Check for updates

DOI: 10.1111/rda.14066

Reproduction in Domestic Animals WILEY

1 | A case of uterine adenomyosis in a bitch: Ultrasonographic appearance

Gabriele Barella[1]; Emanuela Vallesi[1]; Elisabetta Tognacca[2]; Erica Cipolla[2]; Vincenzo Montinaro[2]
[1]*Clinica Veterinaria CMV AniCura, Varese, Italy*; [2]*Clinica Veterinaria Malpensa AniCura, Samarate (VA), Italy*

Uterine adenomyosis (UA) is a pathological condition characterized by the infiltration of endometrial glandular epithelial cells in the myometrium. UA is described in numerous species (bitch, cat, cow, rabbit, guinea pig, mouse, women and non-human primates) and it is often considered an incidental finding. In small animals it is observed with concurrent endometritis, pyometra (P) or cystic endometrial hyperplasia (CEH). Adenomyosis is described in 2.6% of clinically healthy bitches undergoing elective ovariohysterectomy. The presence of abnormal myometrial glandular infiltration can lead to the accumulation of glandular secretion and consequent atrophy of the contiguous myometrial tissue with potential hypofertility to infertility. The pathogenesis of AU in the bitch is not entirely understood, although hormonal imbalances are listed as predisposing factors. In present case a 10-year-old Cavalier King Charles Spaniel was referred for anorexia, polyuria and polydipsia. Neither previous matings nor pregnancies or drug treatments were referred; last heat ended about 2 months before the presentation. The bitch presented hyperthermia (39.9 ° C) and mild abdominal pain. The blood count showed neutrophilic leukocytosis (29,000 K/μl); the biochemical profile was normal. At the radiographic study an increased area of radiopacity, referable to soft tissues, was observed cranio-dorsally to the urinary bladder. The uterine horns appeared slightly fluid filled. The ultrasound examination confirmed the presence of a slight anechoic fluid collection in both uterine horns. The uterine wall was thicker than normal (up to about 4 mm) due to the presence of small multifocal cysts projecting into the lumen. The uterine body presented collapsed and thickened walls (14 mm thickness with a recognizable layering). From the lumen towards the serosa it was possible to identify: the hyperechoic lumen-mucosal interface, the intermediate echogenicity of the endometrium (3 mm), the hypoechoic outermost myometrium that was heterogeneous due to the presence of hyperechoic foci not producing distal attenuation (10 mm) and the hyperechoic external serous layer (<2 mm). The cervix appeared normal. The ovaries appeared normal in size; hypoechoic cystic-like structures to corpora lutea (2 mm) were also observed. Moderate peritoneal reactivity adjacent to the uterine horns and mild parietal abdominal lymphadenopathy were identified. A clinical diagnosis of suspected P associated with metropathy of the uterine body was made. The bitch underwent ovariohysterectomy and histopathological examination reported the presence of P-CEH of uterine horns combined with diffuse glandular infiltration of the myometrium that was suggestive of UA of uterine body. This clinical case describes the ultrasound appearance of UA in a bitch associated with P-CEH. The increase in thickness of the uterine wall with the predominant thickening of myometrium reflects the histopathological description of UA. The ultrasound appearance of UA remind to that of an intestinal wall; however, the anatomical location and the absence of luminal gas help the operator to correctly classify this structure as uterus. Uterine adenomyosis can lead to infertility and pathological rupture of the uterus; therefore, a correct ultrasound characterization of this pathology can be useful especially in screening programs.

2 | A pilot study to evaluate the usefulness of melatonin implants to control the oestrus cycle in bitches

Eva Axnér
Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden

In short-day breeders such as the sheep, melatonin stimulates oestrus activity while a high serum concentration of melatonin inhibits oestrus in long-day breeders such as the cat (1). In contrast to its ancestor, the wolf (Canis lupus), the domestic dog is a non-seasonal breeder with some breeds such as the Basenji, being exceptions with a strong tendency for seasonality. Decreased serum oestradiol concentrations after administration of melatonin in a previous study (2) indicates that melatonin might have a potential to postpone oestrus. In contrast, decreased concentrations of prolactin after administration of melatonin to the wolf (3) could indicate a stimulatory effect of melatonin on the oestrus cycle. The aim of this pilot study was to evaluate if melatonin could be an alternative for short term postponing of oestrus in the bitch.
Nine beagle bitches were observed for three oestrus cycles. One month before the expected time of the third oestrus, based on the previous interoestrus interval, bitches were randomly assigned to control or treatment groups. Long acting melatonin implants (Regulin 18 mg) were placed s.c. in 5 of the bitches (treatment). The other group of 4 bitches were sham implanted. The interoestrus intervals between non-treated cycles and treated cycles were compared with a mixed effects ANOVA after checking distribution of residuals. Bitch was included as a random factor and treatment as a fixed factor. Results are reported as means ± SD. Calculations were

© 2021 The Authors. © 2021 Blackwell Verlag GmbH

made with Minitab® 18 (Minitab Inc. State College, PA, USA). The study was approved by Uppsala ethical committee (C117/15).

The mean intervals between the first and second oestrus were 208.3 ± 36.6 and 212.0 ± 35.7 days for control and treatment groups respectively. Corresponding intervals between second and third oestrus (after treatment) were 208.5 ± 3.7 and 215.8 ± 31.0. The interoestrus intervals differed significantly between bitches (p = 0.045) but were not significantly affected by treatment with melatonin (p = 0.65). The second interoestrus interval of one of the control bitches was 46 days shorter than the first, thus her third oestrus occurred before sham implantation had been planned. No side effects were observed.

There was no effect of treatment with melatonin implants on the oestrus cycle based on the results from this pilot study. The dose of melatonin might be an important factor. Although the dose used in this study has been efficient in altering cyclicity in the sheep and the domestic cat, the dog may require higher doses for an effect. Daily oral melatonin might be more efficient although less practical. It is also possible that melatonin is not involved in regulation of the oestrus cycle in the non-seasonal domestic dog.

Table 1. Interoestrus intervals in individual bitches

Bitch	Group	First interoestrus interval (days)	Second interoestrus interval (days)	Difference in interval length (days)
1	Control	179	207	28
2	Control	194	207	13
3	Control	200	206	6
4	Control	260	214	-46
5	Implant	159	168	9
6	Implant	236	233	-3
7	Implant	234	236	2
8	Implant	191	201	10
9	Implant	240	241	1

The study was supported by Agria and SKK Research Foundation for companion animals (grant number N2015-0017). CEVA Animal Health AB contributed with Regulin®.

[1] Tenorio F, Simoes Mde, J, Teixeira, VW, et al. Revista da Associacao Medica Brasileira (1992) 2015; 61:269–274.

[2] Ashley PF, Frank, LA, Schmeitzel, LP, et al. J Am Vet Med Assoc 1999; 215:1111–1115.

[3] Kreeger TJ, Seal, US, Cohen, Y, et al. Can. J. Zool.-Rev. Can. Zool. 1991; 69:1366–1374.

3 | Caudal-superficial-epigastric axial pattern flap associated with full-thickness buccal mucosa graft for preputial reconstruction in three dogs

Tatihana Ferguson Motheo; A. P. Ribeiro; D. S. Prass; T. B. S. Magalhães; L. D. Lourenço; G. C. Batista; A. Delapícola; N. Freschi; M. F. Fernandes

Federal University of Mato Grosso (UFMT), Cuiabá, MT, Brazil

Clinical cases: Canine prepuce injuries are commonly noticed in small animal practice. The absence of a foreskin caused by trauma or surgical excision leads to chronic penile exposure, dryness, and ulcerations. Severe injuries may require preputial reconstructive surgery to restore function and aesthetics. The present report aims to describe the use of a combined caudal-superficial-epigastric axial pattern flap and full-thickness buccal mucosa graft for single-stage preputial reconstruction in three dogs with major foreskin tissue loss. Three dogs were referred to the Veterinary Hospital of UFMT with severe preputial injuries. Thus, in all cases, single-stage preputial reconstruction using combined caudal-superficial-epigastric axial pattern flap and full-thickness buccal mucosa graft was performed (Figure 1). Cutaneous stitch dehiscence was a common complication noticed in all three cases. In dog 1, dehiscence and tissue necrosis were treated by chemical debridement and healed by second intention after thirty days. The second and third cases were approached differently due to the greater extent of the dehiscence. In dog 2, a half-moon rotation flap was performed, and the wound healed uneventfully. In dog 3, preputial advancement was required to cover the cranial portion of the foreskin. Flap retraction was observed in all three cases. In dogs 1 and 3, a slight retraction with the exposure of a small portion of the penile glans was noticed, however, further interventions were not required. Dog 2 presented a significant retraction that led to partial stenosis of the preputial ostium. Ostium enlargement was obtained by suturing the mucosa to the edge of the wedge. Captons were made with sterile silicone tubes, and sutures were placed in the dorsal and ventral aspects to avoid adhesions of the buccal mucosa graft on the inner part of the new foreskin. After ten days, captons were removed and revealed a good opening of the preputial orifice and adequate penile exposure.

Discussion: Covering of the skin with an axial-type flap of the caudal superficial epigastric region tends to fail since only the graft edges are sutured into the abdominal skin. Thus, the subcutaneous region of the penis is exposed and it becomes in direct contact with urine and the penile mucosa, causing retraction of the flap skin. Free-lip mucosa graft allows the subcutaneous region of the transposed skin flap to create adhesions in the fenestrated regions of the lip tissue through granulation of the tissue buds. The permanence of the penis protects the lower urinary system from infections which are normally observed when performing partial or total penile amputation combined to adjunctive scrotal urethrostomy. The reported cases show that pre-facial reconstruction with an axial skin flap of the caudal superficial epigastric region, combined with free-buccal mucosa graft, is a feasible technique for single-stage foreskin reconstruction. However, in cases which larger portions of the foreskin are removed, the collection of buccal mucosa from both sides of the lips, and the construction of two instead of one fenestrated tunnel, may prevent the occurrence of paraphimosis.

