/mL. Sperm motility was assessed using the CASA system (Hamilton-Thorne Sperm Analyzer, IVOS). Sperm viability was examined by cytometry using the Muse Count&Viability kit. The extended semen was stored for up to 48 h. Analyses were performed after dilution (2 h), and after 24 h and 48 h of storage.

The addition of ZnNPs and MnNPs to the extender had a significant ($p \leq 0.05$) effect on the motility and viability of stored canine spermatozoa. After 24 h of semen storage, a decrease in total motility (TMOT) and progressive motility (PMOT) was observed in the treatments with ZnNPs and MnNPs (100 µg/mL and 200 µg/mL), compared to the control, and these values were 64.0 ± 3.6% and 30.7 ± 4.6%, respectively for ZnNPs (200 µg/mL); 65.7 ± 3.5% and 34.3 ± 2.1%, respectively for MnNPs (200 µg/mL), and 70.8 ± 3.2% and 42.0 ± 3.6% for the control. The addition of MnNPs (50 µg/mL and 25 µg/mL) to the extender induced an increase ($p \leq 0.05$) in TMOT, PMOT, and viability during storage. After 48 h of storage, the values of TMOT, PMOT, and viability were 60.3 ± 6.1%, 40.3 ± 4.9%, and 81.3 ± 3.5%, respectively, for MnNPs (25 µg/mL), and 51.7 ± 3.2%, 26.0 ± 5.7%, and 73.6 ± 4.5%, respectively, for the control.

The effect of ZnNPs and MnNPs on sperm motility and viability depends on their concentration in the Tris-fructose-citric acid extender. The addition of ZnNPs and MnNPs (100 µg/mL and 200 µg/mL) to the extender negatively affects sperm motility. The addition of MnNPs (50 µg/mL and 25 µg/mL) to the extender may improve the quality of canine sperm stored in a liquid state.

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7096 | Ovarian hematoma in a bitch with persistent estrus—A case report

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Ovarian pathologies often lead to an enlargement of the organ and may be hormonally active, like follicular cysts or granulosa cell tumours, inducing persistent estrus (1). Ovarian hematomas as a cause for ovarian enlargement are described in mares and cows (2, 3).

In January 2025, a 1-year-old female Doberman Pinscher mix was presented at our clinic due to prolonged first heat lasting 8 weeks. Gynaecological examination revealed an enlarged vulva with serosanguinous vaginal discharge. Exfoliative vaginal smear indicated proestrus and progesterone concentration was <0.2 ng/mL. Ultrasound examination showed no significant changes in the ovaries and uterus. An attempt was made to induce ovulation with hCG (1 mL Ovogest 300 I.U./ml), but the progesterone level didn't exceed 2.8 ng/mL. However, heat signs diminished, and the owner observed the development further.

Six weeks later the bitch was presented again due to the reoccurrence of serosanguinous vaginal discharge. The gynaecological findings hadn't changed to the first presentation. In the meantime, the bitch had multiple biting incidents with other female dogs, which were treated with surgery and antibiotics. This time, a partially echogenic round structure measuring approximately 4 cm in the scope of the right ovary had been detected during ultrasound examination. Because of the estrous signs a granulosa cell tumour was suspected, but anti-mullerian hormone concentration was 0.11 ng/mL and not elevated as expected.

Based on the ultrasound findings an explorative laparotomy was performed 4 days later and ovariohysterectomy under general anaesthesia (dexmedetomidine 2 µg/kg i.v., ketamine 1 mg/kg i.v., methadone 0.2 mg/kg i.v., propofol 2 mg/kg i.v., isoflurane) was performed. In the days before surgery the bitch developed gastrointestinal problems, diarrhoea and vomitus, which didn't resolve with the surgery, but improved with symptomatic therapy and a four-day inpatient stay.

The histopathological examination revealed a hematoma formation on the right ovary of approximately 4.5 cm in size with compression of the surrounding tissue, but no evidence of inflammatory or neoplastic processes. In addition, there were multifocal haemorrhages of the left ovary and the uterus.

To the author's knowledge there is no description of ovarian hematomas in dogs. In mares and cows, ovarian hematomas result from bleeding into the follicular cavity after ovulation (2, 3). In this case, histopathology could not clearly reveal this aetiology. A possible trauma associated with the reported biting incidences that led to excessive bleeding was discussed. However, the origin of the prolonged heat sings in this bitch remains unclear.

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6365 | Preliminary evaluation results of assessment of left ventricular systolic function by doppler echocardiography in neonate dogs

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The neonatal period in dogs refers to the first 4 weeks of life. During this period, newborn dogs differ from adults in several morphofunctional differences. Understanding these differences is essential for professionals in formulating diagnostic and therapeutic plans for sick newborns (1). Congenital anomalies are commonly observed in newborn dogs, among which, congenital heart disease (CHD) has a high incidence in dogs, approximately 21.7%, as observed in the prevalence of general congenital defects (2). Echocardiography allows obtaining more information about the size and function of the chambers, wall thickness, valve integrity, blood flow patterns and some indicators of ventricular function (3). Depending on the anomaly identified, medical or surgical therapy may be indicated. The objective of this study will be to evaluate and compare ventricular function in newborn patients born by vaginal delivery and caesarean section, at seven, 14, 21 and 35 days of age.

The parameters of ventricular function obtained by two-dimensional and tissue echocardiography will be described and correlated at different times of analysis. Forty dogs will be evaluated in this study. To date, 12 newborn dogs of the Italian Greyhound, Norfolk Terrier and English Bulldog breeds at 7 days of age (Group 1) have been included and evaluated by conventional echocardiographic evaluation. Group 2 consisted of 12 neonates, but at 14 days of age. The data were presented statistically using minimum and maximum medians. For verification, the comparison method was used through the Wilcoxon test.

The neonates were seven males and five females, with a mean weight of 351 g (min: 169; max: 559) (Group 1). Group 2 represented the neonates aged 14 days with a mean weight of 500 g (min: 420; max: 1240)). An increase in left ventricular diastolic diameter (LVDD) (p-value 0.05) and left atrial (LA) diameter (p value 0.01) was observed on conventional echocardiography within 7 days of life of the neonates.

At 7 days of life, conventional echocardiography showed a significant increase in left atrial diameter ($p = 0.01$) and a borderline increase in left ventricular end-diastolic diameter ($p = 0.05$), with preserved systolic function. These changes likely reflect normal cardiovascular adaptation in the neonatal period. However, if persistent, they may suggest volume overload due to conditions such as patent ductus arteriosus or intracardiac shunts, warranting clinical and echocardiographic follow-up.

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6510 | Prevalence and antimicrobial resistance variations of *Escherichia coli* from canine pyometra cases in different geographical regions

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Usually, treatment of pyometra, a common reproductive disease in intact bitches, involves the use of antibiotics (1). In most cases *Escherichia coli* has been identified as being the causative pathogen (2,3). Antimicrobial resistance (AMR) is an increasing problem worldwide and can negatively impact treatment outcomes. The aim of this study was to compare the prevalence and AMR profile of *E. coli* isolated from dogs (intrauterine, intravaginal, urinary or abdominal samples) with pyometra to four commonly used antibiotics: amoxicillin-clavulanic acid (AMCLA), cefpodoxime (CEF), enrofloxacin (ENRO) and trimethoprim-sulfamethoxazole (TMS). Data from two veterinary teaching hospitals in different countries (H1: United States, H2: Switzerland) were used retrospectively using patient records from 2010 to 2024 (H1:2010–2023, 109 dogs; H2:2017–2024, 122 dogs). For the study, AMR was recorded as noted in each hospital's medical records. Data were analysed with Chi-square or Fischer's exact test; $p < 0.05$ was considered significant. *E. coli* was isolated in a similar proportion of patients with available bacterial cultures (60.3% in H1 vs. 65.6% in H2), and the percentage of those *E. coli* isolates in pure culture (91.4% in H1 vs. 87.5% in H2) were also not different ($p > 0.05$). However, AMR of *E. coli* (all isolates) was different between institutions as 82.9% vs. 11.2% of the isolates were resistant to AMCLA ($p < 0.001$) and 22.5% vs. 6.1% were resistant to CEF ($p = 0.012$) in H1 vs. H2. AMR profile for ENRO (9.5% vs. 4.1%) and TMS (10.0% vs. 11.2%) for H1 vs. H2 did not differ significantly ($p > 0.05$).
In conclusion, these preliminary results show similar prevalence of *E. coli* in canine pyometra cases between two geographically distant locations, but a different AMR profile based on retrospective data. Obviously, bacterial cultures with antimicrobial susceptibility testing remain highly important to guide antimicrobial selection for the treatment of canine pyometra. In addition, these data may initiate more projects to assess reasons for these differences and to better guide prudent and reasonable use of antibiotics.
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6539 | Evaluation of biochemical alterations in canine neonatal sepsis

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The incidence of neonatal sepsis in dogs is high, affecting approximately 14.8% of newborns, with a mortality rate of approximately 25.6% (1). Haematological and biochemical indices used together in clinical practice have predictive value and are useful in the diagnosis and prognosis of sepsis. However, there are still no studies in neonatal dogs. In medicine, the main clinical biochemical indexes predictors for the diagnosis and prognosis of sepsis are related to an increase in AST/ALP, ALT, alkaline phosphatase, urea, creatinine and total bilirubin (2). The aim of the study was to evaluate biochemical changes in neonates with diagnosed sepsis.
The study consisted of two groups, one with 14 septic neonates (GS) and the other with 10 healthy animals (GC), evaluated only at the time of care. The group division criterion was according to the presence of leukopenia ($\leq 5 \times 103$ leukocytes) in the blood count at the first visit, in addition to the evaluation of clinical signs (body erythema, omphalitis, diarrhoea, cyanosis or necrosis of the extremities, among others) according to Pereira et al., 2022 (1). For the evaluations, 0.8 mL of blood was collected from each animal to obtain serum and evaluate 14 biochemical parameters analysed in VETSCAN VS2. In this study, mean and standard deviation data of the results were used. The groups were compared and data with parametric distribution were compared with t-test and non-parametric data were compared with Mann–Whitney.
In this preliminary study, the values of six biochemical parameters studied will be exposed. The mean and standard deviation of the septic group and healthy group were respectively: alkaline phosphatase (ALP), 271.29 ± 269.4; 341.8 ± 201.7; Alanine aminotransferase (ALT), 27.5 ± 15.4; 27.2 ± 17.4; Total bilirubin (TBIL), 0.85 ± 1.0; 0.4 ± 0.1; Urea (BUN), 29.4 ± 19.4; 21.7 ± 11; Creatinine (CREA) 0.7 ± 0.7; 0.5 ± 0.3; Glucose (GLU), 78.5 ± 41.6; 127.2 ± 54.7. There was a difference between the groups only in glucose ($p < 0.05$).
Biochemical parameters are of great importance in defining the diagnosis and prognosis of adult patients with sepsis. In neonates, biochemical parameters do not appear to be useful in these situations, probably due to the physiological immaturity of the patient; other biomarkers should be studied. Hypoglycemia, although common in sepsis, is also observed in several situations and should not be related only to this clinical condition.
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A7 | Antenatal resolution of feline hydrops fetalis with suspected streptococcal infection

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Here we discuss a case of feline hydrops fetalis, or anasarca, in a 4-year-old pregnant Birman cat, and a potential link with streptococcal infection.
A 4-year-old Birman cat was presented for her second pregnancy diagnosis. One and a half years ago, the queen underwent a c-section with three kittens, one of them stillborn with anasarca, the second one had enophtalmia and died 1 week after birth and the last one survived. Since this birth, three other females in the same cattery encountered a drop of fertility (not pregnant, resorptions and small litters). A vaginal bacteriological analysis

in one female during estrus was performed, which resulted in a pure and highly positive culture of *Streptococcus canis*, which was multi-drug resistant.
Ultrasonography: Pregnancy diagnosis was performed in the Birman 30 days after mating: two kittens and one resorption were observed. The extrafetal structures were abnormal as if only one placenta was connecting the two foetuses. One of them presented an edema around the skull and the neck, while free fluid was observed in the cranial cavity. Amoxicillin clavulanic acid, 20 mg/kg twice daily was then started until delivery, based on the antibiogram results.
A second ultrasonography 1 week before parturition confirmed an enlarged head (> 3 cm wide) in one fetus while the other fetus seemed normal.
One day before parturition, in order to assess the need for a c-section, the subcutaneous edema that was previously observed was not visible anymore. Progesterone was at 1 ng/mL. No signs of fetal distress were observed with normal heart rate. The cat was discharged to attempt a natural birth at home. The next day, the cat did not give any signs of second stage of labor, and a c-section was performed. Two viable kittens were born, with no visible external abnormalities. Only one larger placenta connecting the two kittens was observed.
Here, spontaneous resolution of hydrops was present as previously described in dogs (1). It was not clear if the streptococcal infection was related with the presence and subsequent resolution of hydrops after treatment. The long term outcome of the kittens is still unknown. The presence of only one placenta may be a result of a true twin pregnancy, or a disruption during placentation. Genetic tests will be performed on both kittens to determine whether they are true twins.
References:
1) Hopper et al., Journal of Small Animal Practice 2004;45: 2–8.

6371/7948 | Factors influencing the monitoring of the rectal temperature to predict onset of parturition in bitches

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A drop of progesteronemia is considered the gold standard for predicting the onset of parturition within 48 h in bitches. Since progesterone is a thermogenic hormone, a drop of rectal temperature could be a potential surrogate and a convenient method for dog breeders (1, 2). However, information about the influence of breed and other factors regarding such method is lacking. This study aimed to analyse variations in rectal temperature in bitches of different breeds in the days preceding whelping and to evaluate the factors that influencing them.
Gestating bitches ($n = 258$) from 12 breeds were recruited in 100+ French breeding facilities over 18 months, representing a range of dog sizes, a variety of dog morphologies and thermoregulatory abilities.
Rectal temperatures were measured at 08:00, 14:00 and 18:00 (±30 min) during the week preceding to the expected whelping date. Simultaneously, environmental parameters were recorded (temperature, hygrometry, date) alongside bitch-related (age, parity, body weight & condition score before and after gestation, diet), litter-related (litter size, number of stillborn, c-section, dystocia, whelping duration) or facility-related (pregnancy number per year, indoor/outdoor housing, isolation for parturition) factors. Factor influences were assessed using a linear-mixed model. A graph illustrating temperature changes over time was created for each pregnancy and reviewed by two readers to confirm the presence (OK) or absence (NOTOK) of a temperature drop within 30 h before the expulsion of the first puppy.
After database cleaning, 230 gestations were analysed, with a median of 25 rectal temperature measures per dog. Dog size had a clear impact on the proportion of temperature drops OK vs. NOTOK such as 35 vs. 65% in extra-small, 48 vs. 52% in small, 65 vs. 35% in medium, 60 vs. 40% in maxi and 40 vs. 60% in giant dogs. Among all the parameters assessed, none influenced the classification of temperature drops between OK and NOTOK, except if the bitches were isolated from the rest of the kennel at the end of gestation.
Among the 119 temperature drops classified as OK over 230, a mean drop of 0.7°C was observed compared to the mean temperature measured in the days before, with a mean duration of 23.2 h, with no differences between dog sizes. For the 10 bitches followed for 2 gestations and 2 bitches for 3 gestations, no pattern regarding OK/NOTOK classification between the gestations was observed.
This field study ran in real-life conditions with a large diversity of dog breeds and breeding facilities demonstrates that a drop of rectal temperature is inconstantly observed before the onset of parturition with important variations between dog breed sizes. No factors that could potentially affect the temperature drop and its characteristics could be found, except for the isolation of the dam before parturition. More investigations are needed to confirm rectal temperature monitoring as a reliable tool for whelping prediction in parturient bitches.
References:
1) Concannon & Hansel, 1977.
2) Grellet et al., EVSSAR (2018).

6274 | Assessment of an anti-GnRH vaccine as an immune contraceptive method in dogs

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Anti-GnRH vaccines, commercially available and used for reproductive control in swine, have been poorly studied in canines. This study aimed to evaluate the effect of the Improvac vaccine on male canine reproductive capacity.
Twenty intact males, aged 2 to 8 and weighing 10 to 40 kg, were used. All animals received a GnRH vaccine (Improvac, Zoetis Belgium SA, 1 mL sc) twice, 4 weeks apart. Treatments were performed on days 0 (D0) and 28 (D28). Day 0 of the study was defined as the day of primary immunisation with the vaccine. Clinical and haematological examinations were performed, and serum testosterone concentrations, testicular volume and sperm production were measured at baseline (D0) and every 4 weeks until week 40. Semen samples were collected using the manual method without an artificial vagina and subjected to the following

seminal evaluation tests: motility (MOT,%), vigour (VI), volume (VOL, mL), sperm concentration (SC, ×106/mL), total sperm count (TSC, ×106), viability (VIA,%) and sperm morphology (SM, % normal). After each semen collection, testicular volume was calculated using the modified sphere equation. A blood sample was also collected to perform a complete blood count and measure testosterone concentrations by electrochemiluminescence immunoassay. All animals were included in a government breeding control program and were surgically castrated at the end of the study. Data were analysed using ANOVA with SAS GLIMMIX.

No significant differences were observed in seminal parameters between days 0 and 28 (MOT: 90.63 ± 3.16 vs. 90.71 ± 2.02; VI: 4.38 ± 0.26 vs. 4.5 ± 0.29; VOL:3.01 ± 0.67 vs. 2.60 ± 0.70; SC:307.5 ± 73.94 vs. 305.71 ± 115.78; TSC: 760.12 ± 162.77 vs. 679 ± 197.12; VIA: 90.38 ± 2.94 vs. 83.78 ± 2.91; SM: 88.21 ± 1.62 vs. 85.23 ± 2.39). Azoospermia was observed on week 8 and persisted until week 36, when sperm reappeared in the ejaculate. By week 40, the seminal parameters showed no significant differences compared to days 0 and 28 (MOT:85 ± 7.64; VI:4.33 ± 0.33; VOL:2.16 ± 0.73; CON:336.66 ± 78.81; ET: 828.33 ± 382.76; VIVOS:86.33 ± 4.18; ME:72.5 ± 2.04). Testicular volume decreased by 30.83% at week 4, 57.47% at week 8, and 13.12% at week 40 compared to the initial value. Additionally, a progressive reduction in serum testosterone concentration was observed from day 0 to week 20, when the lowest values were reached (6.11 ± 0.92 ng/mL; 0.68 ± 0.29 ng/mL, respectively $p < 0.001$). Serum testosterone increased after week 20, reaching a concentration similar to week 4 by week 40 (4.4 ± 0.69 ng/mL; 5.66 ± 1.08 ng/mL, respectively, $p > 0.5$). All haematological parameters remained within the reference range for the species. Regarding testicular morphology all samples showed a high percentage of tubules with tailed spermatids (34.28%) and mature spermatids (45%). The average number of Leydig cells recorded in the microscopic fields used to count 20 cross-sections of tubules was 565.85 ± 37.31.

Administration of two doses of the anti-GnRH vaccine Improvac effectively suppressed spermatogenesis in canines. This effect was achieved by reducing testosterone production, testicular size, and sperm production for longer compared to what was obtained by other authors using a single dose (1).

References:

1) Ajadi y Gazal., Nigerian journal of physiological sciences 2016; 31 (1): 37–42.

6275 | Prolong the contraceptive effect of an anti-GnRH vaccine by revaccination

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Cats present a significant social and economic challenge, contributing to spreading of diseases and their proliferation as they roam through urban and suburban environments. Recent studies showed that Improvac is effective in reducing spermatogenesis and preventing the onset of heat in cats for 10 months (1). This study aimed to evaluate the application of a dose of the anti-GnRH vaccine (Improvac) 10 months after primary immunisation with the vaccine to prolong the suppressive effect of reproductive activity in queens.

Eighteen clinically healthy adult queens, aged 2 to 5 years, were selected from owners seeking reproductive control at the University Veterinary School Hospital. On August, all queens received in estrus or interestrus two doses of anti-GnRH vaccine (Improvac, Zoetis Belgium SA, 0.5 mL sc.) twice 4 weeks apart (primary immunisation), with a booster dose of the same vaccine given 10 months later (third doses [TD]). After the third dose, all queens were monitored every 4 weeks for 16 months. Cats underwent a physical examination, and blood samples were collected in a dry test tube, centrifuged, and serum was stored at—20°C until progesterone and estradiol concentrations were measured by electrochemiluminescence immunoassay; hemogram samples (0.5 mL) were collected into EDTA tubes and processed in a semiautomatic hematologic analyser. Heat behavioural signs (presence/absence) and vaginal cytology were recorded. Vaginal smears were obtained in each queen to determine the stage of the estrous cycle throughout the experiment. The estrous cycle stage was determined according to the percentage and type of cells present. All queens were monitored for pregnancy diagnosis using ultrasound (DP-30 Vet Mindray, Nanshan, China, multifrequency linear probe [5–7.5–10 MHz]). Data were analysed using ANOVA with SAS GLIMMIX.

Between 39 and 41 weeks after the second dose of Improvac, the queens showed estrous vaginal citology and behaviour. Serum oestrogen concentrations were within the physiological estrous concentrations of 39.78 ± 2.08 pg/mL, and serum progesterone concentrations were basal (0.5 ng/mL). Physical and ultrasound examinations were performed, and no pregnancies were recorded. At this time, queens received a third vaccine dose. One month post TD administration, queens displayed anestral vaginal cytology and serum oestrogen concentration of 11.44 ± 0.46 pg/mL. Ten queens resumed cycling 14 months after TD administration, while the remaining eight continued in anestrus until 16 months after TD. During the study, no significant differences were observed in haematocrit, haemoglobin, erythrocytes, leukocytes, total solids, segmented neutrophils, band neutrophils, lymphocytes, monocytes and eosinophils.

Administration of a third dose of the vaccine in queens resulted in a prolonged contraceptive effect lasting more than one reproductive season. These findings suggest that annual contraceptive treatment could be implemented alongside routine administration of the mandatory yearly rabies vaccine, since studies have shown that the rabies vaccine can be administered simultaneously with anti-GnRH vaccines without affecting antibody production (2).

References:

1) Garcia MF et al., Theriogenology 2025;235: 203–09.
2) Novak et al., Frontiers in Veterinary Science 2021; 8:650291.

6294/7786 | X chromosomal monosomy with bilateral testicles in a 6-month-old pitbull

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Sexual differentiation in mammals depends on the consecutive establishment of chromosomal, gonadal and phenotypic sex.