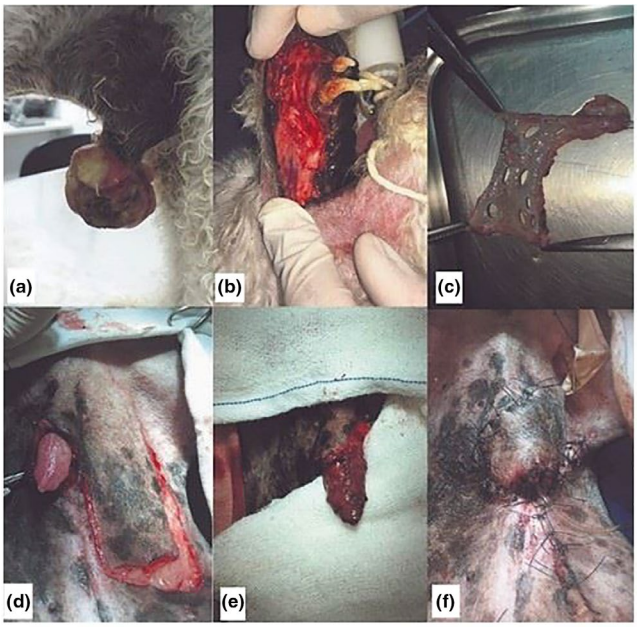


Figure 1 - (**A**) Chronic necrotizing multifocal granulomatous posthitis affecting the cranial portion of the foreskin in dog 1. (**B**) Oral lesion after free buccal mucosa graft collection. (**C**) Fenestration of the free buccal mucosa graft, with its submucosa facing upwards. (**D**) Penile exposure after excision of the foreskin mass and creation of a caudal-superficial-epigastric flap. (**E**) Free buccal mucosa graft after envolving de pênis and being sutured to the edge of the remaining foreskin. (**F**) Final aspect of the skin flap after coating the penis covered by the free buccal mucosa graft and its attachment to the skin margins, creating new preputial ostium.

4 | Evaluation of pre-freeze and post-thaw sperm quality of epididymal canine sperm cooled in situ or extended overnight

Giuseppe Prencipe[1]; F. Marinaro[2]; V. Russo[1]; L. Gónzalez-Fernández[3]; B. Macías-García[4]

[1]*Unit of Basic and Applied Biosciences, Faculty of Bioscience and Agro-Food and Environmental Technology, University of Teramo*; [2]*Stem Cell Therapy Unit, Centro de Cirugía de Mínima Invasión Jesús Usón*; [3]*Department of Biochemistry and Molecular Biology and Genetics, Faculty of Veterinary Sciences, University of Extremadura, Cáceres, Spain*; [4]*Veterinary Teaching Hospital, University of Extremadura, Cáceres, Spain*

Introduction and objectives: Sperm harvesting from the epididymal cauda represents the last chance to obtain progeny in case of an unforeseen decease or castration. However, not all small animal clinics perform sperm cryopreservation and epididymides need to be shipped to specialized facilities. Unfortunately, the lack of technical knowledge regarding its optimal storage and shipping conditions prevents the preservation of canine epididymal sperm. Hence, our study aimed to define the best cooling method for canine epididymal sperm prior cryopreservation at 4 ˚C: within the epididymis or extended.

Materials and methods: Testicles were collected from 11 healthy dogs of different breeds immediately after castration. One epididymis was immediately stored at 4 ˚C for 24 h (Time 0). The contralateral epididymis was flushed with CaniPlus Chill®, extended to reach 150 × 106 sperm/ml and preserved at 4 ˚C. After 24 h of cooling, the stored epididymis was flushed and processed as the former. Samples were centrifuged, and the obtained pellet was resuspended at 150 × 106 sperm/ml in CaniPlus Freeze® medium, plus 20% egg yolk (v/v). Sperm were packed in 0.5 ml straws, cooled at 4 ˚C for 1 h, exposed to liquid nitrogen vapor for 20 min and plunged into liquid nitrogen. Sperm quality was assessed in fresh and frozen-thawed samples at all the time points: 0 and 24 h (fresh samples) and after thawing (37 ˚C for 1 min). Motility was evaluated using a CASA system; viability, mitochondrial membrane potential (MMP) and DNA integrity were assessed by flow cytometry using SYBR-14/PI, JC-1 and sperm chromatin structure respectively. Results are expressed as the mean ± SEM in % comparing epididymal cooling vs. extended sperm. ANOVA and student *t*-test were used to compare normally distributed data; Kruskal-Wallis test and Mood's median test for non-normally distributed data; $p < 0.05$ was considered as significant.

Results: Canine epididymal sperm, cooled for 24 h at 4 ˚C within the epididymis, showed significantly differences compared to the cooled-extended samples respectively for viability (69.9 ± 1.8 vs. 59.6 ± 3.4, mean % ± SEM; $p < 0.05$), higher MMP (64.9 ± 3.7 vs. 51.1 ± 2.7, mean % ± SEM; $p < 0.05$), and beat cross frequency (BCF) (7.5 ± 0.5 vs. 5.6 ± 0.6, mean % ± SEM; $p < 0.05$). After cryopreservation, significant differences were also found in MMP (50.9 ± 2.2 vs. 39.1 ± 4.9, mean % ± SEM; $p < 0.05$) and in viability (48.5 ± 2.7 vs. 39.5 ± 4.2, mean % ± SEM; $p < 0.05$) in samples cooled in the epididymis vs. extended 24 h prior cryopreservation, respectively. DNA fragmentation remained unaffected in all the treatments ($p > 0.05$).

Conclusions: The study demonstrates that epididymal sperm better maintains its quality when cooled within the epididymis prior freezing. This finding opens a new horizon in the small animal clinics procedures to store and/or ship epididymal sperm in dogs prior freezing.

Funding: RYC-2017-21545 (AEI)

5 | The impact of mode of delivery on newborn puppies and their survival

Tanja Plavec[1]; Aleksandra Slapšak[2]; Sara Raspor[2]; Tanja Knific[2]; Barbara Lukanc[2]; Maja Zakošek Pipan[2]

[1]*Small Animal Veterinary Hospital Hofheim, Germany* [2]*University of Ljubljana, Veterinary Faculty, Slovenia*

Birth as a stressful event may influences puppies' neonatal morbidity and mortality. The purpose of this study was to investigate the impact of parturition type on stress in new-born puppies and survival in the first week postpartum.

The study included 123 puppies delivered by either vaginal parturition (VP; $n = 9$, 68 puppies), emergency cesarean section (EMCS; $n = 5$, 17 puppies) or elective cesarean section (ELCS; $n = 8$, 38 puppies). The anesthetic protocol was the same for both CS groups. The day of surgical parturition in EL-CS cases was determined by the average 63-day gestation from ovulation in conjunction with the

information collected by serial monitoring of blood progesterone concentration and fetal ultrasonographic measurements at the end of gestation. Apgar score was assessed at 5, 15, and 60 min postpartum. Lactate and glucose concentrations were measured in amniotic fluid and umbilical blood, and cortisol concentrations were measured in amniotic fluid and puppy's urine.

Ten parturitions occurred in small breed dogs with 44 puppies delivered (four Boston Terriers, two Miniature Schnauzers, and one each Yorkshire Terrier, Maltese, Miniature Poodle and Jack Russell Terrier/Maltese mix). Eight parturitions with 50 puppies were recorded in medium sized dogs (two French Bulldogs and Pembroke Welsh Corgis, and one each English Bulldog, Beagle, Dachshund and Whippet). Four bitches with 29 delivered puppies belonged to the large and giant breeds, each represented by one breed: Golden retriever, Labrador Retriever, German Shepherd and Great Swiss Mountain Dog.

There were nine stillborn puppies, none of the puppies had visible malformations. Apgar score at 5 min was significantly worse in the ELCS group ($p < 0.05$). At 15 min VP puppies had better Apgar scores than ELCS and EMCS puppies ($p < 0.05$), but there was no difference between these two groups. There were no changes between the groups at 60 min. Amniotic fluid was collected from 5863% of all and 6975% of VP puppies, respectively. ELCS and EMCS puppies had significantly lower lactate concentrations in umbilical blood and ELCS puppies also in amniotic fluid when compared to the VP group ($p < 0.05$). A very strong correlation was found between cortisol concentrations in amniotic fluid and urine. Cortisol concentration in amniotic fluid and in urine differed significantly between groups ($p < 0.05$), with the highest cortisol in the EMCS puppies, followed by VP group. Blood glucose concentration in EMCS puppies was significantly lower when compared with VP group. Seven puppies died in the first week postpartum, four were born by ELCS and three by VP. VP puppies had the highest Apgar score in the immediate postpartum period. Analysis of amniotic fluid and/or puppies' urine can serve as good non-invasive predictors of neonatal stress.

6 | Canine vaginal cytology: Towards a tutorial for the definition of vaginal cells

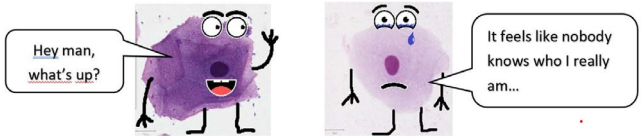
Felix Reckers; Robert Klopfleisch; Vitaly Belik; Johanna Leber; Lisa Riege; Sebastian Arlt
Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany

Introduction and objectives: Vaginal cytology is an often used tool for cycle staging in the bitch. For most practitioners and vet nurses collecting a sample and staining slide are easy to perform procedures. The evaluation of the smears, however, may be challenging as some earlier projects have shown. This may be because of different definitions of canine vaginal cells, especially regarding sizes, cornification and the appearance of the nucleus published by different authors.

To get a deeper insight into the use and interpretation of vaginal cytology in dogs, a survey was distributed via the Café-Repro e-mail list. The overall aim of the project was to develop a tutorial for a more standardized determination of vaginal cells.

Material and methods: The survey was conducted using google forms. Participants were asked to define eight specific cells and answer some questions. Vaginal smears were taken, stained and digitized and evaluated from several bitches in different stages of the estrous cycle to depict all relevant types of vaginal cells.

The tutorial was developed and validated by five raters of different levels of experience, i.e. two students, two veterinarians working in the field of small animal repro, and one Dipl. ECAR.



Results: In total, 16 completed surveys were sent back from veterinarians practicing in eight different countries and with different levels of experience. The agreement of the raters determining the cells was calculated with Fleiss' Kappa (0.0 indicates an agreement not better than chance, values higher than 0.75 indicate good agreement and 1.0 perfect agreement). With $\kappa = 0.398$ the agreement of all 16 raters was poor. A second calculation was made with raters ($n = 8$) who do vaginal cytology in more than 100 bitches per year. This led to a Fleiss' Kappa of $\kappa = 0.501$.

Eleven raters stated that vaginal cytology has a low reliability in the context of ovulation timing. Nevertheless, 13 out of 16 raters state that they use this tool regularly.

The tutorial was developed as a flowchart. When determining a specific cell, the user is guided step by step through an evaluation of specific characteristics. By selecting the appropriate flow lines, with support of some specific illustrations showing cell characteristics, the user ends up at a cell type. The first validation by five participants with difference experience levels led to a Fleiss' Kappa of $\kappa = 0.858$.

Conclusion: Vaginal cytology is a useful diagnostic tool, but it seems helpful to standardize the determination of the cell types. The developed tutorial may support practitioners and clinicians.

7 | Comparison of the acceptance of two postoperative treatment regimens in bitches with malignant mammary tumours—First results

Sebastian Ganz; Maren Sievert; Axel Wehrend
Clinic of Obstetrics, Gynaecology and Andrology of Small and Large Animals with Ambulatory Service, University of Giessen, Giessen, Germany

Introduction and objectives: Mammary tumours of the bitch belong to the most frequent canine neoplasms. Because of their ability to

change in dignity from benign to malignant, surgical removal is the treatment of choice. There are no standardised recommendations and treatment methods regarding postoperative care after removal of malignant proliferations of the mammary gland. At the moment there is in a discussion about the value of homeopathic treatment methods. The aim of this study was to evaluate the acceptance of owners of bitches who have had malignant mammary tumours removed with regard to treatment with a homeopathic and/or therapy with Trocoxil (Mavacoxib, Zoetis Deutschland GmbH, Berlin). The degree of malignancy was neglected in this first step as the owners choice of treatment regimen was the main aim of this survey. In addition, changes in liver and kidney laboratory parameters were monitored during the course. To the best of our knowledge, the present study is the first to address the choice between conventional and homeopathic postoperative care in bitches with mammary tumours.

Material and methods: To date, 50 owners have been offered a homeopathic treatment regimen and a treatment regimen using Trocoxil after the removal of malignant mammary tumours from the respective bitch 10 days post operation. Owners had to decide which of the two regimens they preferred or whether they choose a combination of both approaches. Regardless of the therapy chosen, all dogs were scheduled for a quarterly review of liver and kidney parameters (urea, creatinine, ALT) to analyse any effects of long-term postoperative therapy.

Results: Thirty-seven owners opted for Trocoxil therapy, one owner opted for a homeopathic regimen and 12 owners opted for a combination. In dogs receiving Trocoxil alone, three showed increases in liver or kidney parameters. In one bitch, all parameters were elevated. In the bitch receiving homeopathic treatment alone, there were no laboratory abnormalities. Of the animals receiving a combination, changes in liver parameters were detected in three bitches and in kidney parameters in one. In another bitch, both liver and kidney parameters were elevated.

Conclusion: Even in this small group of patients, it is evident that conventional medical treatment is chosen more often. It should be emphasized that the initial aim of this ongoing study is not to see how different treatment protocols affect the postoperative recurrence rate and survival time, but to record which postoperative treatment procedure owners choose when they are presented with alternatives. The effects of long-term treatment will be evaluated during the course of the study. Furthermore, a representative statement on the extent to which homeopathic postoperative treatment is an alternative for dog owners is to be made on the basis of a larger number of study participants.

A significant proportion of the animals treated with Trocoxil developed changes in liver and kidney values. According to the manufacturer's recommendation, these parameters should be monitored as done in the study.

8 | Influence of meconium-stained amniotic fluid on vitality parameters and hematological variables of term neonatal puppies

Julia L. Sato; Letícia L. Almeida; Roberto R. Rosa Filho; Maíra M. Brito; João V. M. Lopes; Marcela A. Cebim; Thais G. Faustino; Camila I. Vannucchi

Department of Animal Reproduction, University of São Paulo – USP, São Paulo, Brazil

Introduction and objectives: Meconium-stained amniotic fluid (MSAF) is considered a life-threatening condition and widely described in human neonates [1]. Meconium aspiration syndrome has direct consequences on the immediate fetal-to-neonatal transition, interfering with alveolar replacement of fetal fluid to air content at birth, thus causing respiratory obstruction and neonatal hypoxia [2]. Such changes may negatively influence neonatal vitality depending on the degree of meconium-stained amniotic fluid. Therefore, this study aimed to compare the influence of different degrees of meconium-stained amniotic fluid on neonatal vitality and hematological variables in dogs.

Materials and methods: Puppies were born by elective c-section, performed during the first stage of labor (preparatory phase), marked by decline in body temperature, characteristic signs of whelping and progesterone concentration <2.0 ng/ml. Puppies were assigned to three experimental groups according to a subjective analysis of the amniotic fluid: Control Group (translucent amniotic fluid; $n = 5$), Mild-MSAF Group (mild yellowish amniotic fluid; $n = 5$) and MSAF Group (yellowish amniotic fluid; $n = 5$). After hysterotomy and fetal exposure, blood samples were obtained by umbilical cord puncture before clamping, and by jugular vein puncture after 1 h of birth. Both samples were subjected to a complete blood count (CBC). Neonatal vitality (heart rate, respiratory rate, irritability reflex, muscle tone and mucous color), body temperature and non-invasive peripheral oxygen saturation were performed immediately at birth and after 1 h. Variables were analyzed by ANOVA/LSD test, Student T and Spearman correlation ($p \leq 0.05$). Ethical approval CEUA-FMVZ-USP number 1576200519.

Results: No significant difference among groups was verified for neonatal vitality, body temperature and oximetry. Control Group had lower macrocytosis compared to the other groups and higher polychromasia compared to the Mild-MSAF Group, regardless of the moment of evaluation. All neonates had higher monocyte count at birth compared to 1 h of life. For the Control Group, there was a positive correlation between the number of erythroblasts and peripheral oxygenation and negative correlation between erythroblasts and body temperature. In the Mild-MSAF Group, the number of erythroblasts correlated positively with all neonatal vitality parameters.

Conclusions: In conclusion, meconium-stained amniotic fluid influences erythrocyte morphology during the immediate neonatal period and imposes a relationship between the number of erythroblasts and neonatal vitality.

[1] Dargaville PA. The Epidemiology of Meconium Aspiration Syndrome: Incidence, Risk Factors, Therapies, and Outcome. Pediatrics. 2006;117(5), 1712–1721.
[2] Van IY, Beaufort AJ. Why does meconium cause meconium aspiration syndrome? Current concepts of MAS pathophysiology. Early Human Development. 2009;85(10):617–20.

9 | Oestrogen-related dysuria due to ovarian papillary cystadenoma in a Bernese mountain bitch

Emmanuela Vallesi[1,2]; Allessandro Troisi[3]; Angela Polisca[4]; Cristiano Boiti[5]; Marco Russo[6]; Paolo Bargellini[1]; Ricardo Orlandi[1]
[1]*AniCura Tyrus Veterinary Clinic, Terni, Italy*; [2]*AniCura CMV Veterinary Clinic, Varese, Italy*; [3]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*; [4]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy*; [5]*Tyrus Science Foundation, Terni, Italy*; [6]*Department of Veterinary Medicine and Animal Production (DMVPA), Federico II University of Naples, Naples, Italy*

Papillary adenomas and carcinomas, cystadenomas, and cystadenocarcinomas account for approximately 50% of ovarian tumours [1]. These tumours tend to grow up to 20 cm or more and are often functional secreting oestrogens that cause various sexual cycle disorders [2]. On August 2019, a 9 year-old Bernese Mountain bitch was referred at Tyrus Veterinary Clinic for dysuria and haematuria from 90 days not responsive to different antibiotic treatments. Previous oestrus cycles were normal. At clinical examination, manual palpation of caudal vagina allowed to appreciate a tissue proliferation in the vestibulum. The CBC showed mild non-regenerative anemia and leucopenia with 30% hematocrit (reference values 37.3–61.7), 10.6 g/dl hemoglobin (reference values 13.1–20.5), 4.85 M/µl red blood cells (reference values 5.65–8.87), 7.3 /µl reticulocytes (reference values 11–110), 4.88 K/µl leucocytes (reference values 5.05–16.76), and 2.77 K/µl granulocytes (reference values 2.95–11.64). At cytology, vaginal epithelial cells indicated an oestrus status. Chest radiography was negative for metastatic lesions. Ultrasonography evidenced bilateral polycystic ovaries (length 2, width 1.5 cm) and cystic endometrial hyperplasia. Vaginoscopy revealed a mild serumhematic vaginal discharge and presence of a vaginal fold that, arising from the caudal vagina, extended through the cingulum to the vestibule and partially obstructed the external urethral orifice. Based on history, clinical, and instrumental tests, we diagnosed ovarian follicular cyst/tumour with persistent heat and secondary vaginal first-degree hyperplasia obstructing the urethral orifice. No hormonal examinations were performed in this case. Following ovariohysterectomy, histopathology confirmed a right polycystic ovary and an ovarian papillary cystadenoma on the left ovary with signs of hydrometra/pyometra outcomes. Two weeks after surgery, the bitch recovered completely. This case report shows that in the bitch polycystic ovarian structures i) cannot be differentiated from neoplasm and ii) can show hormonal activity. Hormonally active ovarian cysts are clinically relevant and despite no hormonal examination was performed in this case, the clinician can suspect an hyperestrogenism in case of one or more signs including nymphomania, symmetric non pruritic alopecia, pendulous abdomen, prolonged estrus, vulvar swelling, weight loss and lethargy and, as in our case, urinary signs.