Abnormalities in the chromosomal sex, such as XXY or XO syndromes, lead to anatomical abnormalities of the reproductive organs.
A 6-month-old non-spayed bitch was referred to our Teaching Hospital because of clitoris hypertrophy and urinary incontinence. The hypertrophy was detected at a very young age, but the owners report a significant increase in size during the last weeks. Concomitantly, the female owner has observed constant jumping behaviour towards her. The patient showed an enlarged penile-shaped clitoris emerging from the vulva. The urethra ended up in the dorsal and caudal aspect of the clitoris, following the anatomic urethral pathway of a male dog. No vestibular/vaginal lumen was present. Additionally, the patient presented two testicles located in each corresponding inguinal region.
The surgical intervention consisted of inguinal castration and clitoris amputation. On the contrary, the permeabilisation and reconstruction of the urethra consisted of anastomosing it to the vaginal vestibule with Poligliconate 4/0 non-traumatic suture material and 360° circumference simple interrupted sutures.
A DNA extraction was performed from a blood sample to determine the patient's genotype. To determine whether the patient carried the Y sex chromosome, PCR amplification was performed on two short fragments on the X (ChrX) and Y (ChrY) chromosomes. The amplified ChrY fragment was designed to be shorter than the ChrX fragment to minimise false negatives due to product length (112 bp vs. 190 bp for the respective primers). The individual's sex was automatically determined by capillary electrophoresis using a fluorescently labelled internal sex-specific primer from each pair. In this case, the amplification of ChrX and the absence of ChrY amplification indicate that the bitch did not carry the Y chromosome, being an XO individual. The karyotype evaluation allowed the diagnosis of an X chromosomal monosomy in this patient. According to the literature, this abnormality is characterised by the presence of an enlarged clitoris, together with female reproductive organs, including ovaries or ovotestes. In the present clinical case, the patient had no female reproductive organs, other than the vulva itself. Additionally, the gonads were fully formed testes, and the urethra ended up on the dorsal aspect at the base of the enlarged clitoris.

6420/7941 | Postpartum depression: Can bitches also be affected?

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Postpartum depression is a well-known and complex condition affecting women during childbirth, characterised by emotional disturbances of varying intensity, which can lead to severe consequences such as suicide and infanticide (1). A multifactorial aetiology encompasses hormonal, psychological, and environmental factors (2,3). In contrast, its incidence in animals is poorly documented, with scientific literature primarily focused on zoo animals. However, maternal behaviours, such as rejection, aggression or even cannibalism towards puppies, have been reported in some dogs during the postpartum period, suggesting the existence of this condition also in the canine species (4). As an altricial species, the lack of maternal instinct of the bitch at birth may compromise the survival of the inept offspring. This study aimed to investigate the occurrence and incidence of postpartum depression-like phenomena in female dogs, focusing on predisposing factors and the most observed clinical and behavioural signs.
Data were collected through an online questionnaire to owners of bitches that had given birth at least once. The questionnaire consisted of five sections dedicated to anamnestic information, animal management, maternal behaviour, and reproductive data with specific reference to pregnancy and parturition. Maternal behaviour was deemed altered if aggression or hyper-attachment to puppies and/or extreme restlessness were recorded. Maternal behaviour (normal or altered) was then correlated with all the collected parameters using the SPSS statistic software ver. 29.0, employing chi-square tests for categorical variables and the Mann–Whitney U-test for continuous variables, with a significance level set at $p \leq 0.05$.
A total of 92 questionnaires were analysed that is, 90 purebred and 2 crossbreed dogs. Maternal behaviour was altered in 13.1% ($n = 12$) of bitches while 86.9% ($n = 80$) showed normal behaviour. Key findings include a higher neonatal mortality in litters of bitches with altered behaviour ($p = 0.025$) who also spent significantly less time in contact with their puppies compared to mothers with normal behaviour ($p = 0.008$). Many other variables such as breed, body weight, age of adoption, origin, environment, temperament, type of birth (natural or caesarean) and nursing (maternal or artificial), litter size and human presence at birth seem to play a role in the development of maternal behavioural disorders in bitches, although no significant differences emerged between mothers with normal and altered behaviour.
This study explored the complexity of factors involved in canine maternal behaviour, highlighting similarities between postpartum depression in women and maternal behaviour disorders in dogs. However, limitations related to the small number of dogs with maternal behavioural problems in our caseload and to the data collection based on the owner's perception, which often considers this topic a taboo, deserve further investigation. Knowing and recognising this disorder would allow to optimise care during pregnancy and parturition, enhancing the well-being of both the mother and the puppies.

References:

1) Gelaye et al., Lancet Psychiatry 2016; 3(10): 973–82.
2) Prendergast and Austin, Australas Psychiatry 2001;9:255–9.
3) Bloch et al., J Clin Psychiatry 2012;73: 235–41.
4) Garcia et al., Int J Vet Sci Med 2019;7(1):20–30.

7009/7854 | A large cystic granulosa cell tumour and segmental aplasia of the uterus in a golden retriever

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A 4-year-old Golden retriever bitch was referred to the University Animal Hospital (UDS) for ultrasound due to urinary problems.

It was not known when the bitch was last in heat. Ultrasound revealed cystitis and abnormal uterus and ovaries.
Surgery: A midline celiotomy was performed and a 8 ×20 cm large winding fluid filled reddish structure was found at the location of the left ovary. The structure was partly located in the right side of the abdomen. The fluid filled structure continued next to the suspensory ligament and left kidney to the abdominal wall and close to vena cava. The right ovary was small, approx. 0.5 cm. Both uterine horns were partly smaller and thinner, from approx. 3 cm from cervix of the right uterine horn, and approx. 5 cm from cervix of the left uterine horn. The uterine body and cervix appeared normal. The uterus and ovaries were removed in according to routine and sent for pathology.
Pathological examination: The large winding structure contained brownish fluid and contained areas of solid structures and some areas with bleeding. The cystic, enlarged ovary consisted of a cystic tumour with proliferation of round to oval shaped cells. The tumour cells formed follicular cystic formations. The tumour cells showed low pleomorphism and the number of mitotses were low to moderate. No signs of malignancy were found. The right ovary was small, approx. 0.5 cm and contained a small cyst with clear fluid. Both uterine horns had areas with very thin wall with a sharp border between normal and abnormally thin uterine horns. The thinner parts of the uterus had no endometrium. The small right ovary was examined histologically. It was inactive, but with normal structures. The normal parts of the uterus had cystic endometrial hyperplasia (CEH). The smaller/thinner parts of the uterine horns consists of a thin layer of smooth muscle and no endometrium (aplasia).
Diagnosis: Granulosa cell tumour. Segmental aplasia of the uterine horns, and CEH of the normal parts of the uterine horns.
Granulosa cell tumours of this size and appearance are not common. Segmental aplasia of the uterus is a rare finding in dogs, and other urogenital malformations may be found at the same time. This dog had recurrent urinary problems but no malformations of the urinary tract were detected. No more diagnostic tests were performed. The dog recovered uneventfully from surgery.

References:

1) Troisi et al., Theriogenology 2023;15:210:227–33.
2) Kim et al., J Vet Med Sci 2012;74(3):385–9.

7069/7862 | Tick-borne encephalitis virus associated with foetal death in a bitch

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In May 2022, a six-year-old beagle bitch experienced fetal death diagnosed by ultrasonography from day 49 after last mating. She had had two previous litters after normal parturitions, the last one 2½ years previously. By caesarean section on day 56, one live pup and three dead foetuses in different stages of resorption were delivered. Black mucoid, non-smelling fetal membranes surrounded the dead foetuses. The live-born pup died shortly after birth and was sent for autopsy together with the placenta. No malformations or inflammatory changes were present in the pup. Scattered areas of necrosis were present in the placenta, with dystrophic mineralisation and suspected microthrombi. Selective bacteriological cultures for *Brucella canis* from blood, vagina and the pup were all negative. Samples from the pup were analysed with PCR for canine herpes virus (CHV) and found negative. Viral metagenomics analysis was performed from placental tissue and from kidney, liver, lung and spleen of the pup. Tick-borne encephalitis virus (TBEV) was identified in the placental tissue. In situ hybridisation for TBEV was performed on placental tissue and TBEV was identified in the trophoblasts. A serological investigation using an ELISA for flaviviruses and a virus neutralisation test for TBEV was performed on a blood sample from the bitch, taken at the time of the caesarean section, and found positive.
For the first time, a case of TBEV in a dog associated with fetal death in late pregnancy is described. Different stages of pregnancy arrest indicate that there was a vertical transmission of the virus, in contrast to a systemic infection, where the dam and the foetuses are affected at the same time. In dogs, infections with TBEV are often subclinical but TBEV is considered an important infectious cause of neurological disease in endemic areas (1).
The main transmission route for TBEV is via ticks. *Ixodes ricinus* is an endemic tick in Sweden and it increases in abundance and also expands geographically. The owner described that the bitch had been repeatedly infested with ticks, thus this is the most likely route of infection, with following intrauterine transmission to the pups. The bitch was mated again 1½ year after the caesarean section and delivered five healthy pups. The general condition of the bitch continued to be good, with no further signs of disease.
The aetiology of abortions and stillbirths in dogs is often not identified, and the role of TBEV in these cases is not known. With the spread of both ticks and TBEV, infection with TBEV may be an increasingly common cause of fetal death in the dog. However, even if vertical transmission of TBEV leading to reproductive disturbances may occur, it is possible that in areas where TBEV is endemic, most dogs would be exposed and immune before their first pregnancy.

Reference

1) Kleeb et al., Front Vet Sci 2021; 8:782044.

7165/7882 | Neonatal health in puppies: A retrospective evaluation of congenital malformations and survival outcomes at Vetmeduni Vienna, Austria in the last 23 years

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Congenital malformations in neonatal puppies affect survival and long-term health. In this study, the prevalence, risk factors, and trends in neonatal malformations of dogs over a 23-year period were retrospectively analysed. We aimed to assess the impact of breed, litter size, mode of delivery, and foetal age at presentation on the occurrence of malformation. It was

hypothesised that improved diagnostic methods have increased the detection rate and that mortality increases in larger litters.
Data were collected from the Animal Hospital Information System (TIS) at Vetmeduni Vienna and were recorded between 2001 and 2024. Of the 5050 puppies identified, 780 (15.4%) were excluded because they were not presented to the hospital (e.g. healthy littermates of diseased puppies). The remaining 4270 puppies were categorised according to being presented because of illness ($n=954$, 18.9%), assisted vaginal birth ($n=709$, 14.0%) or birth by Caesarean section ($n=2602$, 51.5%). The puppies belonged to 161 different breeds including mixed breed, with Chihuahua ($n=363$, 7.2%) being the most common breed, followed American Staffordshire Terrier ($n=235$, 4.7%) and Cocker Spaniel ($n=224$, 4.4%). The analysis focused on congenital malformations and related survival outcomes.
Of the 4270 puppies, 138 (3.2%) had congenital malformations, consistent with existing literature on puppies (1). A total of 34 malformations were found, with cleft palates being most frequent ($n=44$; 1.0%) followed by gastroschisis ($n=14$; 0.3%) and cleft lip ($n=11$; 0.3%). The percentage of malformed puppies differed by breed ($p<0.001$; only breeds with >20 puppies included) and was the highest with 16.3% (7/43) in Bullmastiffs, 8.3% (2/24) in Cane Corso Italiano and 6.9% (4/58) in Malinois. Among the 1344 litters analysed, 120 (8.9%) had at least one affected puppy. Of the 138 affected puppies, 101 (73.2%) died or were euthanised, with 78 (53.1%) dying on the day of birth.
This study confirms the significant role of congenital malformations in neonatal puppy mortality. The findings also highlight the importance of recognising breed predispositions. Based on these results, it is recommended that owners submit any deceased puppies to pathological examination to determine the cause of death and any breed-specific predispositions. Veterinarians should thoroughly examine puppies for malformations, and data should be regularly analysed to improve early detection and intervention strategies, eventually reducing neonatal mortality.

Reference

1) Pereira et al., Theriogenology 2019;140:52–57.

6523/7787 | A case of hydrocolpos secondary to an imperforate hymen in a queen

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Hydrocolpos is an uncommon condition in dogs and is extremely rare in cats. It results from embryological anomalies, such as imperforate hymen, vaginal atresia, aplasia or occlusion by masses and polyps, further leading to fluid retention. Developmental defects of the paramesonephric ducts are rare in felines, with most cases involving uterine segmental aplasia. Literature on congenital anatomical defects in the feline reproductive tract is limited. This report describes a case of hydrocolpos and hydrometra in a queen due to an imperforate hymen, successfully treated with laser ablation.
A 3-year-old intact female European Shorthair cat was referred due to anorexia and poor body condition. The cat had been rescued from the street, and its medical history was unknown. On presentation, the cat had a 3/9 body condition score, nausea, and 5% dehydration. Vaginal inspection and palpation did not reveal anatomical abnormalities. Haematology showed mild neutrophilic leukocytosis with Döhle bodies and monocytosis. Biochemistry was unremarkable, while urinalysis documented a specific gravity of 1.033 and >100 red blood cells per high-power field. Abdominal ultrasound revealed anechoic content within both uterine horns, compatible with pyo-, hydro, muco- or hemometra, and a tubular structure extending ventrally to the colon and dorsally to the urinary bladder, suggestive of hydrocolpos. A computed tomography scan confirmed generalised distension of the uterine horns and vagina with hypoattenuating content. Caudal distension was evident up to the vestibule without visible obstruction, although a soft tissue attenuation band could not be excluded. On the day of surgery, a vaginoscopy with a 2.7 mm optic was performed and revealed the urinary meatus in a dorsal position and an imperforate vaginal wall ventrally. A 550-µm diode laser was introduced with a power and pulse rate of 7 W until the vestibulo-vaginal junction opened. A continuous flow of semitransparent liquid confirmed permeabilisation. Microbiological cultures of the vaginal and uterine content were performed, yielding a negative result. The analysis was consistent with an aseptic exudate, showing 0.02/0.02 M/uL erythrocytes, 13.79/14.07 K/uL nucleated cells, 6.42/7.75 K/uL granulocytes (46.6/55.1%), and 7.13/6.13 K/uL agranulocytes (51.7/43.6%) for uterine and vaginal content respectively. Cytology was also performed. Both samples were characterised by a clear background with abundant squamous superficial cells, followed by intermediate and some basal cells. No neutrophils or erythrocyte cells were seen.
This case describes congenital hydrocolpos in a queen due to the retention of epithelial fibrous tissue at the vestibular-vaginal junction (imperforated hymen). The similarities between vaginal and uterine fluids demonstrate that the hydrometra was a consequence of the hydrocolpos. Given these results, hydrocolpos should be considered in the differential diagnosis of uterine content accumulation in female cats.

6547/7798 | Atypical metastases of feline mammary carcinoma in the reproductive organs

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Two 13 year-old non-neutered European Shorthair female cats with history of contraceptive administration were presented

to the veterinary medical care center due to the presence of mammary masses. Tissue samples from mammary gland and reproductive organs (ovaries and uterus) were submitted for histopathological analysis. One queen presented a grade III comedocarcinoma in the mammary gland with vascular invasion and with micropapillary metastases in one of the ovaries and in one uterine horn. Multiple cysts were also observed in both ovaries, and grade I endometrial cystic hyperplasia and polyps were found in the endometrium. Histopathology of the other queen revealed presence of different histotypes of mammary carcinomas, namely grade II tubulopapillary carcinoma, grade I tubular carcinoma and grade III micropapillary carcinoma (comedocarcinoma type), with vascular invasion and lymph node and unilateral ovarian metastasis. Bilateral ovarian cysts and grade I endometrial cystic hyperplasia were also observed. Discussion Mammary gland tumours are among the most common neoplasms in cats, with 80 to 90% being malignant and showing metastatic potential. Radical mastectomy (associated or not with adjuvant chemotherapy) is the treatment of choice in most cases of feline mammary tumours, and ovariohysterectomy/ovariectomy is frequently performed in the same surgical procedure. Nonetheless, reproductive organs are not routinely submitted to histopathology analysis, unless macroscopic changes are observed. Feline mammary carcinoma metastasis (stage IV) occurs predominantly in lungs and pleura, and reports addressing atypical metastatic locations and their clinical implications remain scarce. Metastasis of mammary tumours to the reproductive organs is rarely documented in veterinary literature, underscoring the relevance of analysing cases that illustrate this occurrence. The reported cases demonstrate the possibility of metastasis of feline mammary carcinoma to the reproductive organs, an uncommon finding that should be considered in the diagnostic and therapeutic approach of these tumours. This report also emphasises the importance of submitting all sampled reproductive tissue for histopathological examination in female cats diagnosed with mammary tumours. Further studies are needed to better understand the mechanism of tumour dissemination and its clinical implications.

7064/7863 | A suspected case of venereal transmission of *Babesia canis* in dog: A case report

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A 7-year-old, intact male Border Collie presented with progressive signs of weakness, pallor, icterus and dark red urine. Anaemia, leukopenia, elevated ALT and CRP in addition to high bilirubin in serum and urine were the most significant findings in blood and urine analysis. Diagnosis of large babesia was made by microscopic examination of a blood smear stained with modified May-Grünwald Giemsa and further confirmed with PCR targeting the 18 s rRNA.
The dog had no travel history to areas where *B. canis* is endemic for 2 months and was on both acaricidal and repellent compounds. However, the only physical contact that the dog had was natural mating with a Border Collie bitch from Austria both 6 and 5 days prior to symptoms. According to the owner of the bitch, mating was normal, but the male dog had slight bleeding from the penis while attempting penetration prior to copulation. The bitch started presenting same signs of disease 3 days later and was found also to be positive for large babesia in both microscopical examination of a blood smear and in PCR. Both dogs made a full recovery after treatment with imidocarb dipropionate two times, 2 weeks apart. In addition, gestation was affected neither by the disease nor by the medications. The bitch gave birth to six healthy puppies. A blood sample was taken from two of the puppies before the age of 3 months and it was negative for babesia in both blood smear and PCR.
Babesiosis is a hemoprotozoan illness widely distributed in Europe (1). Main vector of *Babesia canis* in central Europe is an ornate dog tick *Dermacentor reticulatus* (2). Other known modes of transmission for protozoans of genus Babesia are transmission directly by blood transfusion or by direct contact between dogs through wounds (3). There have also been a few cases of vertical transmission documented (4) but no venereal transmission has been reported in any species to this date.
Based on the timing of acute *B. canis* infection on both dogs we suspect a venereal transmission. Although we cannot rule out simultaneous tick borne babesiosis of both dogs we consider it highly unlikely since neither *B. canis* nor the main vector *D. reticulatus* is endemic to Finland. The probability of acquiring simultaneous infection from two different *B. canis*-infected *D. reticulatus* ticks is very low even if both dogs would have been in endemic region of Austria. Compared to this situation where the bitch would have gotten babesiosis from Austria and brought another tick to Finland transferring it to the male dog it is even less likely. The incubation period from the tick bite to the symptoms in the male dog would also have been uncharacteristically short (5 to 6 days). Therefore, it is more likely that this is a case of venereal transmission of Babesiosis following a natural mating.

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7166/7883 | Neonatal health in kittens: A retrospective evaluation of congenital malformations and survival outcomes at Vetmeduni Vienna, Austria in the last 23 years

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Congenital malformations in neonatal kittens affect survival and long-term health. In this study the prevalence, risk factors, and trends in neonatal malformations of cats over a 23-year period were analysed retrospectively. We aimed to assess the impact of breed, litter size, mode of delivery, and foetal age at presentation on the occurrence of malformation. It was hypothesised that improved diagnostic methods have increased detection rate and that mortality is increased in larger litters. Data were

collected from the Animal Hospital Information System (TIS) at Vetmeduni Vienna and were recorded between 2001 and 2024. Of the 1261 kittens identified, 335 (26.6%) were excluded because they were not presented at the hospital (e.g. healthy littermates of diseased kittens). The remaining 926 kittens were categorised according to being presented because of illness ($n=377$; 29.9%), assisted vaginal birth ($n=66$, 5.2%) or birth by Caesarean section ($n=483$; 38.3%). The kittens belonged to 22 different breeds including mongrels, with European Shorthair ($n=434$; 34.4%) being the most common breed, followed by British Shorthair ($n=224$; 17.8%) and Maine Coon ($n=205$; 16.3%). The analysis focused on congenital malformations and related survival outcomes. Of the 926 kittens, 45 (4.9%) had congenital malformations, consistent with existing literature in puppies (1). A total of 23 malformations were found, with cleft palates being most frequent ($n=13$; 1.4%) followed by gastroschisis ($n=6$; 0.6%) and dysmelia ($n=5$; 0.5%). The percentage of malformed kittens differed by breed ($p<0.001$; only breeds with > 20 kittens included) and ranged from 8.6% (14/163) in British Shorthair cats to 0.0% (0/28) in Birman cats. Among the 369 litters analysed, 50 (13.6%) had at least one affected kitten. Of the 57 affected kittens, 45 (78.9%) died or were euthanised, with 33 (57.9%) dying on the day of birth. Kitten mortality was not significantly affected by litter size. The percentage of diagnosed malformed kittens increased over time with 2.4% in 2001–2013 and 7.0% in 2020–2024.

This study confirms the significant role of congenital malformations in neonatal kitten mortality. The findings also highlight the importance of recognising breed predispositions. Based on these results, it is recommended that owners should submit any deceased kittens to pathological examination to determine the cause of death and any breed-specific predispositions. An increased detection rate over time indicates that veterinarians should thoroughly examine kittens for malformations. Such data should be regularly analysed to improve early detection and intervention strategies, eventually reducing neonatal mortality.

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6002/7769 | Antioxidant characterisation of zinc–binding proteins isolated from canine seminal plasma

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Numerous studies have demonstrated that zinc (Zn2+) possesses antioxidant properties and plays a crucial role in scavenging reactive oxygen species (ROS) (1,2). Zinc-binding proteins (ZnBPs) have been identified in canine seminal plasma (SP) (3). Previous analyses confirmed that the addition of ZnBPs isolated from dog SP to an incubation mixture of ejaculated dog sperm, stored at 5°C, contributed to the maintenance of motility parameters and provided protective effects on the sperm membranes subjected to cold shock conditions (4). Based on these observations, we hypothesised that ZnBPs possess antioxidant properties. Therefore, the objective of this study was to examine whether ZnBPs of the canine SP exhibit antioxidant properties. This was achieved through the assessment of the following: a) superoxide dismutase (SOD) activity and b) glutathione peroxidase (GPx) activity with the use of a Randox kit, c) total antioxidant status (TAS) with the use of a TAS assay kit (Randox Laboratories Ltd., Crumlin, UK; Randox Laboratories Ltd., London, UK).

The study material consisted of 12 semen samples collected from six mixed-breed dogs (aged 4–6 years) with the use of the manual method. The SP was obtained through centrifugation of the semen twice: first at 3000 rpm for 5 min, then at 10,000 rpm for 10 min. ZnBPs were isolated with the use of affinity chromatography on Chelating Sepharose Fast Flow-Zn^{2+} bed, according to Mogielnicka-Brzozowska et al. (3). The antioxidant activity was characterised in both the seminal plasma and ZnBPs isolated from the dog SP with the use of spectrophotometric methods with a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter Inc., USA). All results were expressed as means ± standard error of the mean (SEM) and were considered statistically significant at $p<0.05$ (Tukey multiple comparison test) with the use of the Statistica software package (StatSoft Inc., Tulsa, OK, USA).

It was demonstrated that ZnBPs isolated from the dog SP exhibit enzymatic activity of SOD and GPx (26.0 ± 0.2 U/mL and 0.015 ± 0.003 U/mL, respectively), as well as a total antioxidant status (TAS) of 274 ± 0.01 µM. These values were statistically significantly different ($p<0.05$) from those obtained in whole SP, which were as follows: SOD: 52.0 ± 4.1 U/mL; GPx: 4.27 ± 0.2 U/mL; and TAS: 460 ± 6.0 µM.

The results obtained suggest that: (I) ZnBPs in the dog semen may play a crucial role in protecting sperm from oxidative stress, which is one of the primary factors affecting their viability, motility and fertilising capacity; (II) The differences in antioxidant content are primarily due to the distinct biochemical composition of whole seminal plasma and the isolated ZnBPs.

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6326/7824 | Surgical management of acquired phimosis in a 6-month old leonberger puppy: A case report

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Phimosis is a rare condition in dogs, characterised by the inability to exteriorise the penis due to a narrow or absent preputial ostium. This condition can be congenital, resulting from abnormal preputial development, or acquired, as a consequence of inflammatory, neoplastic, cicatricial, or traumatic processes (1). In neonates, acquired phimosis may develop due to preputial trauma, often caused by excessive maternal licking or mutual genital suction among littermates (2). If left untreated, phimosis can lead to severe complications, such as urinary retention and preputial distension, necessitating surgical intervention. The

treatment of choice for phimosis is the surgical restoration of the preputial ostium (3). This case report aims to document and highlight the importance of early diagnosis and surgical management of acquired phimosis.

A 7-week-old male Leonberger puppy was presented to the reproduction service with a history of stranguria and a preputial wound. The puppies were being bottle-fed and frequently manifested genital suction among littermates. A surgery was proposed but the owner opted to manage it by voiding the prepuce at home using a catheter. The puppy was presented again only at the age of 6 months with an excessive granulation tissue formation, stenosis of the preputial ostium, and significant distension of the preputial cavity. Catheterisation became increasingly difficult, although the patient remained clinically stable, with no haematological or systemic alterations. A surgical excision of the excessive granulation tissue was performed, followed by posthioplasty for reconstruction of the preputial ostium. Under the surgery, the puppy did not present any adhesions between the penis and preputial mucosa.

The puppy was examined 1 month after the surgery, being in good general condition, with no difficulty in urinating or exteriorising the penis. The surgical procedure was successful in restoring normal preputial function.

This case highlights the rapid progression of acquired phimosis in neonates and underscores the importance of veterinary monitoring, particularly in orphaned or bottle-fed litters, to prevent complications. Early identification and correction of congenital or acquired alterations, such as excessive maternal licking or sibling-induced suction, is crucial. Surgical intervention, although relatively simple, must be performed cautiously to avoid excessive tissue removal, which may lead to paraphimosis.

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6324 | Ovarian remnant syndrome in dogs: A case emphasising the need for a comprehensive diagnostic approach

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Ovarian remnant syndrome (ORS) refers to failure of removing complete ovarian tissue during the gonadectomy of a bitch, characterised by clinical signs of the estrous cycle similar to that of intact females. A 2-year-old German shepherd female of 34.4 kg was presented with recurrent vaginal blood loss. She was spayed at the age of one, due to vulvar blood loss for 4 weeks. An ovariohysterectomy (OVH) was performed as an abnormally enlarged uterus was noted during surgery. However, serosanguineous vulvar discharge was again noted 8 months after the OVH. At first, she received antimicrobial treatment, and the discharge stopped after 2–3 weeks. However, due to recurrence again 7 months later and pollakiuria, she was referred to a local veterinary hospital. On clinical exam no abnormalities were detected, only a small amount of vaginal discharge. Blood analysis was performed and the results of the haematological and biochemical examinations showed all values within reference ranges. A urine sample was collected by cystocentesis which turned out to be normal. As results were unremarkable, abdominal ultrasound was performed and revealed a lobulated hypoechogenic structure of 1.5 cm diameter caudal to the left kidney. To confirm the suspicion of ORS, blood was collected and went to an external laboratory for Anti-Müllerian Hormone (AMH) assessment. Unexpectedly, AMH serum concentration was below 0.03 ng/mL, a value associated with the absence of ovarian tissue. The female dog was then referred to the Veterinary Teaching Hospital. By this time vaginal bleeding was present for about 20 days. During the clinical examination, she had a temperature of 38.1°C, a body condition score (BCS) of 3/5, with a heart rate of 112 beats per minute, all within normal limits. Abdominal palpation revealed no abnormalities, and the mammary glands were not swollen or lactating. The vulva appeared within normal dimensions with a mild vulvar discharge. A deep vaginal swab was collected and stained using Diff-Quick. It showed keratinised epithelial cells (20% nucleated and 80% anuclear), with some red blood cells, while the serum progesterone level was 2.98 ng/mL. During laparoscopy bilateral stump tissue was removed. Histological examination confirmed the removed tissue caudal of the left kidney to be ovarian tissue.

Discussion: Diagnosing ORS remains a significant challenge in clinical practice, particularly when serum AMH levels seem inconsistent with the presented clinical signs or other diagnostic test results. Surgeries performed under suboptimal circumstances may contribute to deficiencies in the spaying technique, which has been recognised as the most significant factor in the development of ORS. In this clinical case, a diagnosis of oestrus due to ovarian remnant syndrome was made using hormonal tests, vaginal cytology and medical imaging. This case report calls attention to the importance of a thorough anamnesis with clinical work up including different tests, as a single parameter, such as AMH concentration, may provide misleading results. Specifically, it emphasises the need for further research into diagnostic tests and their threshold values to ensure a definitive diagnosis in cases where hormonal test results are inconsistent or unclear.

5357/7773 | Female domestic cats do not cease reproductive cycling under a prolonged 6:18 L/D lighting program

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Manipulation of the reproductive activity of the domestic cat (*Felis silvetris catus*) depends on a thorough understanding of the effect of exogenous light. The objective of this study was to test the reproductive effect of a prolonged short photoperiod (SP) in queens.

Five 3 years of age, short—hair female cats were housed free in a windowless, enriched room of 15 m2 with LED lamps. Light was automatically provided with an intensity at cat's eye level of 250

luxes. After a 10:12 L/D adaptation period of 3 months, the females were exposed to a SP of 6:18 L/D for 6 months. The queens underwent a reproductive follow-up which included 2 h per day observation of sexual behaviour (0,+,++), every other day vaginal cytology (> or < 80% superficial cells). The beginning and the end of the heats, defined as > + and > 80% superficial cells, were recorded. The findings of two time- windows (TW) of 3 months each were compared by Student's t and Chi2 tests. This project was approved by the institutional IACUC (123–3-22 T).

Overall number of days in heat (40.20 ± 9.80 vs. 54.40 ± 12.16; $p > 0.1$), the number of heats (8.00 ± 1.58 vs. 7.00 ± 0.63; $p > 0.1$) and the duration of those heats (5.16 ± 0.68 vs. 7.77 ± 1.44; $p > 0.1$) were not different between the first and second TW. Conversely, the intensity of the heats increased in the second TW (1.33 ± 0.03 vs. 1.53 ± 0.03; $p < 0.01$). Contrary to what was expected, reproductive cyclicity was maintained under 6 h of light per day.

These results refute the previous feline paradigm that 8 h of light per day are insufficient for reproductive activity (1,2) and suggest the presence of an internal reproductive cycle as it has been described in ewes (3) and also hypothesised in cats. Further assessment of ovulatory capacity and fertility under this SP is guaranteed.

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5430/7314 | Melatonin serum concentrations using an ELISA kit in the domestic cat

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Melatonin (N-acetyl-5-methoxytryptamine), a hormone primarily produced by the pineal gland, plays a central role in regulating mammalian seasonal reproduction. Circadian melatonin serum concentrations in domestic cats (*Felis catus*) were first reported 40 years ago using radioimmunoassay (RIA; 1)." Since then, limited research has explored the role of melatonin in feline reproduction (2). More recently, RIA has been being replaced by safer immunoassays like enzyme-linked immunosorbent assays (ELISA) for hormone measurements. Since melatonin is highly conserved across species, various multispecies ELISA kits are commercially available. The aim of this experiment was to report melatonin serum concentrations in domestic cats using one of these kits.

Sixty-one female and 18 male short-hair cats of 1.5 to 2.5 y of age, belonging our institutional cat colony were included. The females and males were housed separately and free in light -proof, rooms with LED lamps (1200 lm, 6400 K, AR111, dimmable, Argentina). Light was automatically provided from 6:00 AM with an intensity measured by a digital luxmeter (Schwyz SC105, Argentina) at cat's eye level, of 250 luxes. The animals were fed premium cat food and water ad libitum. This project was approved by the institutional IACUC (123–3-22 T). The cats were exposed to different photoperiods ranging from 6:18 L/D to 16/8 L/D during a period of sampling.

Blood samples were drawn in lateral recumbency by peripheral venipuncture between 5 and 11 h am, under a red monochromatic light of < 5 luxes (Phillips PF712E 15 W) when in the dark phase. Serum was obtained after centrifugation of the samples at 3200 rpm (600 g) for 15 min at 4°C and stored frozen at −70°C until melatonin measurements.

Melatonin determination was performed by a multispecies ELISA colorimetric competitive immunoassay (USBiological, Tucker, GA, USA) according to the manufacturer instructions. The assay was validated for the measurement of domestic cat melatonin by serially diluting cat serum sample in the kit diluent and demonstrating parallelism with the standard curve. The sensitivity and the intraassay CV of the kit were 4.53 pg/mL and < 10%, respectively. Normality of data distribution was tested using the Shapiro–Wilk test and homogeneity of variance by Levene's test. Descriptive statistics was carried out and expressed as mean ± SEM.

Mean melatonin serum concentrations in all the females sampled were 977.77 ± 35.41 pg/mL with a range of 492.91 to 1483.51 pg/mL. The same values for males were 882.68 ± 28.17 pg/mL and 663.40 to 1092.73 pg/mL, respectively.

This study provides preliminary data on domestic cat melatonin concentrations using a commercial ELISA assay. Further standardisation of modern measurement methods and establishment of reference values under different reproductive stages and environments are still necessary for this hormone in this species.

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6176/7489 | Gene expression of Anti Müllerian hormone in queens during breeding and non-breeding seasons

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Anti-Müllerian hormone (AMH) is a 140 kDa glycoprotein belonging to the transforming growth factor-β (TGF-β) superfamily (1). It is synthesised by preantral and small antral follicles in the ovaries (2). In seasonal breeding species, photoperiod may influence AMH concentrations and expression (3,4); however, its seasonal regulation in cats remains unknown. This study aimed to investigate AMH gene expression in feline ovarian tissue during the breeding and non-breeding seasons. Twelve healthy, non-pregnant female cats (5–18 months old) undergoing routine ovariohysterectomy at veterinary centers between October 2023 and May 2024 were included. Ovaries were categorised based on the season of collection: non-breeding season (autumn-winter) ($n = 14$) and breeding season (spring–summer) ($n = 10$). Total RNA was extracted using the GeneJET RNA Purification Kit and reverse transcribed into cDNA with the

SuperScript First-Strand Synthesis System. Relative AMH gene expression was analysed using quantitative PCR (qPCR), with species-specific primers (5). The expression levels were compared between seasons using an unpaired two-tailed Student's t-test ($p < 0.05$).

AMH was expressed in ovarian tissue throughout all the seasons, but its expression was significantly higher in the non-breeding than the breeding season ($p < 0.05$).

This is the first study to evaluate AMH expression in domestic cats using qPCR, providing new insights into the transcriptional regulation of AMH in response to seasonal changes. These findings show that AMH gene expression diminishes during the breeding season probably due to the predominance of antral follicles which do not express this hormone.

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7067/7839 | A vertical vaginal septum extending almost the entire length of the vagina in a three-year-old Polish Hunting Spaniel: A case report

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A 3-year-old Polish Hunting Spaniel bitch was presented to the Department of Reproduction, Clinic for Small Animals in Wroclaw in July 2024. The reason for the appointment was repeated failed matings, both natural and via vaginal insemination, despite the procedure being performed at the optimal time based on progesterone level monitoring. During the interview, the owner reported that the bitch experienced pain during natural mating attempts. Ultrasonographic examination revealed a clearly visible cervix located under the bladder. In the transverse section, the cervix appeared in such a way that a mirror image was observed below. The uterus displayed a typical anatomical course, with a visible uterine body, bifurcation, and both uterine horns. However, the ovaries were difficult to visualise. To further investigate the cause of infertility, an endoscopic examination was performed. This revealed the presence of a vaginal septum at the transition from the vaginal vestibule to the proper vagina, near the urethral opening. The septum divided the vaginal canal into two separate passages. Both vaginal canals appeared similar, with well-defined medial dorsal folds and distinct cervical openings. An MRI was performed for a more accurate diagnosis. At the transition from the vaginal vestibule to the proper vagina, a septum separating the vaginal canal into two was observed as in the endoscopic examination. Right part of the canal was wider than the left part. The width of the fold completely dividing the vagina into two canals was about 27 mm. Caudally towards the cervix the fold passed into a partial septum on the dorsal side, in the midline.

No other abnormalities affecting the reproductive system were observed. Given these findings, an ovariohysterectomy is scheduled for April 2025 to prevent potential complications and improve the bitch's quality of life. Because of the size of the septum, removal will be considered if signs of inflammation or urinary incontinence appear.

Typically, the thin membrane separating the vagina from the vestibule regresses during puberty (1). However, in some cases, remnants of the hymen persist, leading to partial or complete obstruction of the vaginal lumen. Vaginal septa can result in varying degrees of clinical symptoms, ranging from mild discomfort to severe reproductive and urinary issues, including dysuria, urinary incontinence, recurrent vaginitis, urinary tract infections, natural breeding difficulties, infertility and dystocia (3). Some vaginal septa can be easily detected through routine vaginal examination, while others require vaginoscopy for accurate diagnosis and measurement of the septum (2). In cases where the septum is extensive, appearing to create a double vagina, advanced diagnostic imaging techniques such as MRI are necessary for a thorough assessment.

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6533 | The value of risk factors, clinical signs, blood profiles, estradiol levels, ultrasonography, and histopathology in canine testicular disorders

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Testicular tumours are the most common testicular disorders and reproductive tumours in male dogs. Other abnormalities include orchitis and testicular atrophy. Tumours are categorised into interstitial cell (Leydig) tumours, Sertoli cell tumours and seminomas, originating from Leydig, Sertoli and germ cells, respectively (1). Multiple tumour types may co-occur. Though the cause remains unclear, factors like age, breed and cryptorchidism increase tumour risk (2). Diagnosis involves history, physical examination, laboratory tests, ultrasonography and histopathology. Ultrasonography is vital for evaluating size, location and structure of testicular masses (3). This study aims to assess associations among risk factors, clinical signs, haematological findings, estradiol levels and ultrasonographic finding with tumour types.

A retrospective analysis was conducted at the Theriogenology Center, Kasetsart University Veterinary Teaching Hospital, from 1 January 2019 to 31 December 2021 (ACKU67-VET-042). Risk factors, age, breed, cryptorchidism, clinical signs, blood profiles, estradiol levels, ultrasound findings, and histopathology, were evaluated using descriptive and statistical analysis. A total of 305 dogs showed ultrasonographic testicular abnormalities, including anechogenicity, hypoechogenicity, hyperechogenicity or heterogeneous parenchyma. Histopathology classified

cases into non-tumour, benign and malignant tumours and also types of tumour; interstitial cell tumours, Sertoli cell tumours, seminomas and mixed-type tumours. Tumour types, clinical signs, haematology and estradiol levels were analysed descriptively. Chi-square and Fisher's exact tests assessed correlations with significance at $p < 0.05$ using R Studio.

Interstitial cell tumours were most common, followed by seminomas, non-tumour cases, mixed-type tumours and Sertoli cell tumours. Median ages were 11 years for tumour cases and 8 for non-tumour cases. Age and cryptorchidism were significantly associated with tumour types ($p < 0.05$). Sertoli cell tumours occurred mostly in dogs ≤ 7 years, while interstitial cell tumours and seminomas were seen in dogs > 7 years. Cryptorchidism, including both inguinal and abdominal forms, was significantly associated with the development of seminoma ($p < 0.05$). Interstitial cell tumours were frequently associated with prostatitis (48/132; 36.36%) and prostate enlargement (60/160; 37.50%). Non-regenerative anaemia was more common in tumour cases; regenerative anaemia was seen mostly in non-tumour dogs. Elevated estradiol levels in the grey zone (10–15 pg/mL; 3/8, 37.5%) and hyperestrogen range (> 15 pg/mL; 7/22, 31.8%) were mainly found in interstitial cell tumours. No significant association was found between breed or ultrasonographic findings and tumour types ($p > 0.05$).

Dogs ≥ 7 years showed the highest incidence of interstitial cell tumours and fewer non-tumour diagnoses. Dogs without cryptorchidism were more likely to have interstitial cell tumours (4), while seminomas were more frequent in dogs with cryptorchidism, both inguinal and abdominal (5).

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6529/7795 | Development and evaluation of a canine pyometra simulator for clinical training of veterinary student

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Pyometra is the most common gynaecological disorder in intact bitches and can be life-threatening if left untreated. Surgery is the treatment of choice. Despite ovariohysterectomy is trained with students, ovariohysterectomy in case of pyometra is more complicated and requires specific skills. Due to the emergency nature of surgery, the possibility for training students to assist or to perform a pyometra surgery is rare. For this and animal welfare reasons, simulators are gaining importance for practical training of students. Practicing with simulators has some advantages, such as increasing the student's skills and confidence without the need for living animals and/or cadavers. Although simulators for neutering male and female dogs and cats as well as for caesarean sections already exist and are regularly used for student training, the special requirements for pyometra surgery are not met by now. Consequently, we aimed to develop a pyometra simulator, affordable and simple to use for practical training of students and to validate it for its usefulness in teaching students' knowledge and practical skills required for pyometra surgery.

A low-fidelity simulator was developed modifying and optimising the SimSpay for pyometra “purpose”. Afterwards, the simulator was evaluated by veterinarians and by students on a four-point Likert scale (1 for strongly agree, 2 for agree, 3 for disagree and 4 for strongly disagree). The same survey was used for both groups and included 12 questions. Mean answer scores were calculated for each question using Microsoft Excel and compared between groups. The chi-square-test was used to analyse whether there is a significant difference between the answers given by veterinarians and students.

In total, 21 veterinarians and 77 students answered the questionnaire at the time of submission. Mean scores varied between 1.27 and 2.11 in students, with the lowest score for the question if opening of the abdomen is realistic, and 1.29 and 2.05 in veterinarians, with the lowest score for realistic palpation and haptic of the model. In general, both groups agreed that the simulator is easy to use (vet/student: 1.33/1.32), suitable to train and understand the different steps of surgery (1.38/1.36), suitable for student training in general (1.29/1.27) and helpful to use before doing it in living animals (1.43/1.30). The chi-square-test showed that with the current results there is no significant difference between the two groups.

In conclusion this study showed that practicing skills on our pyometra simulator improves students´ confidence and skills required to perform pyometra surgery and that students as well as veterinarians appreciate the simulator. More answers are required for a final conclusion whether veterinarians' and students' appreciation differs. Nevertheless, our pyometra simulator is a useful addition to teaching the theoretical learning content only.

7168/7877 | The Cat's Path: A board game about feline reproduction—A preliminary study

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Research findings suggest that games cater to diverse learning styles, promote self-directed learning, enhance active participation and socialisation and offer opportunities to reinforce existing knowledge while introducing new concepts (1). However, there is a paucity of research on the development and integration of educational games into veterinary curricula (2, 3). The aim is to develop of a board game about feline reproduction for undergraduate veterinary students for training and testing their knowledge, practical skills and team work. “The Cat's Path” is organised as a board game that features a path with different colours with corresponding cards, and objective is to be the first to reach the conclude the path. Cards have two difficulty levels, one for students from the 1st to 3rd years and another for students in the 4th and 5th years. Players move their game pieces along the board by rolling a dice, and the colour of the tiles they land dictates the type of challenge. This includes multiple-choice quizzes, short-answers, true or false questions and more demanding tasks, such as performing practical skills using

various simulation models included in the game. The game is designed to be played in small groups under the guidance of a game master who facilitates the discussion and ensures that the learning objectives are met. To provide a preliminary assessment, a focus-group of six students (comprising 1st to 5th year veterinary students) evaluated this board game. They played the game and completed an anonymous questionnaire assessing their prior knowledge, engagement, perceived learning outcomes, and suggestions for improvement.

The game was rated highly for clarity, engagement, and enjoyment, with students highlighting the interactive and collaborative nature of game. Suggested improvements included refining the graphical interface, increasing the variety of questions and further enhancing interactivity. All participants stated they would use the game as a study method and would recommend it to fellow veterinary students. The overall rating provided by focus-group students was 5 out of 5.

Focus-group students highlighted the engagement and enjoyment provided by "The Cat's Path", which seems to be an effective educational tool for feline theriogenology. Considering these promising preliminary results and the minor adjustments suggested, future studies with a large population of students of different academic years will follow. These will fully elucidate the usefulness of this game-based learning activity for teaching theriogenology.