10 | Endoscopic resection of acquired vaginal stenosis causing infertility in a German Shepherd

Riccardo Orlandi[1]; Emanuela Vallesi[1]; Angela Polisca[2]; Cristiano Boiti[3]; Davide De Lorenzi[4]; Paolo Bargellini[1]; Alessandro Troisi[2]
[1]*Tyrus Veterinary Clinic*; [2]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy*; [3]*Tyrus Science Foundation, Terni, Italy*; [4]*Veterinary Hospital I Portoni Rossi*

Vaginal endoscopy is a part of genital tract evaluation in cases of infertility or other abnormal conditions [1]. Moreover, it can be used to observe changes of vaginal mucosa during oestrous cycle as well as for practicing artificial transcervical insemination with chilled or frozen-thawed semen [2].
On July 2019, a 4 year-old, multiparous German Shepherd bitch was referred at Tyrus Veterinary Clinic for infertility related to the last oestrus. The bitch had a normal reproductive history with regular inter-oestrus intervals. During the previous oestrus, the day of ovulation was determined by vaginal cytology, genital ultrasound, and progesteronemia. Despite the follicular phase progressed normally, the owner referred incomplete male penetration during the mating, 24 and 72 h after the ovulation, respectively.
At the time of consultation, we performed ultrasound examination and vaginoscopy. The bitch was in dioestrus (25 days after ovulation) and not pregnant. Vaginal endoscopy showed a vestibulovaginal stenosis due to a membrane in the cingulum region that involved almost the entire vestibulovaginal passage leaving a hole of few millimetres. Based on history, the stenosis likely resulted from a scarring reparation process linked to a trauma occurring during the last delivery. Once identified, a dilation of the stenosis was carried out trough endoscopy using the laser technique under general anaesthesia to remove the membrane and dilate the vestibulovaginal passage. Three months later the bitch was mated again and she whelped 6 viable pups normally.
Vaginoscopy was a fast and non-invasive procedure, which was helpful for i) a correct evaluation of the acquired condition and ii) additional treatments to restore fertility.

[1] Johnson CA. Diagnosis and treatment of chronic vaginitis in the bitch. Veterinary Clinics of North America Small Animal Practice 1991; 21(3):523–531.
[2] Levy X. Videovaginoscopy of the canine vagina. Reprod Dom Anim 2016;51(Suppl. 1):31–36.

11 | Diagnosis of uterine sarcoma by contrast-enhanced ultrasound in a Rottweiler bitch 35 days after whelping

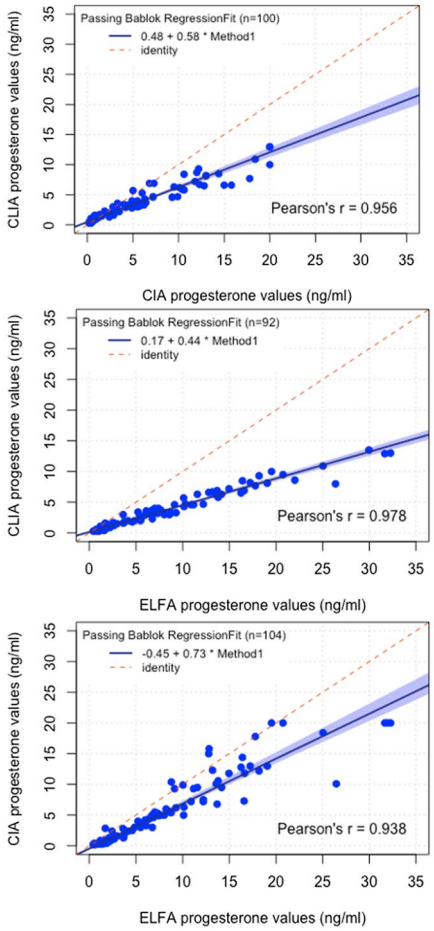
Emmanuela Vallesi[1,2]; Angela Polisca[3]; Allessandro Troisi[4]; Cristiano Boiti[5]; Paolo Bargellini[1]; Ricardo Orlandi[1]
[1]*AniCura Tyrus Veterinary Clinic, Terni, Italy;* [2]*AniCura CMV Veterinary Clinic, Varese, Italy;* [3]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy;* [4]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy;* [5]*Tyrus Science Foundation, Terni, Italy*

Neoplasia of canine uterus rarely occurred in veterinary medicine, accounting for 0.3% to 0.4% of all canine tumours [1]. The most frequent uterine tumour is fibroleiomyoma, which may cause haemorrhage with sanguineous vulvar discharge [2]. Imaging techniques, in particular ultrasonography, are important for the diagnosis of the uterine mass.
On November 2019, a 4-years old Rottweiler bitch, that whelped normally 35 days earlier, was presented at Tyrus Veterinary Clinic for weakness and general malaise. The puppies did not show any signs of illness. At the time of clinical examination, the bitch had fever (39.5°C), profound weakness, slight non regenerative anaemia with neutrophilic leucocytosis (granulocyte neutrophils, 1.29 K/µl), and severe hypernatremia (Na > 180 mmol/L). Ultrasonographic scans evidenced anechoic liquid and an isoechoic structure within the uterine lumen with a suspect of residual foetal structures. Contrast-enhanced ultrasound following Sonovue injection (0.03 ml/kg) evidenced the presence of abnormal vascularization together with non-vascularized necrotic areas. Subsequent ovariohysterectomy revealed the presence of a uterine neoformation; histopathology confirmed that it was a sarcoma (leiomyosarcoma vs fibrosarcoma). Four days after surgery the bitch was discharged with normal biochemical analyses. The presence of uterine structures after whelping can be misdiagnosed with a sub-involution of placental sites (SIPS) or abnormal foetal membranes or haemorrhagic structures. In this case, the use of contrast medium differentiated uterine neoformation from other conditions, even if histopathology remains the gold standard to make a definitive diagnosis.
[1] Brodey RS. Canine and feline neoplasia. Adv Vet Sci Comp Med 1970;14:309–354.
[2] England GCW. Infertility in the bitch and queen. In: Noakes DE, Parkinson TJ, England GCW. Veterinary Reproduction and Obstetrics Elsevier 2019;33:595–596.

12 | Comparison of three different analyzers to measure canine serum progesterone

Trevor Hisanaga; Michelle Kutzler
Department of Integrative Biology, Oregon State University's Honors College, USA

Introduction: The canine estrous cycle is unique compared to other domestic animal species. During estrus, serum progesterone concentrations ([P4]) rise two days prior to ovulation [1]. In fact, the luteinization of pre-ovulatory follicles resulting in this initial increase in [P4] (1.5–2.5 ng/ml) cannot be temporally dissociated from the onset of the surge in luteinizing hormone [1,2]. For this reason, measuring progesterone from daily blood samples is commonly used to determine the optimal breeding day in female dogs [3]. In addition, the fall in [P4] (<2 ng/ml) prior to parturition can be used for the purposes of determining the timing of an elective C-section in dogs [4].
Objective: The objective of this research was to compare [P4] measured on three different progesterone analyzers (enzyme linked fluorescent assay (ELFA), colorimetric immunoassay (CIA), and chemiluminescent immunoassay (CLIA)). We hypothesized that irrespective of analyzer used, the [P4] measurement will be reliable for determining timing for breeding or C-section.
Materials and methods: Venous blood samples (n = 116) were collected from privately-owned female dogs (n = 44) during routine reproductive procedures at the Waipahu Waikele Pet Hospital in Honolulu, Hawaii. Nineteen breeds were represented, and the mean age of dogs was 4.0 ± 1.8 years. Dogs were fasted 6–8 h prior to each blood collection and blood samples were collected into tubes containing a clot activator but not serum separating gel. Blood samples were allowed to clot for 30–60 min and then were centrifuged for five minutes at 3,500 rpm. [P4] was determined from three commercially available progesterone analyzers in accordance with the manufacturer's instructions. The Passing-Bablok regression was used to determine the Pearson correlation coefficient (r) and a residuals analysis was used to analyze the data.
Results: The Pearson correlation coefficients for CIA vs ELFA, CLIA vs CIA, and CLIA vs ELFA, were 0.938, 0.956, and 0.978, respectively (Left column of figure). However, analysis of the residuals showed an increase in the spread as the [P4] increased (Right column of figure).
Conclusions: The results from this study show that comparison of [P4] between the analyzers is accurate at lower values (≤5 ng/ml) but the variability in [P4] increases as the value increases.



References:

[1] Kim Y, Travis AJ, Meyers-Wallen VN. Parturition prediction and timing of canine pregnancy. Theriogenology 2007;68:1177–1182.

[2] Concannon PW. Canine pregnancy and parturition. Vet Clin North Am Small Anim Pract 1986;16:453–475.

[3] Goodman M. Ovulation timing: Concepts and controversies. Vet Clin North Am Small Anim Pract 2001;31:219–235.

[4] Concannon PW, Butler WR, Hansel W, et al. Parturition and lactation in the bitch: Serum progesterone, cortisol and prolactin. Biol Reprod 1978;19:1113–1118.

13 | Effect of age on sperm acrosome associated 3 protein expression in the feline ovary

Ann Ramsey; Michelle Kutzler

Oregon State University, USA

Introduction: Sperm acrosome associated 3 (SPACA3) is a lysozyme-like protein reported to have a role in sperm-egg plasma membrane adhesion and fusion during fertilization [1]. In 5 to 7.5-month-old cats (n = 6), SPACA3 was found in all ovarian follicle stages and was localized to the ooplasm, granulosa, and theca cells [1]. However, SPACA3 expression was not quantified. In murine testes, SPACA3 expression in sperm acrosomes was shown to decline with age as fertility reached a maximum [2]. A preliminary study with young (2 months, n = 3) and adult (>12 months, n = 3) cats found that SPACA3 expression decreased with age [3].

Objectives: The objective was to quantify SPACA3 expression in different cell types from different follicular stages in young (3.0 ± 0.9 months, n = 11) and adult (10.4 ± 2.8 months, n = 11) queens. Based upon previous research, it was hypothesized that SPACA3 expression would decrease with age.

Materials and methods: Routine immunohistochemistry was performed on formalin-fixed, paraffin-embedded feline ovarian sections. Briefly, heat-induced epitope retrieval with sodium citrate (pH 6.1) was used. Anti-SPACA3 polyclonal antibody (#HPA023633, Atlas Antibodies) was applied at 1:200 dilution. Immunostaining specificity was verified by replacing the primary antibody with negative control rabbit serum on adjacent sections. Sections were reacted with one-step horseradish peroxidase-conjugated polymer anti-rabbit IgG (IH-8064-custom-OrSU, ImmunoBioScience) followed by a NovaRED peroxidase substrate (#SK-4800, Vector Labs). Representative images of each follicle stage from each ovary were digitally captured using QCapturePro by a single observer at 200× magnification. Cellular expression of SPACA3 was quantified in primordial, primary, secondary, and tertiary follicles using FIJI image analysis software's RGB stack and manual thresholding functions [4]. The oocyte nucleus, ooplasm, granulosa cells, and theca cells were outlined using the freehand selection tool and mean gray value was measured. Age (in months) was compared between experimental groups using a Student's t-test (Microsoft Excel). Results from each cellular location were compared between age groups using a Student's t-test and between follicle stage using an analysis of variance (GraphPad Prism 8 software). Significance was defined as $p < 0.05$.

Results: Young and adult groups differed significantly by age (p = 0.00). Feline ovarian SPACA3 expression is summarized in Figure 1. There was significantly greater SPACA3 expression in the oocyte nucleus (primordial, primary, secondary follicles) of young queens compared to adult queens, granulosa cells (primary, secondary, tertiary follicles), and theca cells (secondary, tertiary follicles) ($p < 0.05$). However, SPACA3 expression within the ooplasm did not differ significantly between age groups.

Conclusions: Similar to the sperm acrosome,[2] ovarian SPACA3 expression is lower in adult compared to young queens. This is confounding given the proposed role of SPACA3 in sperm-egg plasma membrane adhesion and fusion during fertilization, since expression would be lower at the ages when fertilization would most likely occur. More research is needed to determine what role SPACA3 may play in female fertility as well as what mechanisms regulate ovarian SPACA3 expression over time.

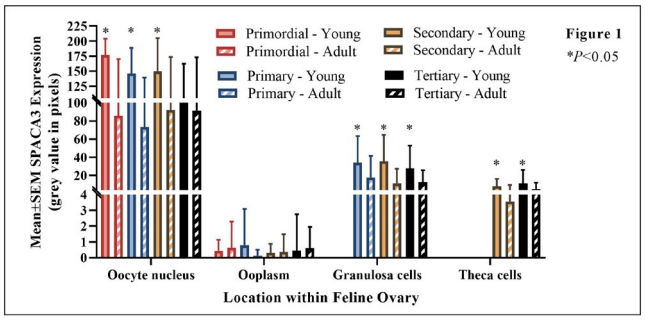


Figure 1 *$P<0.05$

References:
[1] Wagner A, et al: Reprod Sci 2015;22:452–461.
[2] Wei J, et al. Asian J Androl 2013;15(6):824–830.
[3] Ramsey A, et al. Clin Theriogenology 2021;13(3).
[4] Schindelin J, et al: Nat Methods 2012;9(7),676–682.

14 | Analysis of putative stem cell markers in dogs with spontaneous immune mediated orchitis

Larena Reifarth; Sandra Goericke-Pesch; Hanna Körber
Reproductive Unit – Clinic For Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Acquired non-obstructive azoospermia (NOA) is the most common diagnosis in infertile male dogs. Previous studies and our recent data indicate that autoimmune orchitis, better named "immune-mediated orchitis", is the cause of NOA in the dog. With onset of infertility, a poor prognosis and irreversible damage to the testicular tissue must be assumed, and previous studies have failed to demonstrate successful treatment options with significant changes in fertility outcome. Nevertheless, there have been no attempts to evaluate a stem cell-based therapeutic option in case of treating male dogs suffering from AIO until now, nor are there any data on the survival of viable stem cell populations in the respective canine testicular tissues. Consequently, the aim of our study was to detect spermatogonial stem cells (SSCs) using various stem cell markers in canine AIO affected tissue (TT). Testicular biopsies from both testes were taken from 12 dogs with AIO and 10 adult, healthy dogs with normospermic ejaculates (CG). Following histological assessment, CG was considered to represent normal spermatogenesis and AIO samples were divided into two groups of spermatogonial arrest: Early arrest (n = 5) with SCO or spermatogenesis arrested on spermatogonia; and late arrest (n = 7) with spermatogenesis arrested with spermatocytes, round, elongating or elongated spermatids as most developed germ cells. Immunohistochemistry (IHC) using the established post-meiotic marker CREM confirmed our classification. Furthermore, IHCs were conducted in all testicular specimens using stem cell markers, previously described to be specific for the dog, namely DAZL, PGP9.5, FOXO1 and c-kit. Appropriate positive and negative controls were included in the runs. IHC were evaluated descriptively. Besides, the numbers of immunopositive signals (NIP) for DAZL, PGP9.5 and FOXO1 were counted in 30 seminiferous tubules (ST) and 20 fields of vision (F) at 200× magnification. Statistical analysis was performed to compare NIP in AIO and CG using GraphPad Prism v. 7.02 (t-test; GraphPad Software. Inc., San Diego, CA, USA) and $p < 0.05$ was considered statistically significant. Both, DAZL- and PGP9.5 IHC revealed immunopositive signals in spermatogonia near the basal membrane of the ST in both groups. Specific immunopositive staining against FOXO1 was revealed in a small subset of spermatogonia near the basal membrane, namely undifferentiated spermatogonia. Immunohistochemical expression of the c-Kit protein (cd117) was located in the cytoplasm of differentiating spermatogonia. Regarding quantitative evaluation, DAZL (ST $p < 0.0001$; F $p < 0.0001$), PGP9.5 (ST $p < 0.0001$; F $p = 0.0003$) and FOXO1 (ST $p < 0.0001$; F $p < 0.0001$) expression was significantly lower in AIO samples. Our results confirm previous findings and contribute additional evidence suggesting that SSCs with the potential of self-renewal and differentiation survive in the damaged tissue despite severe inflammatory changes and immune cell infiltration. Further on, these findings have significant implications for the understanding of spermatogonial subpopulations and their detectability in canine testis. Further research is required to shed light on the mechanisms underlying the disturbance of the stem cell niche and to establish stem cell-based therapeutic options to (re-) initialize spermatogenesis.
This work is supported by the AfT.

15 | Scrotal hypospadias repair in an English bulldog – Case report

H. S. Camargo[1]; Silvia E. Crusco[2,3]; J. C. P. Monteiro[1]; R. C. P. Monteiro[1]
[1]*USJT*; [2]*Anhanguera University*; [3]*UNIP, SP, Brazil*

A 7-month-old male English Bulldog was referred with the complaint of urine spraying from an orifice near the scrotum. During the clinical examination, it was discovered that the penis was a failure of closure of the preputial leaves. The urethral ostium was ectopic, placed near the scrotum. To correct the defect, the dog was generally anesthetized, and a urinary catheter was placed for two days to prevent clots. A bilateral orchiectomy and a pre-scrotal urethrostomy was performed. The pre-scrotal location was chosen for the urethrostomy because the urethra is wider in the scrotal region [1], thereby improving urine flow and decreasing stenosis risk. Briefly, a cutaneous incision was made around the penis and prepuce and penectomy was performed. The retractor muscle of the penis was transected, and two sutures were used to close the tunica albuginea. A single Cushing suture was used to close the subcutaneous and urethral mucosa. The dog was treated post-operatively with scopolamine butyl bromide (25 mg/kg IM), tramadol (4 mg/kg SC), meloxicam (0.1 mg/kg IM) and amoxicillin with clavulanate (200 mg/kg). Home medication was done with amoxicillin with clavulanate (22 mg/kg every 12 h for 15 days); meloxicam (0.1 mg/kg every 24 h for 4 days); dipyrone (28 mg/kg every 8 h for 5 days); tramadol hydrochloride (3 mg/kg every 12 h for 4 days); omeprazole (0.5 mg/kg every 24 h for 15 days in the morning), all was administered via oral. The wound was cleaned post-operatively with chlorhexidine 2% and rifamycin 1% spray. Two weeks after surgery, the dog presented with no complications and normal healing and the sutures were removed. However, four weeks after surgery, the dog presented with pollakiuria and pain during urination. A granuloma had formed from a suture reaction that resulted in a urethral stenosis. The granuloma was surgically removed and treated topically with 0.9% sodium chloride and topical rifamycin twice daily until the dog completely recovered.

[1] MACPHAIL C. M. Surgery of the Bladder and Urethra. In: FOSSUM, T.W. (Ed) Small Animal Surgery. 4th ed. St. Louis: Elsevier Mosby, 2013, v. 1, p. 735–779.

16 | The intrinsic pathway of apoptosis: Is it involved in case of spontaneous immune-mediated orchitis in dogs? – Preliminary results

Judith Morawietz; Hanna Körber; Sandra Goericke-Pesch
Reproductive Unit of the Clinics – Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Non-obstructive azoospermia (NOA) is a common cause of infertility in dogs. According to our recent research, spontaneous autoimmune or immune-mediated orchitis (AIO) seems to be an important, if not the cause for acquired NOA in the dog. Since only little is known about the underlying causes of AIO in dogs, we aimed to identify whether apoptosis is a key factor in the severeness, progression and maintenance of the disease. We focused on the intrinsic (mitochondrial) pathway of apoptosis with Caspase-3 and -9 as effector enzymes as well as Bcl-2, as antiapoptotic, and Bax, as proapoptotic modulating factors. Furthermore, to detect apoptotic DNA fragments, TUNEL method was applied.

Testicular biopsies of dogs with AIO and normospermic controls were included. Time since onset of infertility was variable in AIO dogs, ranging from months to years. RT-qPCR was performed using specific canine primers against Bax, Bcl-2, Caspase-3 and -9 to study mRNA expression of the intrinsic pathway of apoptosis having tissues available of 9 dogs with AIO and 5 controls. Immunohistochemistry (IHC) was performed to proof protein expression of Caspase-3 and Bcl-2 protein expression and localization (AIO: n = 12, controls: n = 10). Western blotting was used to verify the specificity of the antibodies. To identify apoptotic cells, TUNEL method was applied using the ApopTag® Peroxidase Detection Kit S7100. For TUNEL evaluation, all positive signals were counted in 30 randomly selected round tubuli and 20 random visual fields. Statistical analysis of mRNA expressions and number of TUNEL-positive signals was performed using GraphPad Prism v. 7.02 (GraphPad Software. Inc., San Diego, CA, USA) and $p < 0.05$ was considered statistically significant. Ratios (mRNA expressions) differed significantly between AIO and controls for Bax and Bcl-2 ($p < 0.01$ each), Caspase-3 and -9 ($p < 0.05$ each). IHC revealed a specific staining of Bcl-2 in the cytoplasm of Sertoli cells (in controls and AIO), some peritubular (in controls and AIO) and immune cells (AIO only). Staining for Caspase 3 was only seen sporadically in the interstitial and tubular compartment in AIO, but not in controls. The number of TUNEL-positive apoptotic cells was significantly higher in AIO dogs compared to controls regarding tubuli ($p < 0.001$) and visual fields ($p = <0.0001$).