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7034/7805 | The persisting mesonephric duct: A case report in a male labrador retriever

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Cryptorchidism, renal hypoplasia/dysplasia, and ectopic ureter are distinct congenital abnormalities that can significantly impact the canine genitourinary system. While often observed independently, their simultaneous occurrence poses a complex diagnostic challenge requiring multidisciplinary management. A 20-month-old male Labrador Retriever (36.5 kg) was presented for evaluation due to left-side cryptorchidism, without other clinical signs. The ultrasound revealed not only the testis retained in the abdomen but also several left kidney, ureter and bladder anomalies. On computer-tomography, findings were consistent with complex left-sided genitourinary malformation, including: severe renal atrophy, renal artery aplasia and renal venous malformation; severe hydroureter (attributed to ureteral ectopia); vesical compartment or ureterocele; left testicular ectopia and hypoplasia. Despite these anomalies, the patient remained asymptomatic, and all laboratory parameters (hemogram, blood biochemistry, urinalysis, protein-creatinine ratio) were within normal reference ranges. At surgery, severe hydronephrosis and hydroureter were identified. Despite adhesions to the urinary bladder, careful dissection of the ureter revealed no communication between both structures. The ureter was dissected up to the prostate, where it was ligated with vascular clips. The prostatic attachment was thin, with no obvious communication with the prostatic urethra, although there was no dissection through the prostate. Due to severe hydronephrosis and renal distortion, standard nephrectomy was performed. Orchiectomy was performed, with double ligation with transfixation of the spermatic cord. Palpation of the urinary bladder confirmed a clear communication between both 'compartments', so no intervention was performed. Recovery form surgery was uneventful. On histopathology, the testis was moderately atrophied. All seminiferous tubules observed were exclusively populated by Sertoli cells, with complete absence of sperm cells. No changes in the numbers and morphology of Leydig cells were evident. The epididymis was apparently normal and connected to a segment of vas deferens which drained into the 'ureter'. In the left kidney, the renal papilla and medulla were extensively replaced by interstitial fibrosis and immature mesenchyme. A focally extensive area of persistent metanephric ducts was also evident in the medulla. In both renal poles, the cortex was composed of immature mesenchyme populated by small arterioles, intermixed with moderate numbers of immature glomeruli. In both the dysplastic and normal cortex, there was extensive interstitial haemorrhage. No normal ureter was observed. The structure identified as 'ureter' during surgery revealed to be a duct with a wall partially composed of immature mesenchyme populated by moderate numbers of immature glomeruli, compatible with a segment of incompletely involuted mesonephros and undifferentiated mesonephric duct, connected to both the metanephric kidney and the partially formed vas deferens. Instead of fully differentiating into a normal vas deferens, this segment of mesonephric duct persisted and developed its prostatic connection, but with no true patency into the lower urinary tract. Three months after surgery, the dog remained asymptomatic. Long-term monitoring is required to assess normal urinary function. This case underscores the importance of a comprehensive multidisciplinary approach in identifying clinically silent congenital abnormalities.

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7157/7827 | Testicular tumour in a cryptorchid tomcat

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An 8-year-old European shorthair was presented to the intensive care unit due to anorexia, adipsia, depression and no bowel movements for 2 days. Clinical examination was otherwise normal, except for painful and tense abdominal palpation. The X-ray revealed an irregular band of fatty opacity dorsal to the sternum possibly a mass approximately 11 mm long and 4.3 mm high. Ultrasound showed the presence of bilateral abdominal testes. Right testis reduced in size (5 mm), heterogeneous hyperechogenic, located lateral to the bladder. Left testis replaced by a large hypoechoic mass measuring 5.5 cm in width and 3.8 cm

in height, with a cluster of mineralisation of approximately 3.3 mm in the region of the spermatic cord. The spermatic cord was thickened, hypoechoic appearance with whirl sign and Doppler signal. A mass at the caudal aorta was also identified. Ultrasound-guided fine needle aspiration was performed of the peri-aortic mass, left testis and peritoneal effusion. Cytology results indicated a necrotic round cell tumour consistent with necrotic seminoma of the left testis and a round cell tumour compatible with seminoma of the peri-aortic mass. The tomcat was castrated 1 week later. Histopathology confirmed a diffuse seminoma with extension to the tunica albuginea and epididymis and the presence of numerous vascular emboli. The purpose of this report is to describe a seminoma in an abdominal left testis of a 8-year-old European shorthair tomcat.

Since testicular tumours are rare in cats (1), it is important to report cases to be aware as a possible cause of abdominal pain in tomcats without history of castration.

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6745/7822 | Constipation and urinary retention due to vaginal fibroma in a queen—A case report

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Vaginal tumours in cats are rare (1,2). To prevent unwanted sexual behaviour, female cats are often spayed prepubertal, which leaves the incidence of hormonal induced vaginal tumours in them uncertain. Few publications about vaginal neoplasms have described leiomyomas, adenocarcinomas, polyps and fibromas (3).

In October 2024, a 6-year-old intact female Domestic Shorthair was presented to the emergency service of our clinic due to recurrent constipation, as well as current stranguria and pollakisuria. Additionally, a protruding mass from the vagina was observed. All vital parameters were within normal limits. However, during palpation of the caudal abdomen at the level of the bladder, a mass measuring approximately 3.5 × 5.5 cm was detected, which was confirmed by ultrasound and X-ray.

Surgical removal was performed under general anaesthesia (Methadon 0.2 mg/kg i.v., Dexmedetomedine 2 µg/kg i.v., Ketamin 1 mg/kg i.v., Propofol 6 mg/kg i.v., Isoflurane) via exploratory laparotomy. During surgery, the mass was identified as a firm structure caudal to the bifurcation of the uterus, located within the vagina and dorsal to the urinary bladder. A vaginotomy was executed to remove the mass, and an ovariohysterectomy was performed afterwards. The postoperative recovery was uneventful. The cat resumed normal defecation after the application of a single enema and had normal urination the day after surgery. Histopathological examination revealed glandular cystic hyperplasia of the endometrium, along with various functional structures in the ovaries. Special staining with Masson's trichrome demonstrated a high collagen fibre content within the stroma of the vaginal mass, suggesting a fibroma as the most likely diagnosis.

In female dogs, vaginal tumours are commonly reported and often benign. In cats however, they are very rare. Differential diagnoses for the presented symptoms include colitis, strictures, perineal hernia, hypervitaminosis A, colorectal tumours, rectal diverticulum, lymphadenopathy, megacolon, cystitis, urolithiasis and urinary tract tumours for example (4).

This case shows that even though vaginal tumours are rare in queens, they should be included in the differential diagnostic workup for symptoms like urinary retention and constipation.

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7152/7771 | Hormonal profile and effect of a deslorelin implant administration on gonadotropin and steroid hormones secretion in long-term gonadectomized male dogs

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Surgical castration is commonly used for fertility control and behavioural management in male dogs. However, after gonadectomy, the loss of testicular negative feedback leads to increased luteinising hormone (LH) secretion. Deslorelin implants (Suprelorin, Virbac) have been marketed for temporary infertility induction in adult male dogs since 2007. After subcutaneous administration, deslorelin (a GnRH agonist) is sustainably released, leading to GnRH receptor desensitisation in the pituitary gland and suppression of gonadotropin secretion. Consequently, testosterone production drops, and the dog becomes temporarily infertile (1). Yet, its effect on gonadotropin secretion in already gonadectomised males has not been investigated. This study aimed to characterise the hormonal profile of male dogs gonadectomised for over 3 years and evaluate the effect of a 4.7 mg deslorelin implant on LH and follicle-stimulating hormone (FSH) secretion.

Forty-six adult male dogs, aged between 9 and 72 months and weighing 15 to 40 kg, were included: 34 sexually mature intact dogs and 12 surgically castrated post-pubertally more than 3 years prior to inclusion. At Day 0 (D0), the 12 castrated dogs were blindly and randomly assigned to group 1 (G1), receiving a deslorelin implant ($n=6$) or group 2 (G2), receiving placebo (NaCl, $n=6$). Blood samples were collected at D0, Month 1 (M1), and Month 2 (M2) to assess serum LH and FSH levels using canine-specific ELISA kits, and steroid hormones by GC–MS. Statistical analyses included two-way ANOVA and Mann–Whitney U tests (two-tailed), with $p<0.05$ considered significant. This study was approved by the Virbac Ethical Review Committee (EU-ERC/202312–07).

At D0, LH concentrations were significantly higher in gonadectomised dogs (11.19 ± 9.10 ng/mL) than in intact dogs (3.58 ± 4.17 ng/mL, $p<0.0001$), while FSH levels did not differ significantly (115.4 ± 41.1 vs. 100.7 ± 33.7 ng/mL). Testosterone and DHT were undetectable in all castrated animals. In G2, LH remained elevated throughout D0 (9.02 ± 3.77 ng/mL), M1

(8.47 ± 3.89), M2 (4.00 ± 4.64), while in G1, LH progressively decreased (D0: 13.37 ± 12.52; M1: 2.06 ± 3.78; M2: 0.33 ± 0.22). LH levels were significantly lower in G1 at M1 ($p < 0.05$). At M2, LH became undetectable in G1, though statistical comparison was limited by sample size. FSH concentrations remained stable in both groups. In G2, values ranged from 89.1 ± 14.2 ng/mL (D0) to 83.8 ± 3.7 (M2); in G1, from 112.3 ± 44.4 to 108.8 ± 37.7 ng/mL, with no significant variation.

Dogs castrated over 3 years ago maintained high LH levels, while testosterone and DHT remained suppressed. Deslorelin administration effectively reduced LH in long-term castrated dogs, as similarly reported in another study (2), demonstrating that the hypothalamic–pituitary axis remains sensitive to hormonal modulation long after gonadectomy. In contrast, FSH secretion appeared unaffected by gonadectomy and deslorelin implant administration. To our knowledge, this is the first controlled study to evaluate the hormonal effects of a deslorelin implant in gonadectomized dogs over time.

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6423 | Congenital aplasia cutis in a newborn Cavalier King Charles Spaniel dog—Case report

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Aplasia Cutis Congenita (ACC), also known as Epitheliogenesis imperfecta (EI) is characterised by failure in the development of the epidermis. The most common location is the cranial region (scalp in the midline), with the possibility of reaching the periosteum, skull and dura mater depending on the severity of the malformation. The malformation is described in humans, dogs, cattle, pigs, sheep and cats. In humans, the first report in literature was made in 1767. In 1826, was reported the first case on the scalp, accounting for 84% of the scenarios in humans. In canines, the malformation is considered unusual and rarely described in the literature, with the first case reported in 1975, being described the case of a black Labrador puppy, which presented the malformation (1). Epitheliogenesis imperfecta also reported in Beagle, French Bulldog and kittens. In humans, there are several theories that seek to explain the aetiology of this malformation, which may involve genetic, mechanical (traumatic) factors, vascular alterations and teratogenic agents (benzodiazepines, misoprostol, angiotensin-converting enzyme inhibitors, sodium valproate, among others) (2). The objective of this report is to describe the clinical signs, diagnosis and management of a case of congenital aplasia cutis in a newborn Cavalier King Charles Spaniel. A male Cavalier King Charles Spaniel puppy was presented to the Veterinary Hospital of São Paulo State University (Unesp) with a visible and palpable scalp defect. On initial examination, an ulcerated and crusted skin malformation measuring 0.5 cm in diameter was observed in the cranial frontal region, with exposed periosteum visible at the centre. There were no signs of infections and/or haemorrhages and no skull defects or other abnormalities. The puppy presented good Apgar score and neonatal reflexes at birth. Upon presentation, the puppy weighted 268 g and presented with low body temperature (32.6°C) and blood glucose (43 mg/dL). The mother was multiparous and none of the previously born puppies presented congenital aplasia cutis, as well as no other puppy presented the malformation previously in the kennel. The clinical diagnosis was congenital aplasia cutis and the prescribed topical treatment was dexpanthenol and lanolin-based ointment, and bandage protection in order to avoid exposure of the skin lesion and allow healing. The puppy was evaluated weekly and its development was adequate throughout the neonatal period. The healing process lasted 26 days, with complete recovery of the dermis and coat. In human, the exact mechanism is still not completely understood, although many etiological factors have been incriminated in recent years. There is no consensus regarding the treatment of aplasia cutis. In the case evaluated, only the epidermis was affected and the puppy did not show abnormalities on clinical examination. To these authors´ knowledge, this is the first report of congenital aplasia cutis in the Cavalier King Charles Spaniel breed.

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6540 | Effects of aminophylline, caffeine and doxapram in hypoxic neonatal dogs born by caesarean section—Preliminary results

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The canine neonatal period considered a critical period with a high mortality rate (1). Hypoxia is considered the main consequence of perinatal asphyxia and the cause of more than 60% of neonatal losses in the canine species in the first days of life. Knowledge of physiology, assessment of the vigour of the neonate and rapid intervention, regarding resuscitation, are essential to reduce neonatal mortality (2,3). The aim of this study was to determine the effects of administering the drugs aminophylline, caffeine and doxapram as respiratory support in hypoxic neonates, evaluating the Apgar score, lactate concentrations, blood glucose, O2 saturation, blood gas analysis and cardiac troponin I.

The study was approved by the Institutional Animal Use Ethics Committee (CEUA n°000.103). For this, 22 canine neonates, born by caesarean section, in which the inclusion criteria in the experimental groups were neonates that presented at least three of the following clinical signs: dyspnea, apnea, bradypnea (<15mpm), bradycardia (≤180 bpm), cyanotic mucosa, Apgar less than seven at 5 min (M1). Of these, three received aminophylline (0.2 mL/100 g/sublingual)—Aminophylline Group (AG), four animals received caffeine—Caffeine Group (0.1 mL/100 g/sublingual) (CafG) and five animals Doxapram (0.1 mL/100 g/sublingual) (DG) and 10 animals were included in the control group (CG), in which the neonates did not present respiratory distress and Apgar greater than seven. The neonates were also evaluated at birth and after 10 min of respiratory assistance (M2). At both times, O2 saturation was measured and 0.5 mL of blood was collected through puncture of the jugular

vein, to measure lactate, blood glucose, blood gas analysis and cardiac troponin I. Statistical analyses were performed using the Statistical Package for Social Science software, using PROC MEANS to verify data consistency and descriptive analysis (means and standard deviation) with a significance level of 5%.
The Apgar score remained stable at 8.7 ± 1.16 at baseline, with good recovery in the CafG and DG groups, with DG showing the best response, rising from 5 ± 1.14 (M1) to 8 ± 1 (M2). The pH ranged from 7.02 to 7.28, remaining within the physiological range. The partial pressure of CO_2 decreased in all groups, with a better response in CafG, which went from 84.88 ± 26.99 (M1) to 59 ± 7.48 (M2). Lactate did not show a significant decrease, indicating that the increase in lactate was not rapidly reversed and CafG had the best troponin I reversal (0.1 ± 0.03 to 0 ± 0.78). Other parameters were within normal limits.
All treatment led to stabilisation and recovery of physiological parameters in the first moments after birth, with emphasis on the DG and CafG groups, in which the first showed a significant improvement in the Apgar score and the second a better reversal of troponin I. The pH remained normal and the CO_2 pressure decreased, with Gcaf showing the best response. Lactate did not decrease rapidly, suggesting physiological stress.

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6524 | Puberty delay using an anti-GnRH vaccine in cats

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This study aimed to evaluate the effect of anti-GnRH vaccine on puberty delay in queens and tomcats.
Prepubertal mixed-breed, queens (n = 16) and tomcats (n = 17) between 2 and 5 months of age were used. The animals were divided into two groups: treated group (TRT; queens n = 13 and tomcats n = 15) received 0.5 mL of anti-GnRH vaccine (Improvac), and control group (CON, queens n = 3 and tomcats n = 2) received 0.5 mL of saline solution. Treatments were performed on days 1 (D1) and 28 (D28), with the study followed up to 18 months. Cats underwent a physical examination, and blood samples were collected to study haematological parameters and testosterone, progesterone and estradiol concentrations every 4 weeks starting at D1. Testicular volume, and the presence or absence of penile spines were recorded in males. In queens, signs of heat behaviour (presence/absence), vaginal cytology and uterine ultrasonography were recorded. The experiment was approved by the IACUC of the FCV UNLP (240507–1 and 240305–3). Data were analysed using GLIMMIX procedure of SAS.
CON queens entered estrus between 2 and 5 months after D1 and showed estrus every 15 to 20 days. In CON males, penile spines appeared at 5 months of age and grew progressively. Furthermore, a progressive increase in testicular volume was observed, which was smaller on the first day than at month 12 (53.41 ± 40.84 vs. 2047.01 ± 826.50 mm^3). None of the TRT queens displayed signs of heat behaviour and estrous vaginal cytology during the experiment, and penile spines remained absent in TRT tomcats. In addition, testicular development was absent and testicular volume showed no significant differences when comparing D1 with month 12 (41.22 ± 6.17 vs. 37.47 ± 4.52 mm^3). No significant differences were observed in uterine diameter between the CON and TRT queens at D1 (0.17 ± 0.1 vs. 0.18 ± 0.01 cm, p = 0.39). The TRT queens showed no significant differences in uterine diameter throughout the study. All haematological parameters remained within the reference range for the species. Haematological analysis showed no significant differences between the CON and TRT groups (queens and tomcats) in any of the parameters measured complete blood count throughout the study. Furthermore, no significant differences in haematological parameters were observed between TRT queens and tomcats. In CON tomcats showed a progressive increase in serum testosterone concentrations from D1 until month 12 (0.36 ± 0.3 vs. 4.87 ± 0.7 ng/mL). Serum testosterone and progesterone concentrations at D1 were basal. In addition, the concentration of testosterone and progesterone were basal throughout the study in TRT groups. In CON queens serum E2 concentration at day 2 of heat was 29.72 ± 2.23 pg/mL. During the physical examination, a mobile, painless subcutaneous reaction was palpated at the injection site in one TRT tomcat and seven TRT queens at month three. This reaction did not interfere with the animal's behaviour and subsequently disappeared.
Our study shows that applying two doses of Improvac delayed puberty in cats. Further studies are necessary to assess the duration of immunocontraception and fertility after treatment.

6441 | Analysis of canine epididymal sperm proteins as motility biomarkers

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Epididymal semen is a good alternative to ejaculated semen and can be used in cases of unexpected death of the male, required castration for medical or behavioural reasons, neuropathic conditions, erectile dysfunction, and ejaculation problems. In addition to other biochemical components, proteins in the epididymal spermatozoa (ES) have a significant effect on sperm physiology. These proteins regulate the maturation processes of the sperm cells in the epididymis and their acquisition of the ability to move. In this study proteomic analysis was performed to evaluate whether the canine ES could be a source of motility biomarkers.
The ES were obtained from epididymal tissues from 19 mixed-breed dogs kept at the Shelter for Homeless Animals in Tomaryny (Poland). The dog's testicles were removed by a veterinarian. An automatic pipette was used to aspirate the effluent of the epididymal semen with modification. The ES motion parameters were assessed using a computer-assisted semen analysis (CASA). The ES were separated from epididymal fluid by centrifugation. The ES samples were divided into two groups: good sperm motility (GSM) with progressive motility (PMOT%) ≥ 55% and poor sperm

motility (PSM), PMOT < 55%. Mass spectrometry analysis using NanoUPLC-Q-TOF/MS was used to identify the ES proteins.
Significant statistical differences were demonstrated between the GSM and PSM groups for the TMOT ($p = 0.039$) and PMOT ($p < 0.001$). The TMOT ranged as follows (values were shown as mean ± SEM): GSM—from 90 to 95% (92.20 ± 0.49) and PSM—from 71 to 93% (87.38 ± 2.56). The PMOT ranged as follows: GSM—from 55 to 72% (62.50 ± 1.83) and PSM—from 36 to 53% (45.29 ± 2.26). Using the sperm protein quantitative analysis, five proteins were common in both analysed groups: actin cytoplasmic (ACTB), ce10 protein (ce10), cysteine rich secretory protein 2 (CRISP2), lactotransferrin (LTF), and intracellular cholesterol transporter 2 (NPC2). Protein abundance was markedly higher in the GSM group than in the PSM group (values were shown as mean ± SEM): ACTB (GSM—$2347 \pm 453.4 \times 10^5$, PSM—$1681 \pm 222.3 \times 10^5$), CRISP2 (GSM—$148.5 \pm 46.11 \times 10^5$, PSM—$53.78 \pm 35.77 \times 10^5$) and LTF (GSM—$5425 \pm 1121 \times 10^5$, PSM—$3548 \pm 1034 \times 10^5$). Statistically significant differences were observed for two proteins: ce10 (GSM—$1754 \pm 168.4 \times 10^5$, PSM—$391.4 \pm 209.6 \times 10^5$; $p < 0.001$) and NPC2 (GSM—$1857 \pm 327.6 \times 10^5$, PSM—$281 \pm 96.72 \times 10^5$; $p < 0.01$). Significant positive correlations ($p < 0.05$) were also detected for LTF or NPC2, between the sperm motion parameters.
It can be suggested that differences in the abundance of proteins of the analysed groups indicate their important role in the progressive motility of canine epididymal sperm. We suggest that the finding of our study may serve as basis for future research to gain more insights into the role of the epididymal proteins as biomarkers of sperm motility.
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A13 | Vaginal and urinary bladder leiomyoma in a 12-year-old female dog

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A 12-year-old intact, nulliparous female Cocker Spaniel was brought in for consultation, exhibiting signs of abdominal pain and a large mass protruding from the vaginal area. Vaginal cytology was suggestive of anestrus. Clinically, the dog presented pollakiuria, dysuria, hematuria, blood-tinged vaginal discharge and tenesmus.
After non-conclusive ultrasound scans, a computerised tomography (CT) exam was performed. Three separated large mass lesions (around 5 cm in diameter each) were found throughout the vagina, exerting a severe mass effect on adjacent structures, particularly the rectum, causing partial obstruction. There were multiple ovarian cysts identified in both ovaries with no evidence of mass lesions or associated peritoneal reactivity. The uterine horns and cervix showed cystic endometrial hyperplasia. Inside the bladder, a large urinary calculus of approximately 2.5 cm in diameter was noted. Caudally to it, there was a content suggestive of sediment or hematoma. A nodular lesion was also identified in the left mammary chain, at the level of the fifth gland, approximately 9 mm in diameter. Additionally, four nodular lesions in the liver were spotted, approximately 7 mm in diameter.
The selected treatment was surgical, by performing ovariohysterectomy, episiotomy and cystotomy. Samples of the vaginal mass, the bladder mass, the mass in the uterine horn, the cervix and the ovary were taken for histopathological analysis. On histopathology, both the vaginal and bladder mass samples showed a population of spindle cells arranged in bundles, supported by a collagenous and lax swollen stroma in the vaginal mass and the bladder mass, respectively. The uterine horns showed hyperplasia of the endometrial glands and purulent exudate within, similar to the cervix. The ovarian specimen showed proliferation of spindle-shaped cells, arranged in multidirectional bundles, supported by a collagenous stroma, an inflammatory infiltrate rich in neutrophils, oedema and foci of congestion. Results confirmed the presence of vaginal leiomyoma, bladder leiomyoma, cystic endometrial hyperplasia and pyometra, uterine adenomyosis and luteal cysts, respectively. The prognosis was good. After the surgery, the dog made a good recovery and was discharged home.
We report the combination of multiple masses and alterations in multiple organs demonstrating the complexity of some clinical cases. Although the majority of vaginal tumours are benign and around 80% are leiomyomas, it is rare to report a concomitant bladder tumour (1). The ovarian cysts are most likely related with the aetiology of the vaginal leiomyoma, as this tumour has been suggested to have hormone-dependent characteristics and ovarian cysts can deregulate oestrogen levels (2). In this case, the different anatomical masses were probably interrelated. In addition, this case supports the need to use advanced imaging tests (CT) for accurate diagnosis in geriatric patients with abdominal and pelvic masses when ultrasound or radiography are not sufficient.