Our results clearly indicate that apoptosis is involved in progression and maintenance of AIO as expression of proapoptotic Bax and effector enzymes Caspase-3 and -9, but also the number of TUNEL-positive cells were significantly increased in AIO. The significantly increased Bcl-2 expression, as antiapoptotic factor, might indicate a possible counterregulatory mechanism that deserves further investigation, but is obviously not able to stop progression of the disease.

This work is supported by the GkF (Society for cynological research).

17 | Which role does PTGS2 play in testicular tissue of dogs with non-obstructive azoospermia?

Judith Morawietz, Hanna Körber, Sandra Goericke-Pesch
Reproductive Unit – Clinic For Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Infertility of the stud dog is often challenging for the veterinarian and the breeder. Non-obstructive azoospermia (NOA) is a common cause of canine infertility, however, the causative etiology is often not easy to identify. Recently, we identified that acquired non-obstructive azoospermia (NOA) in dogs is not only associated with a severe impairment of spermatogenesis, but also with a significant infiltration of immune cells, namely lymphocytes and plasma cells, indicating spontaneous autoimmune or immune-mediated orchitis (AIO). The prostaglandin endoperoxide synthase (PTGS) and the prostaglandins play an important role in physiological spermatogenesis and steroidogenesis, but also in inflammation and pain. In men, PTGS-2 expression is induced in Leydig and immune cells in case of impaired spermatogenesis. Therefore, the aim of our study was to investigate whether PTGS2 as a pro-inflammatory enzyme plays an important role for development and maintenance of spontaneous immune-mediated orchitis in dogs resulting in NOA. In order to specify if altered PTGS2 expression might be associated with changes in steroidogenesis, expression of the steroidogenic acute regulatory (StAR) protein was also investigated. Testicular biopsies of 11 dogs with AIO and samples of 5 normospermic control dogs (control) were assessed. RT-qPCR was performed using a specific canine primer against PTGS2 to study mRNA expression. For the protein localization, immunohistochemistry (IHC) was performed using a PTGS2 monoclonal antibody and a StAR polyclonal antibody previously described to be specific for the dog. For IHC evaluation, a software was used to determine the percentage of immunopositive area (PIA) and staining intensity (MGS) in the tubular and interstitial compartment, respectively. Statistical analysis was performed using GraphPad Prism v. 7.02 (GraphPad Software. Inc., San Diego, CA, USA) and $p < 0.05$ was considered statistically significant. PTGS2 mRNA expression was significantly higher in all AIO samples compared to CG. IHC revealed a specific staining of PTGS2 in the cytoplasm of Leydig, Sertoli cells and some peritubular cells. For StAR a specific staining was only seen in the cytoplasm of Leydig cells and some peritubular cells. PIA and MGS of PTGS2 differed significantly between groups (AIO-CG) in the interstitial (PIA: $p < 0.0001$; MGS: $p < 0.01$) and tubular compartment (PIA: $p < 0.01$; MGS: $p < 0.05$), whereas for StAR only a significantly higher PIA was revealed in the interstitial compartment (PIA: $p = 0.001$; MGS not significant). Our results indicate that PTGS2 and StAR appear to play a role in

inflammation and possibly steroidogenesis in dogs with AIO. Further investigations are required to figure out whether administration of anti-inflammatory drugs, better specific PTGS2 inhibitors, such as robenacoxib, might be an interesting therapeutic approach in early cases of NOA to potentially prevent development of immune-mediated orchitis in dogs or to stop progression of AIO and to ensure their use as breeding dogs.

This work is supported by the GkF (Society for cynological research).

18 | Progesterone-antibodies may contribute to dermatitis in bitches – A case report

Sebastian Arlt[1]; Jenny Uhlmann[2]; Julia Schraven[3]; Ralf Einspanier[4]
[1]*Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany*; [2]*Veterinary Clinic for Small Animal Reproduction, Bernau, Germany*; [3]*Department of Radiology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin*; [4]*Institute of Veterinary Biochemistry, Freie Universität Berlin, Berlin, Germany*

Clinical case: A seven-years-old female Sheltie with blue Merle color was presented because of recurrent dermatitis, mainly at the paws but also at the head. The lesions regularly occurred during the weeks after heat and then disappeared again. Histopathological examination of a biopsy revealed a severe inflammation with unclear cause. During the phases of skin disease, the dog was treated with Oclacitinib (Apoquel, Zoetis, Switzerland) which seemed to help according to the owner.

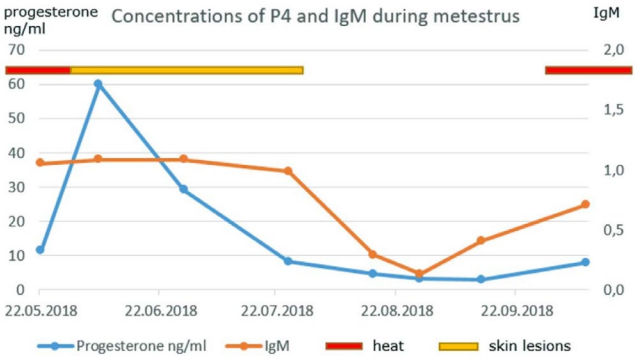
Because of the remarkable pattern of the occurrence of the skin disorders, progesterone was measured in the blood serum in a commercial laboratory using chemiluminescence (Immulite 2000 XPi, Siemens Healthcare GmbH, Erlangen, Germany). In addition, blood serum was tested with an uncertified ELISA essay for progesterone antibodies (1). The amount of immunoglobulins in the serum was determined using ELISA plates coated with 100 µl of progesterone-bovine serum albumin.

Anti-progesterone-IgE was not found in most of the samples. The amount of anti-progesterone-IgM, however, might have corresponded to high progesterone concentrations and dermatitis as their concentrations were higher in the luteal phase.

The dog was neutered and never developed a dermatitis thereafter.

Discussion: The results of this case report must be interpreted very cautiously as the assay is uncertified, and only one dog was studied during a single sexual cycle. Nevertheless, the results indicate that it might be worthwhile to investigate a potential individual autoimmune response to progesterone in dogs as it has been reported in humans (2). More than 50 case reports about autoimmune progesterone reactions in humans during the luteal phase with a variety of presentations including erythema multiforme, eczema, urticaria, angioedema, and progesterone-induced anaphylaxis, have been published. The underlying etiology, however, is still not clear.

A similar approach has been described by Krachudel et al. 2013 (1) in the context of a potential etiology of canine hypoluteoidism. It can be hypothesized that anti-progesterone-IgM-antibodies may play a role in the observed cycle stage-dependent occurrence of dermatitis in this dog.



References:
[1] Krachudel et al (2013): Theriogenology 79:9. 1278–1283.
[2] Ljubojević Hadžavdić, S et al. (2018) Acta Dermatovenerol Croat. 26:3.276–277.

19 | Semen quality in Bernese mountain dogs in Sweden

Ida Hallberg, Angus Lau, Stina Wallander, Hannah Olsson, Anna Snell, B. S. Holst
Swedish University of Agricultural Sciences, Sweden

The Swedish Mountain Dog Breed Club considers work for improved fertility within the Bernese mountain dog (BMD) a prioritized area. The pregnancy rate within the breed is low, 65%. In a collaboration between the breed club and the university, fertility parameters within the BMD were investigated. The aim of the present study was to describe the semen quality of BMD.

Dogs were recruited through collaboration with The Swedish Mountain Dog Breed Club and through social media. An ethical permission was obtained from the regional animal ethical committee. All owners gave their informed written consent. Inclusion criteria were intact BMD 16 months and older not treated with hormones.

All males underwent a clinical examination, including determination of body condition score (BCS, 1–9 scale), size and consistency of the testes, and rectal palpation of prostates.

One semen sample per dog was collected through manual stimulation in the presence of a teaser bitch in oestrus (if possible). Colour, volume, and any abnormality of the ejaculate were recorded. Sperm motility was subjectively assessed on a prewarmed microscope slide under a phase-contrast microscope (100× and 200×) immediately after sample collection. The sperm concentration was measured using a Bürker chamber. Sperm morphology was evaluated using standard procedures in wet preparations of semen fixed in buffered formalin and in air-dried smears stained with carbolfuchsin–eosin. Stepwise linear regression was used to predict semen quality

based on dog characteristics (Minitab, 19.2010.1). Differences with $p < 0.05$ were considered statistically significant.

Semen samples from 65 male BMD were included. Their mean ±standard deviation age was 53 ± 31 months, BCS 5 ±0.8 and weight 49 ± 5 kg.

Testicle palpation revealed normal consistency in 63% of the dogs (39/62) whereas 37% had at least one testicle with abnormal consistency. Age was positively associated with abnormal consistency ($p = 0.043$). Of the semen samples, 57% (37/65) had normal colour, the other samples were clear (1), red-brown (17/65) or yellow (10/65). There were no significant associations between semen colour or testicle size and sperm or dog variables. Two semen samples were azoospermic with no following sperm assessment.

Mean sperm motility was 61 ± 27%. Motility was negatively associated with age ($p = 0.049$). Total number of sperm per ejaculate $1.03\times10^9\pm0.82\times10^9$. Mean per cent of morphologically normal sperm (MNS) per ejaculate was 47 ± 26%. The percentage of MNS was significantly lower in dogs with abnormal testicle consistency ($p < 0.001$). For detailed information on semen quality, see Table 1. Dogs with abnormal testicle consistency had significantly higher percentage of spermatozoa with pathological heads ($p = 0.002$), proximal droplets ($p = 0.008$), knobbed acrosomes ($p = 0.008$) and vacuoles ($p = 0.004$). Age was significantly associated with percentage of proximal droplets ($p = 0.009$) and detached heads ($p = 0.004$).