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7040 | Contrast-enhanced ultrasound of the feline reproductive system

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Ultrasonography is the imaging modality of choice to assess the reproductive system. While B-mode imaging provides

information regarding morphology and Doppler ultrasound about vascularisation, they cannot provide information regarding tissue perfusion, which is a key feature for adequate function. Contrast-enhanced ultrasound (CEUS) is a technique that can provide real-time information regarding tissue perfusion (1), however, few studies have been published in literature regarding the use of this technique to assess the feline reproductive system (2). This pilot study aimed to describe the qualitative and quantitative features of the perfusion of the feline reproductive system using CEUS.

Twelve intact cats (six males, four non-pregnant females and two pregnant females) undergoing routine neutering for population control, were evaluated. Prior to sonographic examination, sedation was induced with medetomidine (0.1 mg/kg) and ketamine (0.01 mg/kg), and a three-way valved catheter was placed in the cephalic vein for contrast agent administration. Imaging was conducted using a Vetus E7 (Mindray) ultrasound platform with a linear multifrequency transducer. B-mode ultrasound was performed to assess morphological characteristics, size, echogenicity, and echotexture of the testicles and ovaries. CEUS was then performed using SonoVue (0.03 mg/mL), with a mechanical index of 0.06. Perfusion was recorded for 90 s and analysed quantitatively using time-intensity curve (TIC) to obtain parameters of Goodness of Fit (GOF); Base Intensity (BI), Arrival Time (AT), Time to Peak (TTP), Peak Intensity (PI), Ascending Slope (AS), 1/2 Descending Time (DT/2), Descending Slope (DS), Area Under the Curve (AUC) and Mean Transit Time (MTT). Data were assessed for normality (Shapiro–Wilk). Statistical comparisons were made using paired t-tests and Wilcoxon signed-rank tests, where applicable. Significancy was found if $p < 0.05$.

B-mode imaging revealed normal testicular and ovarian morphology in all cats. CEUS visualised tissue perfusion patterns, with contrast enhancement first appearing in subcapsular arteries before diffusing homogeneously into the parenchyma. For testicular CEUS, no significant differences were found in GOF ($p = 0.1868$), BI ($p = 0.7220$), AT ($p = 0.5603$), TTP ($p = 0.3242$), PI ($p = 0.0611$), DT/2 ($p = 0.1055$), DS ($p = 0.0806$), AUC ($p = 0.6875$), or MTT ($p = 0.1757$). However, AS was significantly higher in the left testicle (1.1 ± 0.32) compared to the right (0.74 ± 0.31, $p = 0.0224$). In female cats, ovarian CEUS demonstrated homogeneous enhancement, and follicles were distinctly outlined due to their lack of enhancement. No significant differences were found between the right and left ovary perfusion parameters ($p > 0.05$). Qualitative assessment of the ovaries of pregnant females demonstrated a strong peripheral-to-parenchymal enhancement pattern.

CEUS was successfully standardised for the evaluation of feline reproductive structures, providing detailed perfusion assessment in testicles and ovaries. This study establishes a foundation for further research on CEUS applications in reproductive imaging, with future steps involving larger sample sizes and multicentric validation to assess technique reproducibility across different ultrasound platforms.

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6493 | The role of intravaginal administration of feline seminal plasma and llama β-NGF in feline ovarian function

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In some induced ovulatory species, as camelids, the seminal plasma protein β-NGF can trigger ovulation when administered parenterally (1). This study aimed to investigate the effect of intravaginal administration of cat seminal plasma or purified llama β-NGF on ovarian function in queens.

Six clinically healthy, sexually mature queens (2–4 years) housed under controlled conditions (12 h light:12 h dark) were used in a crossover study. Each queen received all three treatments, separated by three estrous cycles. Vaginal cytology was performed every other day to determine the stage of estrous cycle. Forty-eight hours post-cytological estrus, queens were randomly assigned to one of three intravaginal treatments: 1) 0.25 mL saline (negative control, $n = 6$); 2) 0.25 mL cat seminal plasma (CSP, $n = 6$); 3) 35 µg of β-NGF purified from llama seminal plasma (NGF, $n = 6$). Serum samples were collected during estrus and on days 1, 24, 31, 38 and 45 of cytologic interestrus (IE) and stored at −20°C for estradiol (E2) and progesterone (P4) analysis by chemiluminescence (Elecsys, Roche, Germany). The study was approved by the LACUC. FCV. UNLP (#114–8-21P).

No significant differences were observed in estrus length (10.17 ± 1.7 d) or estradiol (E2) concentrations (36.02 ± 9.97 pg/mL) across the experimental treatments. However, elevated serum progesterone (P4) concentrations, indicative of active corpora lutea (CL), were detected in two cats within the CSP group. These cats showed prolonged IE intervals (43.5 ± 4.5 d), with P4 concentration of 0.34 and 0.24; 32.32 and 20.00; 0.35 and 0.55; 0.23; 0.22 ng/mL at IE days 1, 24, 31, 38, and 45, respectively. This data suggest that CSP administration induced CL formation in 33% (2/6) of the treated queens. In contrast, the IE length of non-ovulating queens in the CSP and NGF groups were similar than those of the Negative Control (5.00 ± 1.4, 8.75 ± 0.6 vs. 6.60 ± 1.2 d). In agreement, serum P4 concentration at IE remained basal (≤0.5 ng/mL). These results are consistent with prior studies demonstrating that subcutaneous administration of cat seminal plasma can induce CL formation (P4: 27.57 ± 8.3 ng/mL at IE day 21) and prolong IE intervals (37 ± 2 d) in 67% of queens. Furthermore, mating with a vasectomised male has been shown to result in extended IE (45 ± 4 d) and active CL (P4: 51.94 ± 6.44 ng/mL at IE day 21) in 100% of queens (2).

The data presented suggest that seminal plasma plays a role in the ovulation mechanism of domestic cats. However, the active component within feline seminal plasma responsible for ovulation induction would differ from the previously identified β-NGF in camelids. This is supported by the lack of prolonged interestrus intervals following vaginal or intramuscular administration of purified llama β-NGF to queens (2). Consequently, further investigations are essential to identify and characterise the specific molecule within feline seminal plasma that induces ovulation.

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6507/7875 | Neonatal mortality related to dystocia in chihuahuas: 72 cases (2009–2024)

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Chihuahuas are predisposed to dystocia (1), but it remains unclear if the extreme features inherent to the breed reflect on the typical presentation of dystocia and affect the expected threshold for intervention during parturition. A retrospective study of dystocia cases aimed to elucidate typical features of dystocia in chihuahuas and evaluate factors related to immediate post-interventional neonatal survival.

Data extraction from the patient records of the University Small Animal Hospital (Helsinki, Finland) found 72 dystocia cases treated in 67 chihuahuas between 2009 and 2024. Collected data included signalment, duration of pregnancy, clinical and obstetrical examination, evaluation of foetal heart rate, blood analysis, onset of expulsion phase, time interval from the last birth at presentation, neonatal characteristics (live or stillborn, born before or after presentation, born apparently healthy or with obvious congenital defects), type of intervention (conservative or surgical) and in cases with c-section, records from surgery and anaesthesia. Retrospective attempt to classify all dystocias into obstructive/non-obstructive or maternal/foetal appeared unreliable, as well as interpretation of which criteria of dystocia were met. Median age of the bitch was 2.8 years (8 months—10 years), representing median parity of 1.8 (1–4).

In total, 211 neonates were born, out of which 24.6% were either stillborn or euthanised due to congenital defects (seen in 9.5% of the neonates). Only 18% were born pre-intervention, and most bitches ($n=49$) were presented with full litter unborn. Most (66%, $n=48$) cases underwent c-section. Stillbirth rate of neonates born pre-intervention was 34.2%, compared to 15.0% post-intervention. Comparison of bitches with full survival (no stillbirths) post-intervention to bitches with ≥1 stillbirths with univariate Pearson chi-square test associated 'all neonates unborn at presentation' ($\chi^2(1)=11.5$, $p<0.001$) and 'intervention type c-section' ($\chi^2(1)=7.0$, $p=0.008$) with full survival. Out of conservatively treated bitches, 54.2% had ≥1 stillbirths, compared to only 22.9% of bitches treated with c-section. Furthermore, independent samples T-test showed greater maternal blood glucose concentration with ≥1 stillbirths (6.91 mmol/L, $n=13$) compared with full survival (5.84 mmol/L, $n=37$, $p=0.036$). Logistic regression model (Nagelkerke $R^2=0.37$) built with glucose concentration, neonatal birth pre-intervention (yes/no) and intervention method c-section (yes/no) maintained significant effect on neonatal mortality from pre-interventional birth (OR 10.4, $p=0.003$), while high glucose concentration showed tendency (OR 1.8, $p=0.035$).

Neonatal mortality related to dystocia in chihuahuas in this study appeared greater than previously reported (2), and comparatively greater number of neonates showed congenital defects (3). Survival was significantly influenced by the progression of parturition prior to presentation, and the higher stillbirth rate among neonates born before intervention indicates a potentially high incidence of unrecognised dystocia. Early intervention with c-section can significantly improve the survival of neonates, whereas depleted energy reservoirs are an unlikely factor associated with dystocia in chihuahuas.

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6506 | Conservative treatment of canine penile necrosis: A case report

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Penile necrosis is a possible severe consequence of canine paraphimosis, with variable clinical presentation and incoherent treatment guidelines. A 9-years old, intact male Jack Russel terrier was presented in September 2024 due to postoperative complications after removal of urinary calculi via cystotomy and urethrotomy. Peri-incisional swelling and subcutaneous fluid accumulation had resisted treatment with aspiration, antimicrobials and non-steroidal anti-inflammatory medication, leading to paraphimosis within 3 days post-surgery. Treatment of paraphimosis with manual reduction, local cooling and topical dextrose had failed and 7 days after surgery (3 days before presentation) both the irreducible penis and the swollen scrotum had shown dark colour. Presentation revealed necrosis of the scrotum and the glans penis, latter extending 3 cm caudally from the tip of the penis on the ventral side, whereas dorsally the level of healthy tissue remained undetected under the prepuce. Penile reposition, urinary catheterisation and partial preputial closure were done under general anaesthesia. Surgery revealed a large subcutaneous hematoma, apparently originating from the urethrotomy incision but extending craniolaterally next to the os penis and caudally until the scrotum, with extensive surrounding necrotic changes. Intraoperative urethroscopy excluded urethral perforation. The hematoma and the adjoining necrotic tissues were resected with concurrent orchiectomy and scrotal ablation. Postoperative hospitalisation included antimicrobials (amoxicillin-clavulanic acid, enrofloxacin), analgesia (meloxicam, acetaminophen, levomethadone), urethral relaxant (prazosin) and flushing of the preputial pouch with saline. After 4 days the dog, having shown normal micturition and stable general demeanour without signs of pain, was discharged with continuing antimicrobials, analgesia (gabapentin, acetaminophen), prazosin and local flushing. Control visits at 6-to-7-day intervals showed gradual demarcation of the necrotic tissues and decreasing penile swelling, until eventual emergence of vital, pink tissue below the necrotic layer along the glans penis. Preputial sutures were removed without recurrence of paraphimosis 17 days post-surgery. Last control 73 days post-surgery revealed almost full recovery, with only minor scar-type, pigmented mucosal lesions on both sides of the penis.

Compromised local circulation associated with haemorrhage and the resulting hematoma-related pressure after urethrotomy appeared as the most likely aetiology for penile necrosis. However, multifactorial background related to venous congestion (paraphimosis), local vasoconstriction (cold-treatment) and tissue irritation (dextrose-treatment) could not be excluded.

Total or partial penile amputation to treat penile necrosis is described in the literature (1), but despite reported good prognosis (2), the procedure is highly invasive and carries a risk of complications (3). Although severe necrotic changes were observed in our case, amputation remained unnecessary. However, the vitality of urethral tissues may have supported the success of conservative treatment, possibly indicating uncompromised deeper vasculature, while unproblematic micturition, lack of pain and unaltered general condition were essential for conservative treatment.

Conservative treatment may be a suitable treatment method for penile necrosis when predisposing factors are resolvable. Postponement of penile amputation should be considered when not mandatory due to pain, progressive necrosis or problems of micturition.

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7008 | Fetal expulsion without complete pregnancy loss: A case of luteal insufficiency in a pregnant bitch

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A 26-month-old German Shepherd Bitch with a known history of fetal resorption in a previous pregnancy was presented at 6 weeks of gestation for evaluation of a greenish vaginal discharge. The owner had noticed the discharge for approximately 2 days, prompting immediate veterinary attention. During initial examination, transabdominal ultrasonography revealed that two foetuses had died evidenced by the absence of detectable heartbeats while the remaining foetuses were still viable with normal cardiac activity. A serum progesterone assay performed concurrently showed a level of 4.13 ng/mL, which raised concern for potential luteal insufficiency. Over the next 2 days, the bitch spontaneously expelled the two nonviable foetuses, after which the greenish discharge ceased within a week. No progesterone supplementation was administered as the clinical decision was made to monitor the bitch's natural hormonal progression and assess whether she could maintain the surviving foetuses without medical intervention. Serial ultrasound examinations were carried out weekly to assess the viability of the remaining foetuses and observe any further signs of fetal distress. At 7 weeks of gestation, a repeat progesterone assay indicated that the level had dropped to 2.01 ng/mL yet the bitch showed no sign of additional fetal expulsion. Ultrasonography confirmed that the surviving foetuses remained active with normal heart rates and adequate amniotic fluid. Over the following week, the progesterone levels continued to decline steadily intensifying concerns about monitoring pregnancy to term. Given the history of fetal loss, progressively declining progesterone, and the risk of further compromise to the surviving foetuses, an elective caesarian section was scheduled. During the caesarian section, four healthy puppies were delivered successfully. The dam recovered uneventfully and the puppies thrived under postnatal care In conclusion, this case highlights the importance of close monitoring of progesterone levels especially in bitches with a history of pregnancy loss or signs of impending abortion. Also, this scenario suggested that despite a marked decline in systemic progesterone, there were some compensatory mechanisms possibly local progesterone production within the placental tissues or uterus. Hence, further research into local progesterone synthesis may offer new insights into how pregnancies can be partially sustained despite declining systemic hormonal levels.

7007 | Foetal mummification and survival of littermates in a pregnant boerboel with luteal insufficiency

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A 15-month old, primiparous, pedigree Boerboel was presented at 6 weeks of gestation with persistent vulvar bleeding, which continued until an elective caesarian section was performed at term. The owner also reported blood in urine during urination, and from 7 weeks of gestation, a mucus plug mixed with blood was observed, pregnancy had been previously confirmed by ultrasound at 4 weeks, showing normal fetal development.

At 6 weeks of gestation (estimated from pre-mating progesterone assay performed to determine ovulation date), ultrasonography confirmed that one fetus had died, while the remaining foetuses exhibited normal heart rates ranging from 230 to 245 bpm. A progesterone assay at this time revealed a level 5.12 ng/mL, prompting immediate supplementation with intramuscular progesterone in oil at 2 mg/kg every 72 h. After initial treatment, progesterone rose to 6.22 ng/mL, but subsequently dropped to 1.03 ng/mL by 7 weeks and 2 days, despite continued supplementation. This persistently low progesterone level raised concerns about pregnancy maintenance. At this point the bitch was hospitalised and serial ultrasonographic monitoring carried out every other day to confirm viability in the surviving foetuses. Given the persistent bleeding, progesterone decline and the risk of further foetal loss, an elective C-section was performed 62 days from the ovulation date. At surgery, two mummified foetuses were discovered, suggesting foetal demise occurred at approximately 6 weeks of gestation, while the remaining eight puppies were delivered healthy and viable.

Fetal Mummification in dogs is uncommon and typically associated with hormonal insufficiency, placental failure, or infection. This s in agreement with the works of McDonald and Nicols, (2012), Schafer-Somi, (2017), Decaro and Buonavoglia, (2024) In this case, the ultrasound confirmation of foetal death at 6 weeks coincided with a Progesterone decline to 5.12 ng/mL, indicating luteal insufficiency as a likely cause of foetal demise. While Progesterone supplementation with intramuscular progesterone in oil (2 mg/kg every 72 h) initially stabilised levels at 6.22 ng/mL, the subsequent sharp decline to 1.03 ng/at 7 weeks 2 days, despite continued treatment, suggests an underlying failure of progesterone production or responsiveness.

The sharp and sustained drop in systemic progesterone likely compromised uterine support, leading to the mummification of affected fetus. However, the survival of the remaining puppies raises questions of whether localised progesterone production within the uterus, placenta or foetal membranes contributed to sustaining pregnancy. While systemic progesterone levels

influence overall pregnancy stability, some studies in other species suggest that local sources of progesterone may help maintain foetal viability in certain regions of the uterus. The selective loss of a fetus while others survived even when systemic progesterone was inadequate underscores the complexity of progesterone- dependent pregnancy in canines and indicates a need for further research into local hormonal mechanism and selective foetal viability.

Clinically, this case highlights the importance of early progesterone monitoring and intervention in pregnant bitches with unexplained vaginal bleeding. The failure to maintain stable progesterone levels despite supplementation suggests that some cases of luteal insufficiency may not be fully responsive to treatment.

7035 | Fetal resorption in canine pregnancy: Understanding the hormonal and the demographic risk factors

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Foetal resorption is a major cause of pregnancy loss in bitches, with multiple underlying factors including hormonal imbalance, infectious agents (*Brucella canis*, canine herpes virus, etc.), genetic predisposition, uterine pathology and environmental stressors. This study investigates the incidence of foetal resorption in different breeds, the role of progesterone levels and whether age and parity influence the likelihood of foetal resorption.

A retrospective study was conducted from January 2023 to March 2025 on 185 bitches presented for pregnancy confirmation at 4–5 weeks using ultrasonography. The breeds examined included Boerboel (39), Rottweiler (20), German Shepherd (21), Lhasa apso (23), Eskimo (31) and Caucasian (51). Pregnancy status and foetal resorption were confirmed via ultrasound. In 30 out of the 41 bitches with foetal resorption, paired progesterone values were documented prior to mating and at the time of foetal resorption confirmed through ultrasonosography. Statistical analysis, including Chi-square tests, was performed using SPSS to assess the association between foetal resorption, breed, age and parity.

Among the 185 bitches examined, 86 were pregnant without resorption, 41 had foetal resorption, and 58 were not pregnant. The mean progesterone level significantly declined from 9.63 ± 3.23 ng/mL prior to mating to 3.84 ± 1.71 ng/mL ($p < 0.001$) at the time of confirmation of foetal resorption. This statistically significant decline in serum progesterone levels strongly supports the association between low progesterone and foetal resorption. Foetal resorption was observed in 30 of 125 bitches (24%) aged 1–3 years and in 11 of 60 bitches (18.3%) aged 4 years and above. Statistical analysis revealed no significant association between age group and occurrence of foetal resorption (Chi-square = 0.46, $p = 0.497$). Parity was categorised as Nulliparous ($n = 30$), Primiparous ($n = 70$) and Multiparous ($n = 85$). Foetal resorption was observed in 10(33.3%), 16(22.9%) and 15(17.6%) bitches in each respective group. Statistical analysis revealed no significant association between parity and foetal resorption (Chi-square = 3.19, $p = 0.202$), though the highest incidence was observed among nulliparous bitches. Although Boerboels (15/39) and Rottweilers (8/20) had higher incidence among the breeds studied, statistical analysis revealed no significant breed predisposition to foetal resorption.

Among parameters studied, only progesterone concentration showed a significant association with foetal resorption as progesterone levels were consistently lower in all the affected This however does not establish causality. It remains unclear whether the progesterone drop precedes and causes foetal loss or a consequence of resorption due to other causes such as infections or embryonic abnormalities. Early post-breeding progesterone assays is essential in detecting hormonal deficiencies that could lead to foetal resorption and that would improve early detection and management of high-risk pregnancies. The lack of significant association between foetal resorption and age, parity and breeds shows these may be less predictive. Nonetheless, higher numerical resorption rates in nulliparous and younger bitches may reflect subtle physiological or uterine maturity differences, that could merit further investigation with a large sample size.

7036 | Persistent progesterone and failure of parturition in pregnant bitches

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Parturition in bitches is triggered by a prepartum drop in progesterone, which facilitates uterine contractions and cervical dilatation. Failure of progesterone to decline can lead to prolonged gestation, risking foetal and maternal health. This case series describes four instances recorded between January 2024 and March 2025, where pregnant bitches failed to initiate labour due to persistently high progesterone levels (≥ 7 ng/ml at term), necessitating surgical intervention. A 17-month-old primiparous Caucasian bitch presented with prolonged gestation and no signs of labor initiation. Her progesterone remained elevated at term, requiring an elective caesarian section. A 5-year-old pedigree Boerboel with a a history of three prior elective caesarian sections and treated endometritis-pyometra also failed to enter labour at term, necessitating surgical intervention Her 14-month-old daughter, a primiparous pedigree Boerboel, exhibited the same condition, with persistent progesterone levels preventing labour onset requiring a caesarian section. A 15 m-month-old primiparous German Shepherd with silent heat, initially unnoticed by the owner. She presented with almost insignificant brownish vulvar discharge and lacked typical estrous signs. Progesterone assay confirmed estrous state (5.86 ng/mL). At term, her progesterone remained elevated, and she failed to initiate labour, necessitating surgical delivery. Discussion The persistence of high progesterone at term across all cases suggests an underlying defect in luteolysis, potentially due to inadequate prostaglandin F2 alpha release, excessive luteal support or endocrine dysfunction. The Boerboel with a history of reproductive pathology may have had an altered uterine environment, impairing Prostaglandin F2 alpha synthesis. The recurrence in her daughter suggests a possible hereditary or breed disposition to prolonged luteal function. Given that all bitches had progesterone levels ≥ 7 ng/mL at term, hormonal profiling particularly progesterone monitoring should be integral in cases of

prolonged gestation. Further research is needed to explore the mechanism behind luteolysis failure, the impact of oestrogen on parturition, and potential genetic predispositions.
In conclusion, performing progesterone assays before mating is critical for accurately estimating the expected due date (EDD) particularly in bitches predisposed to elevated progesterone levels at term. Breeders and pet owners who neglect progesterone testing risk miscalculating gestational timelines, leading to delayed intervention. This often results in foetal demise and, in severe cases, endangers the bitch's life.

7063/7799 | Sticker sarcoma in a female dog in Western Europe

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A 4-year-old German Shepherd bitch was presented to our clinical centre in January 2025 with a history of persistent vaginal hemorrhagic discharge following a mating 3 months before. Since the mating, the owner reported a daily blood-stained vaginal discharge. After a series of checks by the referring vet, including ultrasound and progesterone levels, the clinical suspicion was raised for a luteal insufficiency (progesterone: 13.9 ng/mL at around 25 days of pregnancy), leading to progesterone supplementation. Despite this, the bitch delivered four stillborn puppies in November 2024. Further examination by the referring vet revealed the presence of a hemorrhagic vaginal mass. It was the reason of referring this bitch to our faculty. Upon clinical examination at our clinical centre, general condition appeared normal, with no signs of systemic illness. Vaginal examinations with a speculum revealed hemorrhagic discharge with an irregular mass of approximately 1 cm3 at the vaginal floor, which was suspected to be a sarcoma, particularly the canine transmissible venereal tumour (CTVT), commonly known as the Sticker's sarcoma. This was confirmed as a CTVT based on histological and cytological analysis. Both histology and cytology confirmed the diagnosis. A treatment regimen of Vincristine (0.7 mg/m^2, IV, q7days) chemotherapy was initiated. Weekly treatments resulted in progressive improvement, including a reduction in hemorrhagic discharge and tumour size, as confirmed by both physical examination and endoscopic findings. After four chemotherapy sessions, no further signs of bleeding, and a significant reduction in the mass were noted. Discussion Sticker's sarcoma is a transmissible tumour primarily affecting sexually active dogs, most commonly between 2 and 7 years of age. The tumour is spread through direct contact, often during mating, but can also be transmitted by interactions such as licking, sniffing or biting. The disease is endemic in tropical and subtropical regions, although cases have been reported in Western Europe, often linked to imported animals (1). To the best of our knowledge, no recent case has been described recently in dogs born in France, and, as such, this case is exceptional. The source of contamination seems to be the stud male German Shepherd, but it remains unclear. Treatment with Vincristine, a chemotherapeutic agent, is considered the gold standard for treating CTVT, with high success rates, especially when administered early. This case shows that with proper treatment, the prognosis for recovery is excellent, with most dogs returning to normal health and reproductive function. The treatment is well-tolerated, with side effects such as neutropenia being manageable through careful monitoring before each session (2). This case highlights the importance of early diagnosis and intervention, as well as the need for careful consideration of potential risks of the mating, particularly regarding the transmission of transmissible diseases like CTVT. Preventive strategies include controlling reproduction, avoiding contact with stray dogs and ensuring proper veterinary screening of animals intended for breeding (1).

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6289/7801 | Biological and biochemical characteristics of semen from dogs over 10 years old

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Age has been found to affect the efficiency of the ejaculation process and the biological and biochemical properties of canine semen (1). So, the aim of this study was to investigate how advanced age affected dogs' semen quality.
The study material consisted of 12 ejaculates collected from nine dogs over 10 years old (age range: 11–14 years) and three dogs aged 5 years (controls). In the present study, the ejaculates of four mixed-breed, one Japanese Akita, three German shepherds, two golden retrievers and two Lhasa Apso dogs were used. Semen was collected manually into 15 mL-sterile tubes, then immediately transported in a thermobox (37°C) to the laboratory. Ejaculates were evaluated for sperm concentration (haemocytometer), morphology assessment (light microscope), motility (CASA), plasma membrane integrity—PMI (SYBR-14/PI dyes) (2), mitochondrial membrane potential—MMP (JC-1/PI dyes) (3), percentage of sperm that generated NO (DAF-2DA) (4) and total protein content (5). After analysing semen parameters, electrophoretic separations of sperm and seminal plasma were made. Statistical analyses were performed using the Statistica program (StatSoft Inc., Tulsa, OK, USA) and two post hoc tests: Tukey HSD (quality parameters) and U Mann–Whitney's (optical density—OD—of protein fractions).
Statistically significant differences in ejaculate volume (1.33 ± 0.86 vs. 5.67 ± 2.08 mL), sperm concentration (217.8 ± 200 vs. $740 \pm 103 \times 109$ sperm/ml), sperm progressive movement (26.4 ± 16.2 vs. $66.3 \pm 4.16\%$), plasmalemma membrane integrity (58.1 ± 17.8 vs. $92 \pm 4.62\%$) and total protein content in seminal plasma (8.82 ± 0.78 vs. 7.17 ± 0.15 mg/mL) were demonstrated between old and young dogs, respectively. There were no significant differences in total sperm motility, mitochondrial membrane potential, percentage of sperm that generated nitric oxide and total protein content in sperm. Primary and secondary defects of sperm (i.e. sperm with double heads, proximal cytoplasmic droplets and folded tails) were ascertained in four ejaculates derived from old dogs. Moreover, three of them had a reddish coloration due to the presence of erythrocytes. Protein profiles of sperm included approximately 17 protein fractions (i.e. 76, 71, 66, 54, 49, 34, 31, 30, 28, 25, 21, 18, 16, 15, 13, 11, 10 and 9 kDa)

in both examined groups. On the contrary, old and young seminal plasma protein profiles were largely convergent (76, 66, 54, 49, 30, 28 and 21 kDa) except for two proteins, that is, 25 and 18 kDa. The former protein was specific for old dogs, whereas the latter for young dogs. Nevertheless, statistically significant differences in OD of 76, 66, 54, 49, 30, 28 and 21 kDa proteins in seminal plasma from old (> 10 years) and young (5 years) dogs were demonstrated.

The advanced age of dogs significantly affects the biological and biochemical properties of semen and its protein profile.

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6527/7819 | Keep it simple—How accurate are testicular volumes in tomcats?

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The testicular volume is an important tool in the initial assessment of gonadal function, since it correlates well with various indices of testicular function in cats (1), men (2) and other mammalian species (3). Whereas body weight-dependent reference ranges are available for dogs, data for cats is missing, although more and more cats are presented at veterinary reproduction specialists due to fertility issues. Testicular dimensions can be obtained using manual or digital callipers, measuring tapes, ultrasound or—after castration—water displacement. The aim of this study was to test different measuring modalities for practicality and accuracy.

Left and right testis of 20 male cats were measured prior to routine castration by one single veterinarian, three times with a digital calliper and twice with a measuring tape (length, height, width, scrotum circumference) and weighed together with the epididymis after castration. Additionally, the water displacement was measured twice. According to the histological stage of spermatogenesis, tomcats were defined as juvenile, pubertal or adult. Testicular volume of each testis was calculated with means using the formulas 'ellipsoid' testicular volume = L*W*H*0.5236 (4) and with the formula 'Lambert' testicular volume = L*W*H*0.71 (5). Normally distributed length, height and width were compared with paired t-tests. Due to non-normal distribution, calculated testicular volumes and water displacement of all cats were compared using the Friedman test. Separate comparisons of calculated volumes for each age group were conducted with the Friedman test (juveniles and adults) or ANOVA for pubertal cats. Additionally, Spearman correlations were carried out between volumes, circumference, testis weight, body weight and age in months.

Dimensions obtained with the measuring tape were larger than with the calliper (length and height $p < 0.0001$, width $p = 0.0003$). Accordingly, calculated volumes were higher for the measuring tape (ellipsoid and Lambert both $p < 0.0001$). As expected, the Lambert formula produced larger volumes as well (calliper $p = 0.0005$, measuring tape $p = 0.0004$). Most differences between the measuring modalities occurred in the adult tomcats ($n = 10$), followed by juveniles ($n = 7$) and pubertal tomcats ($n = 3$). Calliper with Lambert and measuring tape with the ellipsoid formula, however, did not differ in any of the age groups. Water displacement appeared to be least accurate for feline testes. All volumes, as well as testis circumference, water displacement, testis weight, body weight and age in months showed strong positive correlations ($r > 0.78$; $p < 0.0001$).

As expected, juvenile testes were smaller, promoting the gonadosomatic index as a body weight-dependent reference for an estimation of fertility in tomcats, taking the difficulty of sperm collection in tomcats into account. According to our data, the use of a simple measuring tape with the ellipsoid formula produces sufficiently accurate volumes. Further studies are necessary correlating semen parameters with testicular dimension.

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6514/7940 | Laboratorial parameters of septic neonate dogs after adjuvant treatment with fresh frozen plasma

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Sepsis is the leading cause of mortality in dogs in the first 3 weeks of life (1, 2). The high mortality rates (approximately 25%) may be associated with the immunoincompetence of dogs at birth, which reduces their ability to respond to infections (1–4). The aim of this study was to evaluate laboratory parameters of neonatal dogs after the use of fresh frozen plasma (FFP) as passive immunotherapy for the treatment of sepsis.

Sixteen septic neonatal dogs were included in the experiment: seven in the plasma group (PG), which received FFP subcutaneously (2 mL per 100 g of body weight, single dose) on the first day of treatment, together with antibiotic therapy (ceftriaxone 50 mg/kg), every 12 h, intravenously and subsequently subcutaneously; and nine neonates in the control group (CG), which received only antibiotic therapy. The total leukocyte count (103 μL), lactate (mmol/L) and blood glucose (mg/dL) were used for laboratory analyses. The groups were evaluated at the first appointment (M0), after 24 h (M24), 48 h (M48) and at the end of treatment (MF). The variables were analysed by the least squares method, using the GLM procedure of the SAS statistical program version 9.2. The normality of the data was verified by the Kolmogorov–Smirnov test. The comparison of the means between the groups was performed by ANOVA/Kruskal-Wallis for each moment.

At M0, the parameters of the PG and CG were, respectively (mean ± SD): leukocytes count (leukopenia), 3.4 ± 1.4 and

3.9 ± 1.6; blood glucose, 80.5 ± 34.9 and 75.8 ± 52.2; and lactate, 4.7 ± 1.2 and 5.4 ± 2.0. At M24, leukocyte (6.8 ± 3.7; 4.5 ± 2.0) and blood glucose (122.2 ± 15.9; 89.1 ± 28.9) levels were significantly ($p < 0.05$) higher (parameters improvement) in the PG compared to the CG. At M48, the blood glucose level was 123.8 ± 15.9; 82.1 ± 37.8 and leukocyte count was 7.2 ± 3.2; 4.95 ± 0.7. Lactate was significantly ($p < 0.05$) only in MF, with lower levels in PG compared to CG (1.9 ± 0.37; 3.0 ± 1.2). Four neonates in the PG presented leukograms without alterations after 24 h of treatment, evolving from leukopenia to the reference standard for age. In the CG group, leukograms without alterations were observed only after 72 h of treatment. The mortality rate was 22% in CG patients, with no mortality in the PG neonates.

Blood plasma had a significant impact on clinical improvement of septic neonates, especially after 24 h of administration, which is possibly associated with the greater efficacy of immunoglobulins in combating infection. Blood plasma is an effective additional therapeutic strategy for the treatment of neonatal sepsis in dogs.

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A12 | Congenital intrahepatic arteriovenous fistulae and extrahepatic portosystemic shunt in a 1-month-old male beagle

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A 1-month-old Beagle pup was presented due to depressed mental state, anorexia, profuse diarrhoea and repeated vomiting. Blood count and biochemistry evidenced hypoglycemia, slight anaemia and slightly increased transaminases. The dog did not have a history of previous trauma and it was not clear if other littermates presented similar symptoms. He was referred for ultrasound and hospitalisation.

The ultrasound showed severe gastroenterocolitis, severely enlarged mesenteric ganglia, and moderate ascites. The portal vein was dilated and there was a strong suspicion of arteriovenal communications at the hepatic level. Besides, the liver also presented multiple anaechogenic, round structures. Doppler ultrasound showed a reverse blood flow (hepatofugal), which can be a characteristic of arteriovenous fistulas (2).

During hospitalisation, the episodes of diarrhoea and vomiting persisted and the mental state worsened, suggesting an initial stage of hepatic encephalopathy (1). Initially, he was treated symptomatically, using antiemetic drugs, analgesic, antibiotic and serum supplemented with glucose, but there was no positive evolution in the dog's condition. A basal cortisol test was carried out, resulting in no modifications.

A contrast computerised tomography scan (CT) was performed, showing anomalous vascular communication and the presence of a venous blood vessel driving from the portal vein, measuring around 1 cm. This vessel had a tortuous caudal path towards the mesentery and in the middle of the abdomen, it decreased in size, dividing into multiple small vessels (0.1 to 0.05 cm in diameter). In addition, the jugular vein was slightly reduced in size compared to the portal vein and the entire liver was reduced in size. Finally, an extrahepatic shunt associated with arteriovenous malformation was identified. Ultrasound and CT results were highly suggestive of arterio-venous malformations: complex surgery was required to approach the condition, with poor prognosis. Ultimately, surgery was refused by the owners and euthanasia was performed on the pup.

The relevance of this case lies not only in providing an appropriate medical approach to the onset of clinical signs, but also to prove the importance of advanced imaging techniques, such as ultrasound and CT, which is considered the new gold standard imaging modality, for diagnostic confirmation, especially in these congenital defects.

To improve the description of the case, it would have been interesting to perform histopathology on the liver, the free fluid in the abdominal cavity should have been collected and cultured for analysis, as well as the execution of an angiography. Besides that, type II urine tests, C-protein and C-reactive protein assays could also have been carried out (1).

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7037/7816 | Feline testosterone assay: Analytical and clinical validation in intact and castrated tomcats

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High testosterone concentrations allow to distinguish intact from castrated tomcats. Empirically, as in dogs, intact tomcats are assumed to have testosterone concentrations above 1 ng/mL. However, validated analytical methodologies and reliable cut-off values for feline testosterone quantification remain limited. This study aimed to validate a fluorescence enzyme immunoassay (FEIA) for testosterone measurement, establish reference ranges for testosterone in intact and neutered tomcats, and determine optimal cut-off values.

Serum samples were collected from 80 post-pubertal male cats (40 intact, 40 castrated) admitted to the veterinary hospital between January 2022 and October 2024, excluding cats who were under the effect of any contraceptive drug. Testosterone concentrations were assayed using the Automated Immunoassay Analyzer-360 (AIA-360, Tosoh, Japan). Analytical validation was conducted to assess intra-assay and inter-assay coefficients of variation (CVs) in three serum pools, ensuring the test's

accuracy across different testosterone levels. Clinical validation involved receiver operating characteristic (ROC) curve analysis to establish the most effective testosterone cut-off values for differentiating intact from castrated tomcats. Additionally, a range for testosterone in intact males was determined, and the upper reference limit for castrated cats was calculated within a 95% confidence interval (CI).

At high testosterone concentrations (> 1 ng/mL), intra-assay and inter-assay CVs were 3.93% and 7.9%, respectively. At intermediate levels (0.5–2 ng/mL), intra-assay and inter-assay CVs were 12.08 and 11.78%, respectively. At low concentrations (< 0.15 ng/mL), intra-assay and inter-assay CVs were 17.43 and 9.96%, respectively. The testosterone ranges determined in intact and castrated tomcats were 0.013 to 9.83 ng/mL and 0.012 to 0.27 ng/mL, respectively. The upper reference limit for testosterone in neutered cats was 0.16 ng/mL (95% CI: 0.12–0.20 ng/mL). The most clinically useful cut-off was determined to be 0.27 ng/mL with 100% specificity and 47.5% sensitivity for identifying intact cats, meaning that no castrated cat was incorrectly classified as intact, and all tomcats with testosterone levels above this value are intact. However, 52.5% of intact tomcats have testosterone levels below 0.27 ng/mL.

The analytical validation confirmed the accuracy of the FEIA for testosterone measurement in tomcats. Intra-assay and inter-assay precision were excellent at higher testosterone concentrations, while greater variability was observed at lower levels. All intra-assay and inter-assay CVs were considered acceptable, as they remain below 15%, while for low analyte concentrations, values < 20% are deemed acceptable (1). This study establishes new ranges for testosterone levels in tomcats and defines clinically relevant cut-off values for distinguishing intact from castrated individuals. Testosterone > 0.27 ng/mL confirms intact status with 100% specificity, while values ≤ 0.27 ng/mL may include both intact and castrated tomcats, emphasising the need for complementary diagnostics. The best approach for confirming reproductive status remains combining testosterone measurement with penile spikes assessment or a GnRH stimulation test. This study provides valuable insights for clinical practice, particularly in detecting testicular tissue in suspected cryptorchids.

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6436/7757 | Can canine prostate-specific esterase predict frozen–thawed semen quality?

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Semen cryopreservation is essential in canine reproductive biology for genetic preservation and breeding across time and distance. However, the freezing process can compromise sperm integrity, and the extent of its impact on post-thaw quality remains difficult to predict. Canine prostate-specific esterase (CPSE) is a biomarker of prostatic function, the latter being strongly related to the susceptibility of sperm to cryoinjury (1). This study aimed to assess whether CPSE levels can predict the quality of frozen–thawed semen, regardless of the presence of prostate disorders.

Fifteen adult dogs without visible prostatic or testicular lesions (confirmed via ultrasound) were selected for sperm cryopreservation. Semen was collected by digital manipulation without a teaser bitch. The sperm-rich fraction was extended using a two-step extender method (2) and frozen with a constant-rate freezing device (IceCube 1810, SyLab, Austria) following a previously described curve (3). Fresh and thawed semen samples underwent identical analyses, including total motility (TM) and progressive motility (PM). Kinematic parameters—average path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), wobble (WOB), straightness (STR) and linearity (LIN)—were evaluated using a CASA system (ISASv1, Proiser, Spain). Morphology was assessed via eosin-nigrosin staining. Plasma membrane integrity, acrosome integrity, and mitochondrial membrane potential were analysed using a modified triple fluorescent procedure with propidium-iodide, PNA-FITC and JC-1. Serum CPSE levels were measured using a canine-specific ELISA kit (AFG Bioscience, USA) on samples collected immediately after semen collection and stored at −20°C. Data analysis was conducted using IBM SPSS 26.0 (USA).

The median age of the dogs was 3.5 years (IQR = 3.17), and median CPSE levels were 56.33 ng/mL (IQR = 15.56). CPSE negatively correlated with frozen–thawed TM ($r = -0.854$, $p < 0.001$) and PM ($r = -0.808$, $p < 0.001$). The TM drop after freezing correlated positively with CPSE levels ($r = 0.546$, $p < 0.001$). Linear regression analysis showed CPSE had a significant association with post-thaw TM decrease ($p = 0.035$, $R^2 = 0.298$), with each 1 ng/mL CPSE increase linked to a 0.604% TM drop ($p = 0.035$, 95% CI: 0.048–1.161). CPSE negatively correlated with VAP ($r = -0.546$, $p < 0.05$), LIN ($r = -0.553$, $p < 0.05$), WOB ($r = -0.641$, $p < 0.05$), and BCF ($r = -0.552$, $p < 0.05$). Higher CPSE levels correlated with increased morphological abnormalities in frozen–thawed sperm ($r = 0.675$, $p < 0.01$), particularly major abnormalities ($\rho = 0.567$, $p < 0.05$). No significant correlation was found with plasma membrane integrity, acrosome integrity or mitochondrial membrane potential ($p > 0.05$).

Serum CPSE appears to be a potential predictor of frozen–thawed semen quality, with higher levels associated with reduced freezability, characterised by decreased motility and velocity, as well as increased morphological abnormalities. Larger studies are required to validate these findings.

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6509/7857 | Glycosylation pattern in the endometrium of bitches with endometritis

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Histological endometritis is a condition that may occur in clinically healthy bitches (1), and its pathogenesis continues to be a topic of study in this and other domestic species.

Glycoconjugates of the surface of the endometrial epithelium interact with the embryo during implantation. Previous studies have shown that modifications of glycoconjugates in the canine endometrium are associated with endometrial hyperplasia and pyometra (2,3). The objective was to study the glycosylation pattern of the normal canine endometrium and that of the endometrium with endometritis.

A total of 24 histological sections of uterine horns from clinically healthy mixed-breed bitches, in diestrus, between 1 and 6 years were studied. Samples with normal endometrium ($n=6$), with acute endometritis ($n=6$), with subacute endometritis ($n=6$) and with chronic endometritis ($n=6$) were evaluated according to previous descriptions (1) Histological sections have been deparaffinised and incubated with lectins biotinylated CON-A, SBA, WGA, UEA-I (Lectin Kit Biotinylated BK 1000; Vector Laboratories Inc., Burlingame, CA, USA) following the specifications of the datasheet. Each sample was evaluated in three fields arbitrarily chosen with an optical microscope at ×400. The staining intensity was assessed with a semiquantitative scale: 0, no mark; 1, weak mark; 2, moderate mark; 3, strong mark. Two independent operators performed the evaluations in a double-blind model. The frequencies of the categorical variables were compared using chi-square tests with the statistical program Deducer R, Rv.2.15.0.

WGA lectins showed a strong mark in the apex of the endometrial epithelium in chronic and acute endometritis and a moderate mark in subacute endometritis and normal endometrium. In the cytoplasm of cells of superficial and deep endometrial glands, moderate marking was recorded in chronic endometritis, while the marking was weak in the other groups ($p<0.001$). UEA-I lectin showed a weak mark in the apex of the superficial endometrial epithelium in acute and subacute endometritis and moderate in normal endometrium and chronic endometritis. CON-A lectin showed a moderate mark in the cytoplasm of cells of superficial endometrial glands in normal endometrium and was weak in all endometritis groups. SBA lectin showed a moderate mark in the apex of the superficial endometrial epithelium in normal endometrium and was strong in the endometritis groups ($p<0.001$).

This study identified distinct glycosylation patterns in the endometrium of bitches with endometritis compared to healthy tissue. The changes in the lectin patters of CON-A, SBA, WGA, and UEA-I lectins observed in this study could alter the normal function of the endometrium and interfere with the embryo implantation, causing infertility. Further research is required to elucidate the role of glycosylation in the pathogenesis and progression of canine endometritis.