The mean semen quality of the BMD is lower than considered normal for dogs.[1] Further studies on causes behind the decreased semen quality are warranted to enable strategic measured for increased fertility within the breed to be undertaken.

	N	Mean	StDev	Min	Max
Motility (%)	65	61.43	26.50	0.00	98.00
Total number of sperm (10^9)	65	1.03	0.82	0.00	3.65
MNS (%)	65	46.78	26.05	0.00	87.50
Number of MNS[a] (10^6)	65	522	491.8	0.00	1793.7
Pathological heads (%)	63	7.21	5.93	0.00	37.90
Proximal droplets (%)	63	14.14	14.72	0.50	75.50
Detached heads (%)	63	3.01	5.57	0.00	34.00
Knobbed acrosomes (%)	63	3.69	6.05	0.00	37.50
Abnormal acrosomes (%)	63	0.06	0.20	0.00	1.00
Vacuoles (%)	63	1.11	2.41	0.00	15.50
Midpiece defects (%)	63	3.30	3.51	0.00	26.30
Single bent tails (%)	63	10.13	9.12	0.00	38.00
Coiled tails (%)	63	10.48	10.03	0.50	43.00
Double bent tails (%)	63	5.35	5.32	0.00	24.00

[a]morphologically normal sperm

Funded by: Jan Skogsborgs fund, Thure F and Karin Forsberg fund & Swedish University of Agricultural Sciences.

[1] Johnston, S.D. et al (2001). Canine and feline theriogenology. Philadelphia, PA; Saunders.

20 | Maternal elements in meconium bacteria flora of naturally delivered puppies of two dog breeds

Angela Del Carro[1]; M. Corrò[2]; E. Spagnolo[2]; E. Bertero[3]; P. Banchi[1]; A. P. Del Carro[4]; A. Rota[1]

[1]*Dip Scienze Veterinarie, University of Turin, Grugliasco, Italy*; [2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*; [3]*Practitioner, Turin, Italy*; [4]*Practitioner and ECAR Dip, Clinique Vét S.Hubert-IunoVet, Beausoleil, France*

Limited is the knowledge of meconium microbial flora in newborn puppies and its colonization timing and pathways. Bacteria have been isolated from meconium of naturally born puppies and puppies delivered through caesarean section (CS) [1,2], also in case of elective CS and immediate sampling [2]. As shown in humans [3], also in dogs the delivery mode affects meconium bacterial flora composition [1].

Three dam-litter units for each of two medium size breeds, Appenzeller Cattle Dog (ACD) and Lagotto Romagnolo (LR), housed in the same breeding kennel, were included in the study. No antimicrobials or other drugs or supplements were administered during pregnancy; in the last two weeks, the dams were fed a dry diet (MONGE Medium Puppy & Junior rich in chicken®). Twenty-one puppies were naturally born for each breed [mean birth weight (g)±SD; ACD 410 ± 0.05; LR 240 ± 0.05]. Mini-swabs for bacteriological samples were collected from the newborns' rectum immediately after birth, before maternal care and suckling. Also stillborn puppies were sampled (three ACD and three LR). Swabs were collected from the dams' vagina and rectum at the end of parturition. Culture was performed according to standard lab techniques.

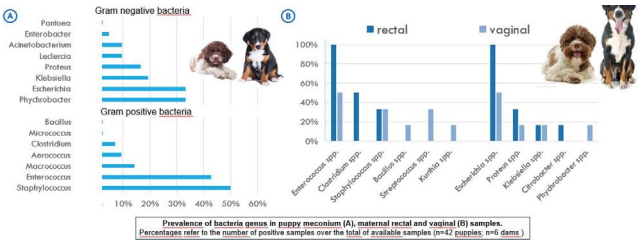
All except one meconium samples resulted in positive cultures (97.6%). Both Gram-positive [coagulase-positive and negative Staphylococci ($N = 21$; 50% of samples), Enterococcus spp. (42.9%), Macrococcus spp. (14.3%), Aerococcus spp. (9.5%), Clostridium spp. (7.1%), Micrococcus spp (2.4%). and Bacillus spp. (2.4%)] and Gram-negative bacteria [Psychrobacter spp. and Escherichia coli (33.3%), Proteus spp. (16.7%), Klebsiella spp. (19.3%), Leclercia spp. (9.5%), Acinetobacter spp. (9.5%), Enterobacter spp. (4.8%), Pantoea spp. (2.4%)] were identified. In general, more puppies of a litter showed the same bacteria species (i.e. E. faecalis, E. coli, K. pneumoniae). More frequently, growth in culture was limited to few colonies, sporadically high growth was detected, especially for E. faecalis. C. perfringens was found only in three puppies of an ACD litter.

All dams harboured E. faecalis and E. coli, in rectum or vagina, suggesting the origin of colonization. For the other bacteria, only occasionally the dams' species coincided with those of their litter. The origin could be the dams' mouth or the environment or bacteria could be already present during foetal life [2]. Two puppies died at 7 and 14 days: the first one was an underweight LR female from which only M. luteus had been isolated; the other one a LR male harbouring E. faecalis. Irrespective of bacteriological findings and of the isolation of potentially pathogen bacteria (haemolytic *E. coli*,

K. pneumoniae, Coagulase-positive Staphylococci, *C. perfringens*), all the other puppies were healthy at weaning.

A richer meconium bacterial flora resulted in a previous work [1], but meconium was sampled later, immediately after colostrum intake to stimulate defecation.

Knowing the normal pattern of gut microbiota development could give a key for detecting dysbiosis and preventing pathological conditions. This pioneer field of research is worth being deeply investigated.



[1] Zakošek Pipan M et al., Theriogenology. 152 (2020) 18–28.
[2] Rota A et al., Animals 11, 1415.
[3] Mueller NT et al., Trends Mol Med. 21 (2015) 109–117.

21 | Omphalocele in a newborn puppy: Treatment and post-mortem investigations

Angela Del Carro[1]; M. Corrò[2]; A. P. Del Carro[3]; A. Nicoletti[1]; A. Rota[1]

[1]*Dip Scienze Veterinarie, University of Turin, Grugliasco, Italy*; [2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*; [3]*Practitioner and ECAR Dip, Clinique Vét S.Hubert- IunoVet, Beausoleil, France*

Omphalocele is a congenital opening of the abdominal wall at the umbilicus. The incidence described in humans is 2/10,000 births. The condition has been occasionally described in cats [1] and dogs [2] but the true incidence in both species is unknown. Pedigree evaluation in cats confirms a familial relationship and suggests an autosomal recessive mode of inheritance [1]. Affected puppies and kittens are commonly euthanized because of the anaesthetic risk and surgical complications associated with repair.

A female newborn Appenzeller Cattle Dog presented with a congenital omphalocele. The litter of five puppies was naturally delivered without complications. No medical treatment had been administered to the dam during pregnancy except dewormer. The affected puppy had an intestinal loop protruding through a 1.5 cm long umbilical opening. There was no membrane covering the intestine. At birth, the APGAR score of the puppy was 9 [3]. Surgery was immediately performed under field conditions. Analgesia and anaesthesia were provided with butorphanol (0.05 mg/kg IM) and alfaxalone (3 mg/kg IM) and local lidocaine (2 mg/kg SC). The umbilical opening was enlarged, the intestine was gently washed with warm saline solution, and replaced into the abdominal cavity. Two layers of monofilament absorbable suture (4/0 Biosyn TM®) were used for abdominal closure. After surgery, the APGAR score was 3, 5 and 9 at 1, 3 and 6 h, respectively. At 6 h, the puppy started suckling colostrum from the dam. Amoxicillin and clavulanic acid (50 mg/kg SC) and metronidazole (15 mg/kg PO) were administered daily for a 7-day course. The puppy steadily gained weight for two weeks. At 14 days, the puppy refused to suckle and developed clinical signs of an acute abdomen. Death occurred in few hours.

Necropsy showed evident signs of chronic peritonitis and pneumonia. The histological analysis confirmed pneumonia and showed severe lymphocyte depletion and septic emboli into the mesenteric vessels. PCR for Canine Herpesvirus was negative. Culture of the abdominal exudate showed a relevant growth of E. coli and S. canis, low numbers of K. pneumoniae and Proteus spp. All isolates were sensitive to the antimicrobials used with the exception of E. coli ('intermediate').

The causes and the incidence of omphalocele in the canine species are unknown although genetic defects and teratogen drugs seem to be likely factors. Immediate surgical reduction of the congenital defect may be an option in case of viable and strong puppies. Alfaxalone and butorphanol provided sufficient anaesthesia, analgesia and myorelaxation. Sensitivity test results showed that a third-generation cephalosporin would have been the best option in this case.

[1] Margolis C, Pipan MJ, Demchur J et al., JFMS Open Rep 2018:2055116918804305.
[2] Kaplan JL, Gunther-Harrington T, Sutton JS et al. BMC Vet Res 2018;14:86.
[3] Veronesi MC, Panzani S, Faustini M, et al. Theriogenology 2009;72:401–407.