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6497/7828 | Histopathologic findings in placental biopsies of liveborn and stillborn puppies

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Canine neonatology is a critical yet underdeveloped field within veterinary medicine. Neonatal mortality and stillborn are frequent reasons for consultation in veterinary clinics, highlighting the need to advance knowledge in this area to improve the weaning rates. In this way, increasing neonatal assessment tools in the peripartum could help reduce neonatal mortality rates in this species. This study aimed to evaluate canine placentas from liveborn and stillborn puppies to identify lesions that could affect the survival of newborn puppies. Sixty-five placentas of bitches between 1 and 10 years old, mixed breeds and of different breeds were used in this study (emergency caesarean, $n=52$, elective caesarean, $n=11$, and natural birth, $n=2$). Thirty-five placentas belonged to live neonates and 30 to stillborn. All the stillborn belonged to the emergency group. The placentas obtained were separated from the extraplacental membranes and washed in physiological solution. Macroscopic congestion and necrosis were assessed with a scale of 0 to 3(1). Then, a biopsy was obtained from the central region, fixed in formalin until histological processing and staining with H&E. Later, the presence of necrosis, congestion, mineralisation, and infiltration of polymorphonuclear neutrophils (PMNN) were evaluated with optical microscopy at ×400 with a scale of 0 to 2(1). The frequencies of the categorical variables of the placentas of live and dead neonates were compared using chi-square tests with the statistical program Deducer R, Rv.2.15.0.

Macroscopic congestion was observed in 29% of placentas from live neonates (10/35; 0=25, 1=4, 2=4, 3=2) and 77% of placentas from stillborn (23/30; 0=7, 1=3, 2=12, 3=8; $p<0.001$); macroscopic necrosis was observed in 11% of placentas from live born (4/35; 0=31.1=4, 2=0, 3=0) and 57% from stillborn (17/30; 0=13, 1=4, 2=6, 3=7; $p<0.001$). In addition, microscopic congestion was the most prevalent lesion in both groups and was observed in 86% of placentas from live birth (31/35, 0=4, 1=23, 2=8) and 93% from stillborn (28/30; 0=2, 1=14, 2=14; $p=0.5$). Microscopic necrosis was observed in 49% of placentas from live neonates (17/35, 0=18, 1=16, 2=1) and 77% from stillborn (23/30, 0=7, 1=18, 2=5; $p=0.02$), infiltration of PMNN in 37% of placentas from live-neonates (13/35, 0=22, 1=12, 2=1) and 73% from stillborn (22/30, 0=8, 1=13, 2=9; $p=0.004$) and mineralisation in 20% (6/30, 0=24, 1=1, 2=5) of stillborn and it was not observed in placentas of live neonates ($p=0.005$).

This study showed significant differences in the placental evaluation between the groups. It also identified placental lesions in live neonates. Although these lesions could be associated with sample handling, future studies will assess whether they may be related to puppies at risk during the neonatal period. Placental assessment could help identify at-risk neonates.

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7162/7784 | Association of ivermectin and vincristine in the treatment of cutaneous canine transmissible venereal tumour (CTVT) in the cervical region: A case report

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Canine Transmissible Venereal Tumour (CTVT) is a common transmissible neoplasia in dogs, usually associated with the genital region but capable of affecting other organs. The standard treatment for CTVT typically consists of chemotherapy, and vincristine is used as the preferred drug due to its effectiveness in most cases. However, resistance to treatment and adverse effects caused by chemotherapy may necessitate the adoption of additional therapeutic approaches. Ivermectin, traditionally used for parasite control, has shown therapeutic potential as an adjuvant in CTVT treatment when combined with vincristine (1). It can inhibit P-glycoprotein, a membrane protein responsible primarily for transmembrane drug transport. Plasmacytoid-type CTVT exhibits greater resistance to vincristine and higher P-glycoprotein expression, making the tumour less sensitive to chemotherapy. Therefore, ivermectin may be a valuable adjunct in treatment. This case report aims to describe the association of ivermectin and vincristine in the treatment of a cutaneous CTVT, highlighting the clinical response to the adopted therapeutic protocol and discussing the efficacy of this combination as an alternative treatment.

Clinical case. A 7-year-old, 5.6 kg, intact male mixed-breed dog was presented with a progressive-growing cutaneous ulcerated mass in the ventral cervical region, left of the midline, measuring 5 × 5 cm. The dog had free access to the street and lived with two other contact dogs. On physical examination, the patient showed signs of dehydration, pale mucous membranes and verrucous nodules on the bulb and body of the penis. Additionally, enlargement of the left prescapular lymph node was noted. Cytological evaluation of the mass was consistent with a plasmacytoid variant of canine transmissible venereal tumour (CTVT-cP). Chemotherapy with vincristine (0.5 mg/m^2, IV) was initiated, alongside antibiotic therapy, analgesics, and anti-inflammatory medication. A second chemotherapy session was performed 1 week later; however, the patient did not return for the third session as scheduled. Seventeen days after the second treatment, the dog returned with marked progression of the cutaneous lesion, now measuring 12 cm in diameter. A complete blood count revealed mild leukocytosis secondary to neutrophilia. A third vincristine session was administered (0.7 mg/m^2, IV). One week later, a significant reduction in mass size was observed. However, haematological evaluation showed notable alterations, including anaemia and leukopenia due to neutropenia. Following the therapeutic protocol, ivermectin was administered at a dose of 0.5 mg/kg SC, along with the fourth vincristine session. One week later, the reduction of the lesion was noted, accompanied by improvement in anaemia and normalisation of the leukogram. Unfortunately, the treatment protocol could not be completed, as the owners failed to return for the subsequent follow-up appointments.

This case illustrates the potential of combining vincristine and ivermectin in the treatment of CTVT-cP, demonstrating a significant reduction in tumour size after four treatment sessions. The administration of ivermectin alongside vincristine led to tumour shrinkage without exacerbating adverse reactions such as leukopenia. This report highlights the importance of adjuvant therapies in the management of CTVT, expanding treatment options and improving clinical outcomes.

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7150/7829 | The prevalence of pathologies of the canine penis and prepuce in a reproduction centre

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Pathologies of the canine penis and prepuce seem to be of low rate in the caseload of theriogenologists. In fact, common lesions in the male reproductive tract of dogs seem to be difficult to determine since they vary from one geographical location to another (1). Nevertheless, some cases are difficult to treat and they are often of poor prognosis in case of stud dogs. The aim of this study was to retrieve cases of penile and preputial problems that motivated a consultation at the reproduction centre in the last 5 years. Furthermore, to characterise the affected population, and to determine possible risk factors in dogs.

The medical records of the centre of reproduction were searched for 1150 cases of male dog consultations from 03/2020 to 03/2025. Cases of penile and preputial pathologies (balanoposthitis, canine transmissible venereal tumour, paraphimosis, phimosis, priapism, urethral prolapse, persistent frenulum and lesions of the penis) with all relevant information were extracted and analysed.

The penile pathologies represented 4% of the andrology caseload. Sixty-five dogs were presented at the reproduction centre with a clinical complain associated to a penile pathology. For 14 dogs (22%), no lesion was observed on the penis/prepuce and the problem described was related to a behavioural problem or normal physiological features of the male reproductive tract (presence of smegma or normal erection). For the pathologies related to erection, 4 presented with priapism, 12 with paraphimosis and one dog with phimosis. Inflammatory lesions, neoplasia, trauma and developmental disorders affected respectively 13, 6, 5 and 3 dogs. Only one dog was diagnosed with canine transmissible venereal tumour. Penile pathologies seem not to have a breed predisposition, except for the urethral prolapse observed in five American bully and one English bulldog. Reproductive status was a risk factor since the majority of dogs (70%, 36/51) presented with penile lesions were intact. The age of the dogs varied from 6 months to 16.5 years with a median of 4.7 years.

Penile and preputial pathologies in dogs are not frequent, no age or predisposition of any specific breed was identified, except for the idiopathic urethral prolapse in English bulldogs and American bullies. Reproductive status seems to be a risk factor.

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7117 | CT and histopathological findings in canine fetal anasarcas

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Fetal anasarca is characterised by generalised edema, resulting in disproportionate body dimensions due to subcutaneous and intracavitary fluid accumulation that is incompatible with life. This condition often leads to obstructive dystocia, necessitating caesarean sections (1). Anasarca may affect one or more puppies within a litter (2) and is commonly reported in brachycephalic breeds, potentially due to inbreeding and the expression of recessive genes (3). Although two-dimensional ultrasound can aid in early diagnosis (4), there are few studies describing necropsy or histopathological findings and none have addressed computed tomography (CT). This study aimed to evaluate fluid distribution in the body, cavities, and organs of affected neonates through morphological and morphometric analyses, while preserving specimens for post-mortem examination and histopathology.

Twenty neonatal canines (18 English Bulldog, 1 German Spitz, and 1 American Bully) diagnosed with foetal anasarca were examined post-mortem. Three puppies underwent CT scanning followed by immediate necropsy, five underwent necropsy only, while the remaining specimens were frozen at −20°C, then thawed, scanned and necropsied. External and internal evaluations were conducted, and organs were collected for histopathology in 10% buffered formalin (excluding those previously frozen).

Maternal history revealed significant risk factors, including multidrug-resistant infections, reproductive disorders, indiscriminate drug use, and inbreeding. CT identified fluid accumulation with attenuation values similar to water density, confirming edema distribution across different body regions. Necropsy and histopathological analyses corroborated the CT findings, revealing pulmonary hypoplasia (20/20; 100%), pleural effusion (20/20; 100%) and cardiac alterations such as hydropic degeneration of cardiomyocytes (7/8; 87.5%). Potential lissencephaly and incomplete myelination (20/20; 100%) were also noted; however, incomplete myelination can occur in healthy newborns and develops fully after 16 weeks of life (5). Therefore, confirmation of lissencephaly and incomplete myelination can only be validated if puppies with anasarca survive until this age. Unfortunately, with the simultaneous occurrence of multiple alterations, the condition typically becomes incompatible with life, resulting in nearly zero survival rates. Lower degrees of edema may respond to treatment with diuretics, and CT can assist in determining the extent of the edema. In human medicine, hydrops has been attributed to congestive heart failure, hypoxia and congenital lymphatic dysplasia. Our necropsy and histopathology findings suggest that hydrops due to heart failure and hypoxia may also be relevant in dogs.

The study identified key pathological changes including pulmonary hypoplasia, pleural effusion, and cardiac alterations. While lower degrees of edema may be alleviated with diuretic treatment, the multifaceted nature of anasarca renders it incompatible with life. CT proved to be a valuable tool for assessing the extent of edema and guiding the selection of candidates for treatment. Overall, these findings underscore the importance of vigilant maternal health monitoring and genetic management to reduce the incidence of anasarca in canine litters.

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6528/7841 | The role of vaginal bacteria and antibiotics on canine fertility

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In canine reproduction, microbiological examinations of the vagina and antimicrobial treatment prior to mating are frequently performed. However, recent studies found no differences in the vaginal microbiota between healthy dogs and those with clinical abnormalities in the reproductive tract, indicating not to treat healthy bitches with antibiotics regardless the bacterial findings (1). Nevertheless, many breeders still request vaginal swab sampling and antibiotic prescription due to their fear of infectious infertility. However, inappropriate use of antibiotics exacerbates antimicrobial resistance (2). This study investigated the pregnancy-outcome of healthy dogs in correlation to bacteriological examination results and antimicrobial use aiming to assess the effect on canine fertility.

We retrospectively analysed bacteriological examination results of vaginal swabs collected from clinically healthy breeding bitches between January 2016 and July 2023. All dogs underwent a general and gynaecological examination, and vaginal swabs were collected with a tube speculum. The specimens were examined using culture-based techniques. Identification was carried out with cultural and biochemical parameters and MALDI-TOF. Subsequent information about antimicrobial treatment and pregnancy-outcome was obtained from the medical records. Microsoft Excel and Graph Pad Prism were applied for data analysis.

A total of 807 samples with 53 different bacterial species were analysed. Of these, 2.6% showed no bacterial growth, 15.9% were monocultures, and 81.5% were mixed cultures. The most frequently isolated bacteria/bacterial groups were alpha-hemolytic streptococci, *Haemophilus haemoglobinophilus, Streptococcus canis/beta-hemolytic streptococci, Escherichia coli* and *Staphylococcus intermedius* group (SIG). Information about antimicrobial use and pregnancy-outcome was available in 376 cases. Fifty-nine animals had confirmed bacterial monocultures. Of those, 11 were treated with confirmed proof

of *Escherichia coli* (3x high-grade), *Streptococcus canis* (3x high-grade, 1x intermediate-grade), SIG (1x intermediate-grade), *Haemophilus haemoglobinophilus* (1x high-grade), *Klebsiella pneumoniae* (1x high-grade) and *Mycoplasma* spp. (1x low-grade). Of those, 9 animals got pregnant, 2 were non-pregnant (*Streptococcus canis* and *Mycoplasma* spp.). Pregnancy was confirmed in 35 of 48 untreated ones, including some with opportunistic pathogens (*Streptococcus canis*: 3x high-grade, 1x low-grade; *Escherichia coli*: 1x intermediate-grade). The remaining 13 were non-pregnant including monocultures of alpha-hemolytic streptococci (3x intermediate-grade, 2x low-grade), *Enterococcus faecalis* (1x low-grade), *Haemophilus haemoglobinophilus* (1x low-grade), coagulase-negative staphylococci (1x low-grade), SIG (3x low-grade), ß-hemolytic streptococci (1x low-grade) and *Escherichia coli* (1x low-grade). Concerning high-grade growth of *Escherichia coli*, 8/8 untreated and 20/22 treated bitches got pregnant with no significant differences between groups ($p > 0.9999$). Similarly with high-grade growth of *Streptococcus canis*, pregnancy was observed in 8/9 untreated and 34/40 treated dogs ($p > 0.9999$). Similarly, no significant differences were identified regarding pregnancy rates in treated (87/107, 81.3%) and untreated bitches (210/269, 78.1%) ($p = 0.5751$).

Our data show that antimicrobial use in healthy bitches does not improve fertility, even in cases of opportunistic pathogens. Consequently, other factors are more likely to influence the pregnancy outcome in healthy bitches clearly confirming that use of antimicrobials should critically evaluated in healthy bitches.

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6385 | Adropin and its potential role in regulating ovarian function in the female dogs

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Adropin is a peptide that plays a role in regulating energy metabolism. It is encoded by the energy homeostasis associated (Enho) gene (1). The potential role of adropin in the female reproductive system is not well understood, especially in female dogs. Research indicates that adropin enhances secretion of progesterone and estradiol, as well as the production of steroidogenic proteins in mouse ovaries. Moreover, adropin may be involved in the formation of the corpus luteum (2). Lower levels of adropin have been observed in women with polycystic ovary syndrome (3). Adropin may also play a key regulatory role in the activity of immune cells and inflammatory factors, since expression of this peptide is reduced in various inflammatory diseases (4).

The aim of the study was to outline the potential role of adropin in healthy ovarian tissue compared to ovarian cysts, by characterising the expression and localisation of adropin in ovarian tissue and its blood concentration in female dogs. Additionally, the study aimed to investigate how disorders affecting the uterus, such as cystic endometrial hyperplasia (CEH) and pyometra, may influence changes in adropin expression in ovarian tissue.

Ovarian fragments were collected from 70 female dogs undergoing ovariohysterectomy. The dogs were categorised into a healthy control group and those with ovarian cysts, or CEH, or pyometra. All animals were in the diestrus or anestrous phase. The analyses used in the study included RT-qPCR, western blot, and immunofluorescent assays. Moreover, in blood plasma adropin and progesterone concentration were detected.

The results showed increased expression of Enho in the ovarian tissue of healthy female dogs compared to animals with healthy gonads and coexisting pathologically changed uterus ($p < 0.05$). However, higher protein signals were shown in healthy ovarian tissue collected from animals affected by CEH and pyometra compared to control group ($p < 0.05$). The development of various types of ovarian cysts reduced Enho mRNA and adropin production within the ovarian tissue ($p < 0.05$). Immunoreactivity of adropin was observed in granulosa cells-in antral follicles, and in the luteal cells of the corpus luteum. There was a positive correlation between adropin and the P4 concentration in dogs' plasma ($r = 0.43$, $p < 0.01$). In ovarian cysts, adropin was detected in the wall of epithelial cysts and corpus luteum cysts, and in follicular cysts albeit with a lower immunoreactivity.

The expression of adropin during development of canine ovarian activity may be changing. The development of ovarian cysts may lead to a decrease in adropin production. This peptide may also provide an immunoprotective effect for ovarian tissue in females suffering from uterine diseases.

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6366 | Successful postoperative management of neonates with gastroschisis

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Gastroschisis is a congenital malformation which makes the abdominal organs protrude outside the body through a defect in the anterior abdominal wall. The standard procedure is surgery, but the postoperative period remains an essential stage that largely determines patients' survival and further quality of life, but it is not described in relevant literature.

Neonates with gastroschisis often face the following complications during and after surgery:

- Hypothermia;
- Hypoglycemia;
- Pain syndrome.

As a result, the gastrointestinal function is disrupted, particularly, peristalsis is lost. The described case follows the treatment of three neonates: one kitten and two puppies from different litters. Surgical treatment was performed immediately after birth for the kitten and one puppy, and on the second day after birth for the other puppy. The defect was closed by skin suturing

(without closing the peritoneum) before surgery immediately after birth. Special attention was paid after surgery to maintain their stable condition.

One of essential tasks was to prevent hypothermia, as body cooling results in subsequent occurrence of the neonatal triad. Hypothermia was corrected with heating pads during and after surgery. One of the most common complications after gastroschisis surgery is intestinal atony. Two patients had the sucking reflex, which allowed for early preventive peristalsis stimulation before hypotonia occurred. For this purpose, domperidone was used (1 mg/kg orally every 8 h). Pain management was performed (metamizole sodium, 25 mg/kg every 8 h) with antibiotic therapy (amoxicillin-clavulanic acid, 25 mg/kg orally every 12 h) to prevent infectious complications. The third patient had no sucking reflex. Intestinal atony was diagnosed after ultrasonography, which required more active treatment. Throughout the day the neonate received intravenous infusion. Feeding did not begin until the intestinal atony was resolved. Hypoglycemia was corrected through sublingual administration of 40% dextrose. After peristalsis was restored, tube feeding was initiated. By the moment of the puppy's discharge the sucking reflex was restored, and breast feeding was continued.

Dehydration was treated via fluid therapy (crystalloid solutions, 1.5 mL/h). To stimulate peristalsis, metoclopramide was used via a continuous infusion (0.5 mg/kg/h), since initial attempts of bolus administration every 4 h were unsuccessful. Monitoring was performed by ultrasonography. Azithromycin (3.5 mg/kg once a day) was included as a prokinetic agent. After discharge from the ICU, the third patient received metoclopramide intramuscularly (0.5 mg/kg every 8 h) with a subsequent transition to oral administration of domperidone (1 mg/kg). In addition, the patient received antibiotics (ceftriaxone, 25 mg/kg every 8 h) intravenously, then orally during outpatient care. The analgesic effect was achieved in the ICU by administering metamizole sodium (25 mg/kg every 8 h) and continuous infusion of lidocaine hydrochloride (25 mcg/kg per minute). One patient was treated in the hospital, the other two received outpatient treatment immediately. All children recovered with 100% survival rate, confirming the importance of thorough postoperative management. Postoperative management of gastroschisis remains a challenging task requiring a multidisciplinary approach. These cases demonstrate that positive outcomes are possible with correct medications and treatment methods even in cases with severe peristaltic disorders and no sucking reflex if proper medications and treatment methods are applied.

6443/7770 | Vaginal bacterial isolates in metoestrous bitches remain similar after natural mating or transcervical insemination

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Artificial insemination using fresh semen is widely used in dog breeding, especially when natural mating (NM) is not feasible. Some breeders prefer artificial insemination to minimise bacterial exchange, and prophylactic antibiotics are used to clean the genital tract and enhance fertility. However, semen carries a distinct microbiome, which is still introduced into the female during artificial insemination. While culture-based studies report up to 10^5 bacteria/mL in semen [1], DNA-based methods reveal an even higher bacterial load. This study compares results of vaginal bacterial culture before and after NM and transcervical insemination (TCI) with fresh semen to assess changes in the number of bacterial isolates per sample and their grades of growth. Vaginal samples from 10 dogs (NM = 4; TCI = 6) were collected using a double-guarded swab and submitted for routine aerobic bacterial culture on Columbia, Columbia Nalidixic Acid, and MacConkey agar. Pre-mating samples were obtained around ovulation, as determined by serum progesterone levels, ovarian ultrasound, and vaginal cytology. Post-mating samples were collected at pregnancy diagnosis, 3 weeks after ovulation. The number of bacterial isolates per sample and their grade of growth were compared pre- and post-mating using the Wilcoxon Signed-Rank test, while differences between NM and TCI were analysed with the Mann–Whitney U-test. Fisher's exact test assessed bacterial species matches before and after mating. Significance was set at $p < 0.05$. Six different bacterial species were isolated across all samples, with *Streptococcus canis* ($n = 10$) and *Haemophilus haemoglobinophilus* ($n = 7$) being the most frequently detected. Number of isolates and growth grades were significantly higher during estrus compared to metestrus in both groups ($p = 0.043$; resp., $p = 0.007$). No significant differences were observed between NM and TCI groups in changes of the number of isolates ($p = 0.610$) or grades of growth before and after mating ($p = 0.406$). No significant difference in species matches was found between NM and TCI groups ($p = 0.24$; match: ≥ one species the same; $p = 0.99$ different: ≥ one species different). Although no significant differences were observed, all NM samples obtained at metestrus were negative ($n = 2$) or monocultures ($n = 2$). In this small-scale study, no significant differences in vaginal bacterial load were observed between NM and TCI. However, penile introduction and exposure to the first and third semen fractions may trigger a stronger post-mating immune response in NM compared to TCI, though further data are needed to confirm this hypothesis. Our findings support previous research showing that bacterial diversity and growth increase during estrus, likely due to hormonal influences and changes in the vaginal environment [2]. More comprehensive studies with larger sample sizes and molecular techniques such as 16S rRNA sequencing could provide deeper insights into potential differences in the vaginal microbiome, as culture-based methods fail to detect over 90% of the microbial community.

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6445 | Effect of an anti-GnRh vaccine on benign prostatic hyperplasia treatment in dogs

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Benign prostatic hyperplasia (BPH) is a common prostatic disease in dogs. In canines, BPH affects approximately 80% of

intact male dogs over 5 years of age and approximately 95% of intact male dogs over 9 years of age. Clinical signs associated with BPH include tenesmus, bloody urethral discharge and hematospermia. In addition, bacterial prostatitis and recurrent urinary tract infections are commonly associated with BPH. The main triggering factor for this disease is an imbalance between testosterone and oestrogen synthesis, leading to an increase in the prostatic androgen receptor and a marked conversion of testosterone to dihydrotestosterone (1). The Improvac vaccine is a GnRH analogue coupled with diphtheria toxoid, which stimulates the production of anti-GnRH antibodies that bind to endogenous GnRH, preventing it from binding to pituitary gonadotropin receptors. As a result, LH, FSH and testosterone levels are reduced. The hypothesis was that the Improvac vaccine produces prostate volume reduction. This work aimed to evaluate the efficacy of the Improvac vaccine in treating BPH.
Male dogs ($n=6$) between 5 and 10 years of age, intact, weighing 10 to 40 kg, with normal renal and hepatic function and with a BPH diagnostic at the study's beginning were used. The animals ate premium, balanced food during the experiment and were subjected to a natural light regime. Subsequently, each animal was administered two 1 mL doses of Improvac vaccine (Zoetis, Belgium) subcutaneously (sc) in the left rib cage on day 1 and day 30. Blood samples were obtained every 30 days for 8 months, and testosterone concentrations were measured by electrochemiluminescence immunoassay (Testosterone II, Elecsys and Cobas e analysers, Roche, Mannheim, Germany). In addition, abdominal ultrasound was performed once a month during the study to obtain prostatic volume and testicular length (a), width (b) and depth (c) (mm) were recorded with a calliper to calculate testicular volume using the equation of a modified sphere (volume [mm3] = 4/3* π *(½ a*½ b*½ c). Data were analysed by ANOVA using SAS GLIMMIX.
Testicular volume decreased by 27.58% at week 4, 59.81% at week 8, and 96.88% at week 12 compared to the initial value. Prostate volume decreased by 66.80% at week 4, 93.68% at week 8, and 94.68% at week 12 compared to the initial value. Testicular and prostate volume reduction is maintained to the end of the study. Serum testosterone concentrations dropped to basal levels 8 weeks after the first immunisation (0.65 ± 0.18 ng/mL). This basal level was maintained until the end of the study ($p < 0.01$).
Our results showed that two doses of the Improvac vaccine could help treat BHP in dogs, especially those with high-risk surgical castration. However, more studies are necessary to determine the length of the vaccine's effect on the prostate.

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6384 | Effect of an anti-GnRH vaccine in bitches: Preliminary results

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The emergence of stray dog populations has a high social and economic impact, especially in marginal neighbourhoods and regions with high population density. These stray animal populations affect the population's health, and the animals suffer from a lack of animal welfare. Hence, contraceptive methods without side effects are needed to control reproduction temporarily. The Improvac vaccine is a GnRH analog linked to a diphtheria toxoid, which stimulates the production of anti-GnRH antibodies that bind to endogenous GnRH, preventing it from binding to the receptors of the pituitary gonadotrophs (1). Consequently, LH and FSH hormones are reduced, failing follicular development and ovulation in females (2). The objective was to evaluate the efficacy of the Improvac vaccine to avoid heat in canines.
Female canine ($n=8$) between 1 and 7 years of age, clinically healthy, in late anestrus, and included in a reproduction control program were used and monitored for 16 months. Each animal underwent a clinical examination to assess its health status and vaginal cytology to confirm anestrus. Subsequently, each animal was administered two 1 mL doses of Improvac vaccine (Zoetis, Belgium) subcutaneously (sc) on day 1 and day 30. Blood samples were obtained for a complete blood count and serum progesterone concentration on the day of entry into the study and then every 30 days until the end of the study or until signs and vaginal cytology compatible with heat were evident. A sample for serum oestrogen concentration was collected during estrus values expressed as mean days± standard deviation. Data were analysed by ANOVA using SAS GLIMMIX.
Haematological and biochemical values (haematocrit [%], haemoglobin [g/dl], erythrocytes [106/ µl], leukocytes [103/ µl], segmented neutrophils [%], immature neutrophils [%], lymphocytes [%], eosinophils [%], monocytes [%], total solids) in all bitches agree within reports for the species. (45.81 ± 0.23, 15.43 ± 0.09, 6966.35 ± 237.04, 10192.66 ± 1294.51, 7.41 ± 0.68, 61.50 ± 0.19, 2.14 ± 0.049, 27.56 ± 0.14, 6.32 ± 0.069, 2.2 ± 0.084). Pre-vaccination interestrus was shorter than post-vaccination interestrus (195.62 ± 37.38 vs. 368.25 ± 89.11; $p \leq 0.05$; values expressed as mean ± standard deviation). Serum progesterone concentration values remained basal during the study (0.5 ng/mL).
Our results showed that two doses of the Improvac vaccine, administered during anestrus, could lengthen the inter-estrous interval and did not affect haematological parameters. Thus, if administered annually, the Improvac vaccine may be an alternative for controlling reproduction in this species.

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6339/7806 | Differences in haematological, hormonal, and semen parameters in dogs with subclinical haemoparasitism under subtropical conditions

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Haemoparasitism is a significant health concern in canines, particularly in subtropical regions, where environmental conditions favour the spread of parasites such as Babesia, Ehrlichia and Anaplasma species. Many infected dogs exhibit subclinical infections with low levels of parasitemia, often remaining undiagnosed (1, 2). The potential role of subclinical infections as an underlying cause of male infertility remains underexplored

(3, 4). The current study aims to address this gap by evaluating the effects of subclinical haemoparasitism on haematological, hormonal and semen parameters in male dogs. Additionally, it seeks to investigate the potential reproductive consequences of such infections and explore their possible role in male infertility.
The study was conducted on 50 adult male dogs, aged 2–6 years, from one local breed (Bully) and two exotic breeds (German Shepherd and Labrador Retriever). All dogs enrolled in the study were clinically healthy, with no signs of illness or disease. Thin blood films were prepared, field-stained, and examined microscopically for the presence or absence of intraerythrocytic parasitic inclusions. A complete blood count was performed using an automated haematology analyser (BC-30Vet, Mindray, China). Serum testosterone and cortisol levels were measured using chemiluminescence immunoassay (MiniVIDAS, Biomerieux, France). Semen analysis included macroscopic, microscopic, and computer-assisted sperm analysis (AndroVision, Minitube, Germany). Differences in the parameters between dogs with and without haemoparasitism were analysed using an independent t-test ($p < 0.05$).
Haemoglobin, red blood cell count, and haematocrit were significantly lower, while monocyte and eosinophil counts were significantly higher in the haemoparasite-positive group ($p < 0.05$). No significant differences were observed in testosterone or cortisol levels ($p > 0.05$). Semen parameters, including total motility, progressive motility, fast motility, curvilinear velocity, average path velocity, concentration, viability, morphology, plasma membrane integrity and mitochondrial membrane integrity, were significantly reduced in the haemoparasite-positive group ($p < 0.05$).
Subclinical haemoparasitism in dogs was associated with significant alterations in haematological parameters and semen quality, suggesting potential implications for male fertility, without clear evidence of involvement of the endocrine pathways. Therefore, screening for haemoparasitism, even in asymptomatic dogs, is recommended as a component of breeding soundness examinations. Such proactive parasite control strategies may contribute to improving both general health and reproductive performance in dogs.

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6943/7639 | Breed-specific prostatic volume variation in dogs: A comparative analysis based on body weight

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The prostate gland is a key anatomical and functional component of the male reproductive system, contributing to seminal fluid production and ejaculate composition. Prostate size in dogs may vary depending on breed, body weight, age, and hormonal status This study aimed to quantitatively analyse the relationship between prostate size and body weight by calculating mean prostate volumes in different dog breeds.
This study was performed with 29 dogs of various breeds. The dogs were divided into two groups according to body weight: Group 1 (30–44 kg, $n = 16$) and Group 2 (≥45 kg, $n = 13$). Prostatic dimensions (length, width, height) were measured via ultrasonography, and volumes were calculated using the ellipsoid formula (length × width × height ×0.523) (1). The arithmetic formulas of volume calculations were determined separately, and arithmetic averages were found for each group.
Following standardised ultrasonographic measurements, the mean prostate volume was $22.98 \pm 9.58\,cm^3$ in Group 1 and $33.72 \pm 12.40\,cm^3$ in Group 2. Among the breeds in Group 1, Malinois (~$17.10cm^3$) and Golden Retrievers (~$16.25cm^3$) had relatively smaller prostate volumes compared to other breeds. In Group 2, larger breeds like Doberman (~$35.10cm^3$) and Turkish Shepherds (~$41.77cm^3$) showed higher prostate volumes than other breeds in the same group. A positive trend was observed between increasing body weight and prostate size (2, 3); however, variations within similar weight categories show that breed-specific anatomical features may also influence prostate size independently of weight.
This study emphasises the importance of considering breed and weight in the assessment of prostatic dimensions. Prostatic size assessments based on these weight ranges may clinically contribute to more accurate diagnosis in canine andrology practice and to assess the general condition of the animal.

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7160/7842 | Treatment outcome and follow-up treatment in dogs with benign prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) is the most common prostatic condition in intact male dogs. Both pharmacological (osaterone acetate, finasteride and deslorelin) and surgical (orchiectomy) approaches are available (1). The aim of this study was to assess therapeutic choices after initial treatment and impact on quality of life of the different treatment modalities.
Clinical records of 51 dogs diagnosed with BPH between 2021 and 2025 were retrospectively assessed. Diagnosis was based on signalment, physical and reproductive examination, hematobiochemistry, and reproductive ultrasound. Treatment choices depended on the severity of symptoms, biochemistry results and owners' intention to keep the dog intact.
The mean age and weight of the subjects was 8.6 ± 3.1 years and 27.4 ± 12.8 kg, respectively. Sixteen/51 cases (31.4%) were asymptomatic. The most common clinical signs were penile blood discharge (23.5%), hematuria (11.8%), tenesmus (11.8%), and hemospermia (9.8%). Six dogs did not undergo treatment. Osaterone acetate was prescribed as a first approach to 35/51 dogs, from which 28 didn't receive any additional treatment. One dog presented partial alopecia on the neck and hips, and another abundant hair shedding accompanied by character change. Over 6 months after administration, 7 dogs experienced

recurrence of symptoms, and required a change in treatment: orchiectomy $n=3$, deslorelin $n=3$ and finasteride $n=1$. Finasteride (0.1–0.5 mg/kg SID) was prescribed to three dogs with intermittent treatment cycles of 3–4 months duration followed by 1–2 months interval. One dog continued the treatment without modifications, while two required alternative therapy, switching to osaterone acetate and deslorelin, the latter showing significant hair loss in the neck and forelimbs.

Deslorelin (4.7 or 9.4 mg—dosage chosen in agreement with the owner based on expected duration of efficacy) was administered as first approach to 4 dogs and repeated in one. In another BPH had not recurred 6 months post-implatation. One dog never returned for control appointments, and another switched to osaterone acetate.

Surgical castration was initially performed in 1 dog. Another 3 dogs later underwent orchiectomy after the failure of pharmacological treatments (Osaterone acetate, Finasteride and Deslorelin) or the emergence of side effects. Thirty-six of the initial 51 dogs were eventually lost to follow-up as they did not return for control appointments, presumably due to absence of symptoms.

All treatment options were effective in eliminating clinical signs, though differences emerged in therapeutic duration and recurrence rates. For 7/34 animals treated with osaterone acetate, recurrence occurred after more than 6 months, and no animal repeated the treatment cycle. In two cases, osaterone acetate led to hair-related side effects. Finasteride was the least preferred approach, probably due to the inconvenience of daily administration for long periods. Alopecia was reported for the first time after deslorelin treatment. Surgical castration prevents recurrence while permanently altering hormonal balance. Treatment choice should be tailored to patient characteristics and owner preferences, considering long-term management goals. Further studies are needed to assess the impact of repeated pharmacological treatments on canine health.

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5966/7780 | Sertoli cell dynamics following during GnRH slow release implant downregulation and subsequent recovery in canine testis

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GnRH agonist slow-release implants (SRI) are widely used to temporarily suppress testicular function (1, 2). Effects are fully reversible, with testosterone recovering within 2–3 weeks after SRI removal. However, complete spermatogenic recovery requires 12–19 weeks (3) or even up to 29 weeks (4). Sertoli cells (SCs) provide structural and paracrine support to spermatogonial stem cells, but their response to hormonal suppression remains poorly understood. This study aimed to evaluate SC markers AR (Androgen Receptor), GDNF (Glial cell line-Derived Neurotrophic Factor), bFGF (basic Fibroblast Growth Factor), BMP4 (Bone Morphogenetic Protein 4), and FSHR (Follicle-Stimulating Hormone Receptor) in dogs treated with three different SRIs, comparing them to untreated controls.

Nineteen male dogs received an 18.5 mg azagly-nafarelin SRI for 5 months, followed by implant removal (W0). Testes were collected at W0 as well as 3, 6, 9, 12, and 24 weeks post-removal, with 3–5 dogs per time point. Additional three dogs each received a 6.3 mg buserelin SRI (PG) or a 4.7 mg deslorelin SRI (SG) and were castrated after 5 months. Untreated controls included five adult (CG) and three juvenile dogs (JG). SC markers (AR, GDNF, bFGF, BMP4, FSHR) were assessed using qPCR and IHC for AR, GDNF, and bFGF, with numbers of immunopositive SCs (NIPSCs) quantified in 20 seminiferous tubules per sample or semiquantitative scoring (AR only). Groupwise comparisons were conducted within the downregulation phase (W0, PG, SG, CG, JG; Gdown) and the recovery phase (W0–W24, CG; Grecov) using ANOVA and Kruskal-Wallis tests. Downregulation significantly reduced SC function (ANOVA-AR: $p<0.001$, Kruskal-Wallis-GDNF/bFGF: $p<0.05$ each), with lowest counts in JG, followed by W0, SG and PG. GDNF and FSHR gene expression were significantly upregulated in JG in Gdown (ANOVA-GDNF: $p<0.05$, FSHR: $p<0.0001$). During recovery, NIPSCs and scores also differed significantly across time points (ANOVA-AR/GDNF: $p<0.05$ each, bFGF: $p<0.001$), with lowest counts/scores at W0. Subsequent increases of GDNF and bFGF above CG-level expression suggest a gradual reactivation of SC function in later weeks. Gene expression in recovery (Grecov) showed no significant differences.

Low and absent AR protein expression during downregulation and in JG confirm the androgen dependency of SC function. The expression was, however, restored during recovery allowing for a rapid response to restored androgen production. Overexpression of GDNF and bFGF protein during recovery highlights their involvement in SC regeneration and spermatogonial stem cells support. SC function appeared largely restored by W12. The high expression of GDNF and FSHR in JG suggests their importance in testicular maturation. Most differences were observed at the protein rather than mRNA level, possibly due to RNA degradation. The lack of cross-reactive antibodies for (BMP4, FSHR) limits protein-level insights.

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6370/7789 | Ultrasonographic evaluation of ovarian morphology and blood flow in the first peripubertal estrous cycles in cats

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Ultrasonographic characterisation of domestic cat ovaries during sexual maturation has only been limited to the first estrous cycle

(1). In other domestic species, ultrasound has shown to be useful to difference peripubertal from mature females (2). The aim of this study was to evaluate two-dimensional and Doppler ultrasonographic changes of feline ovaries in the first three estrous cycles.

Seven 3 months old, mixed-bred female cats were exposed to a 14:10 L:D photoperiod. Puberty (Day 1 of the first estrus) was diagnosed by the first appearance of sexual behaviour with more than 80% keratinised vaginal epithelial cells and a clean background in vaginal smears for at least three successive days (3). Vaginal cytology was carried out daily during the estruses. On Day 4 or 5 of each estrus, two-dimensional ultrasonography, colour-coded and pulsed-wave Doppler evaluation of the ovaries (Toshiba Nemio XG, Japan, 14-MHz linear-array transducer) were carried out. Longitudinal (mm) and transversal (mm) sections of the ovaries were obtained and all anechoic spherical structures were considered to be follicles (4). In each section, the number and diameter of follicles > 1 mm were recorded. The maximum diameter (mm) of the largest follicle was also determined. Peak systolic velocity and end diastolic velocity of intraovarian arteries were measured to automatically calculate the resistance index (RI; 1). Repeated measures ANOVA followed by Tukey test was carried out to evaluate the effect of time on the two-dimensional and Doppler ultrasonographic parameters. The level of significance was set at 0.05.

In all two-dimensional sonographic images, the ovaries appeared ovoid and hypoechoic compared to the surrounding tissues. The mean ovarian longitudinal and transversal diameters did not differ among the three first estrous cycles ($p > 0.1$). In all the animals, multiple submillimetric anechoic spherical structures were detected during estrous ultrasound. The mean number of follicles was similar in the three time points, being 2.3 ± 0.3, 2.9 ± 0.3 and 2.7 ± 0.5, respectively ($p > 0.1$). The corresponding mean follicular diameter was higher in the second and third estrus than in the first one ($p < 0.01$). Similarly, the maximum follicular diameter increased from the first to the second estrus (2.0 ± 0.2 mm vs. 3.0 ± 0.2 mm; $p > 0.01$), while it did not vary in the third estrus (3.0 ± 0.2 mm vs. 3.4 ± 0.2 mm; $p > 0.1$). Conversely to the follicular size, intraovarian arteries RI did not change among the 3 cycles ($p > 0.1$).

These results demonstrated that although ovarian dimensions, number of follicles and intraovarian blood flow remained unchanged, mean and maximum follicular diameter increased from the first to the second estrous cycle in female cats. Two-dimensional ultrasonography of the ovaries proved to be a suitable non-invasive tool for characterisation of sexual maturation in this species.

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7030 | Use of deslorelin acetate in female dogs with prolonged estrus and shortened interestrous intervals

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Prolonged estrus (PE) or shortened interestrous intervals (SIEI) can result from failure to ovulate, abbreviated metestrus or anestrus or hormonally active ovarian cysts or tumours. Prolonged exposure to steroid hormones may lead to subfertility in breeding animals and, more severe, to metropathies or oestrogen intoxication. Deslorelin acetate can be used for estrous induction and estrous suppression. The aim of this case collection was to examine the usability of Deslorelin acetate to treat estrous cycle aberrations.

Nine bitches of different breeds, aged 17 to 175 months, were presented to our clinic due to PE for more than 3 months ($n = 3$) or SIEI of less than 4 months ($n = 6$). Two of the dogs with PE exhibited difficulties terminating their first estrus, while the third had ovarian cysts. Four dogs with SIEI had experienced irregular interestrous intervals since their first estrus were intended to breed. Three of them had a history of subfertility. The remaining two dogs with SIEI were former breeding animals presented at an older age after experiencing a single shortened interestrous, one of them was diagnosed with ovarian cysts. Each dog received a 4.7 mg Deslorelin acetate implant (Virbac, Germany) subcutaneously near the navel with the aim to suppress estrous signs and prolong the interestrous interval. The two young dogs with PE in their first estrus received the implant following repeated examinations and two unsuccessful attempts to induce ovulation using hCG 4 to 6 days apart.

Estrous signs resolved within 8 days in all dogs. Progesterone concentrations did not increase in any of the PE cases. In one young dog with PE, the Deslorelin implant was removed after 6 months what is equivalent to a normal IEI, and the dog ovulated during its subsequent estrus. The other two dogs with PE underwent ovariohysterectomy at the owner's request 1 and 3 months after implant administration, respectively. Among the dogs with SIEI intended for breeding, ovulation was not induced in one case, and this dog has since received three additional implants at 5-month intervals for continued estrous suppression. Another dog exhibited estrous signs 5 months post-treatment but failed to conceive. In the other two dogs, implants were removed because the owner wanted to breed them after five and 18 months, respectively, and both dogs successfully conceived and gave birth to litters of 10 puppies each. The two former breeding dogs with SIEI ovulated following Deslorelin treatment, and the one diagnosed with ovarian cysts was spayed 4 weeks later.

These cases suggest that Deslorelin acetate effectively terminates estrous signs in dogs with PE without inducing ovulation and may help prolong the interestrous interval to address subfertility caused by shortened anestrus. Additionally, its use enables spaying without oestrogen influence, even in older dogs with ovarian cysts. However, the administration of Deslorelin should be carefully considered due to potential adverse effects such as prolonged estrus, metropathies and ovarian tumours (1,2).

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6517 | Dopplerflowmetry in fetal and its correlation with the probable date of delivery and neonatal viability in toy poodle dogs

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Doppler flowmetry is a valuable tool for gestational monitoring, allowing assessment of fetal viability, allowing estimation of delivery time, identification of fetal distress and detection of developmental abnormalities, particularly in breeds predisposed to dystocia (1,2). Poodles are widely popular and distributed globally, with in Poodles Toy being more predisposed to dystocia (3). Thus, this study aimed to evaluate and describe changes in umbilical artery blood flow and fetal hemodynamics, with emphasis on the applicability of the pulsatility index, correlating the findings with proximity of delivery and neonatal viability in Toy Poodles.

Ultrasonographic evaluation was performed on 15 pregnant Toy Poodle bitches, assessing umbilical cord hemodynamics through measurements of minimum fetal heart rate (FCFMIN), maximum fetal heart rate (FCFMAX), heart rate variation coefficient (HRvariation), resistivity index (RI), pulsatility index (PI), systole/diastole ratio (S/D), end-diastolic velocity (EDV), peak systolic velocity (PSV) and the mean of maximum velocities in a cardiac cycle (TAMAX) across three different assessment periods: T1, measurements taken between 20 and 15 days before parturition; T2, measurements taken between 14 and 7 days before parturition; and T3, measurements taken from 7 days before parturition until delivery. Of the 15 bitches, nine were included in the normal foetus (FN) group, in which they had multiple normal foetuses; three in the single fetus (FU) group, in which they were carrying only one fetus; and three in the stillborn group (NT), in which at the time of delivery at least one stillborn fetus was detected, according to the delivery information reported by the owners. All variables presented normal distribution and were compared between groups and different periods using ANOVA one-way followed by Tukey's post hoc using the Statistical Package for Social Science (IBM SPSS) software, with a significance level of 5%.

It was observed that the group (NF, SB and SF) had a significant effect on RI, PI, and S/D ($p < 0.001$), as well as the period (T1, T2, and T3) significantly influencing FCF MIN, HRvariation, RI, S/D, PSV, and TAMAX ($p < 0.01$). When evaluating the correlation between groups (NF, SB, and SF) or periods (T1, T2, and T3), PI was the only parameter that showed a low correlation with the group ($p < 0.05$), while FCF MIN, HRvariation, EDV, PSV, and TAMAX exhibited a moderate correlation with the period ($p < 0.01$).

This study identified differences in Doppler flowmetry indices across gestational age periods (or days before parturition) and in cases of abnormal pregnancies, reinforcing the applicability and importance of these analyses in monitoring gestational development in Toy Poodle bitches. The PI emerged as a promising index for predicting neonatal viability.

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