22 | Influence of the GnRH stimulation test in the concentration of canine prostatic specific esterase (CPSE)

Lluis Ferré Dolcet[1]; Simona Attard[2]; Stefano Romagnoli[3]; Maria Carmela Pisu[2]
[1]*Clinica Veterinaria San Marco;* [2]*Centro di Referenza Veterinario;* [3]*Università degli studi di Padova, Italy*

Introduction and objectives: Usually, during a BSE it is required a GnRH stimulation test to determine the correct testicular production of serum testosterone concentration in order to evaluate infertility problems [1,2]. CPSE has been found to be increased in dogs suffering from benign prostatic hyperplasia and in other prostatic conditions [11] and its concentration has been used as a prostatic disease marker. Even if symptomatic prostatic disorders have higher CPSE concentrations over 90 ng/ml [3], the advantage of CPSE concentration determination is that it is very useful for determining any prostatic condition even if the dog is asymptomatic. To the authors knowledge, no studies between GnRH stimulus test and CPSE secretion have been performed. Thus, the aim of this study is to identify the effect of GnRH stimulation tests on CPSE concentrations in dogs independently of the prostatic condition.
Material and methods: Twenty-eight client-owned intact adult male dogs were included in the study.
All male dogs underwent a clinical examination and an ultrasonographic exam of the prostatic gland. All the subjects presented two scrotal testicles without the presence of masses or abnormalities and no history of ejaculation was recorded during the last week as sexual rest for seven days was mandatory for the inclusion.
Prostatic size and parenchyma of every tested dog was evaluated by ultrasonography in order to discriminate prostatic conditions.
Dogs were divided into 2 groups. Group A included 15 dogs where 50 µg/dog of gonadorelin was administered IM in the posterior limb. Group B included 13 dogs where 0.12 µg/kg of buserelin was administered IV. CPSE concentrations were measured by a laser-induced fluorescence analysis (Speed Reader, Virbac, Milan, Italy). Non-parametric Mann-Whitney test was used to compare treatments (A vs B) in Pre and Post phases. Non-parametric Wilcoxon test was used for pairwise samples between pre and post phases within each treatment. Non-parametric Wilcoxon test was used for pairwise comparison between pre and post phases within each diagnosis (presence or absence of BPH).
Results: No statistically significant differences were reported concerning the use of gonadorelin or buserelin despite age or prostatic condition in the evaluated animals. CPSE concentrations were not altered with both GnRH analogues one hour after the treatment.
Conclusion: One single blood sampling after the use of a GnRH stimulus test is effective in determining both testosterone and CPSE secretion during a breeding soundness examination independently of prostatic status in male dogs.
References:
[1] J. De Gier, J.J.C.W.M. Buijtels, C.H.J. Albers-Wolthers, C.H.Y. Oei, H.S. Kooistra, A.C. Okkens, Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy, Theriogenology. (2012).
[2] B.P. Meij, J.A. Mol, H.A.W. Hazewinkel, M.M. Bevers, A. Rijnberk, Assessment of a combined anterior pituitary function test in beagle dogs: Rapid sequential intravenous administration of four hypothalamic releasing hormones, Domest. Anim. Endocrinol. (1996).
[3] B.S. Holst, E. Holmroos, L. Friling, S. Hanås, L.M. Langborg, M.A. Franko, K. Hansson, The association between the serum concentration of canine prostate specific esterase (CPSE) and the size of the canine prostate, Theriogenology. (2017).

23 | Neutering as a risk factor for joint disorders and cancers in Belgian male dogs

Joke Lannoo, Guillaume Domain, Jeroen Dewulf, Ann Van Soom, Eline Wydooghe
Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium

Introduction and objectives: In recent years, gonadectomy in dogs has become a topic that has been critically discussed and reviewed. Previous studies have demonstrated that orthopedic problems and some non-genital tumors are more prevalent in dogs after gonadectomy, especially if neutered before puberty. These effects cannot be directly linked to the removal of the gonads or the absence of sex steroids. The present study aimed to evaluate the odds of developing certain non-genital conditions after neutering in male dogs treated at the Small Animal Veterinary Teaching Hospital in Merelbeke, Belgium. The pathological conditions evaluated for this study were hip dysplasia (HD) elbow dysplasia (ED) and cranial cruciate ligature rupture (CCR) (joint disorders) and osteosarcoma (OSA), mast cell tumors (MCT), lymphoma (LSA) and hemangiosarcoma (HSA) (cancers).
Materials and methods: A retrospective case-control study was performed on 2014 male dogs. Cases of each condition were selected from the databank of the Hospital and a large group of control animals. Breed, categorized in small breed (<10 kg), medium breed (10–25 kg) and large breed (>25 kg) dogs, age, date of diagnosis, date of first symptoms (if known), castration status (intact or neutered), age at time of neutering: early (<12 months old) and late (>12 months) were catalogued for each dog. Cases and control groups were categorized based on their castration status, intact or castrated male, and time of castration. Body condition scores at time of diagnosis were recorded from the patient records for every dog. Data were analysed by means of a multivariate binary logistic regression model (SPSS 25.0). p value's <0.05 were considered statistically significant.
Results: Early castration was confirmed as a significant risk factor for the development of CCR (Odds Ratio: 2.6) and LSA (Odds Ratio: 2.2) when comparing to intact dogs, whereas late castration was not. No significant effect of castration was seen for the development of HD, ED, HSA, OSA or MCT. Interestingly, we could show that large breed dogs were at higher risk for all the conditions investigated, regardless of their castration status. Medium and small breed dogs

had comparable odds for all conditions except for HSA, where small breed dogs had significantly lower odds than medium breed dogs. Higher body condition scores were seen in the groups with orthopedic disorders in comparison to the control group. Age was a risk factor for all four cancers.

Conclusion: In male dogs it was shown that the seven investigated conditions have a higher incidence in large breed dogs. Higher body condition scores increased the odds of development of cranial cruciate rupture, hip dysplasia and elbow dysplasia. Gonadectomy before the age of 12 months caused an increased risk for lymphoma and cranial cruciate rupture in male dogs of large breeds. It is advised to postpone castration until the age of 1 year old, especially in large breed male dogs. Prepuberal castration should be discouraged.

24 | Germ cell apoptosis induced by tumor necrosis factor (TNF) alpha in spontaneous autoimmune orchitis in dogs is mainly triggered by interaction with TNF receptor 2 on spermatogonia

Caroline Pröbstl, Hanna Körber, Sandra Goericke-Pesch
Reproductive Unit – Clinic for Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Spontaneous autoimmune orchitis (sAIO) is an upcoming underlying cause of azoospermia in dogs. With a prevalence up to 35% in dogs with reproductive problems, non-obstructive azoospermia (NOA) is one of the most important reasons for male dog infertility. In our previous studies on nine dogs with NOA, we demonstrated dominant immune cell infiltration and significantly disturbed spermatogenesis – the two main factors of sAIO. In other species, AIO was shown to be associated with a significantly increased expression of inflammatory markers, as e.g. tumor necrosis factor alpha (TNF-α) and macrophage chemotactic protein 1. TNF-α is acting by its receptors TNFR1 and 2 and known to be the key factor for germ cell apoptosis in AIO in other species. Consequently, this study aimed to investigate TNF-α and its receptors in dogs with sAIO (n = 9) and normospermic controls (n = 5) on mRNA and protein level to gain insights into the underlying mechanisms of germ cell apoptosis in sAIO. RT-qPCR and immunohistochemistry (IHC) using specific canine primers and antibodies against TNF-α, TNFR1 and TNFR2 were performed. IHC evaluation was descriptively. Besides, all immunopositive signals were counted in 40 random fields of vision at 400-fold magnification in each sample with each antibody and the stained cell type was noted. The evaluator was blinded to the sample/group at the time of examination. Statistical analysis was performed using GraphPad Prism 8 version 8.0.1 for windows, GraphPad Software, San Diego, California, USA (www.graphpad.com) to compare the RT-qPCR ratios and the quantity of immunopositive signals for TNF-α, TNFR1 and TNFR2 in sAIO and CG by unpaired t test and Mann Whitney test (exact, two tailed) respectively. On the mRNA level, TNF-α (p = 0.0037) and TNFR2 (p = 0.0443) expressions were significantly higher in sAIO compared to CG, but not TNFR1 (p = 0.6729). On the protein level, TNF-α was expressed in Leydig, Sertoli, and germ cells, mainly spermatogonia, TNFR1 and TNFR2 in Leydig and germ cells, mainly spermatogonia, but not in Sertoli cells. Besides, immune cells in sAIO expressed TNF-α, TNFR1 and 2. Similarly to mRNA expression, the total number of immunopositive cells and the number of immunopositive cells of each population for TNF-α (p = 0.0051), TNFR1 (p = 0.0430) and TNFR2 (p = 0.0160) was significantly increased in sAIO compared to CG, with the absolute numbers of TNFR2 positive cells being higher than TNFR1 positive cells in sAIO. Our data clearly indicate an important role of TNF-α and its receptors in canine sAIO. However, different to other species, TNF-α is not only produced by Sertoli, but also by Leydig cells. Besides, not TNFR1, but TNFR2 on spermatogonia is the crucial interactor for TNF-α in canine sAIO.

25 | Uterine torsion in a pregnant queen

Emmanuela Vallesi[1,2]; M. Cecchetto[2]; R. Orlandi[1]; A. Troisi[3]; R. Gobbi[2]; P. Belli[2]; A. Polisca[4]; A. Boiti[5]
[1]*AniCura Tyrus Veterinary Clinic, Terni, Italy*; [2]*AniCura CMV Veterinary Clinic, Varese, Italy*; [3]*School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy*; [4]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy*; [5]*Tyrus Science Foundation, Terni, Italy*

Uterine torsion (UT) is defined as twisting of the uterus or uterine horn perpendicular to its long axis (1); in small animals, UT more commonly involves a single uterine horn twisted at its base. This condition has been reported in non-pregnant nulliparous animals, even though it typically occurs in multiparous animals late in pregnancy or at the time of parturition. Pyometra may also predispose to UT [2]. The pathogenesis of UT is not well understood, although fetal movement, deficiency of uterine tone, lack of fetal fluids, stretching of the broad ligament and excessive exercise of the mother are listed as contributing factors. The present case is a 5-year-old Maine Coon queen referred at the time of whelping. At presentation, the queen showed hypothermia (37.1°C), 70 mmHg systolic pressure, heart rate of 180 bpm, weak femoral and tarsal pulses. An amniotic sac protruded through the vulva with hematic vulvar discharge. Previous pregnancy and parturition that occurred three months before were normal and no signs of pathology was recorded neither during this last. Biochemical analysis showed a severe regenerative anaemia (PVC 14%, RBC 3.49 M/µl, HGB 5.1 g/dl, reticulocytes 76.8 K/µl), PLT 112 K/µl, a slight increase of azotaemia (46 mg/dl) and total protein (5.4 mg/dl). Ultrasound examination detected two dead and two alive fetuses, with heart rate of 200 and 130 bpm respectively. At celiotomy, a complete 360° clockwise right uterine horn torsion was revealed. The right uterine wall was violet in color, with tortuous and enlarged vessels. After kittens' removal, although the attempt to resuscitate the two alive kittens, none of them survived. Since the complete clockwise right uterine torsion caused an irreversible pathologic condition of the uterus, due to the altered vascularization of uterine wall, ovariohysterectomy was performed. The queen was

hospitalized with supportive treatment and completely recovered within 10 days. Among obstructive causes of dystocia in dogs and cats, UT occurs infrequently in small animals and there are only few reports in pregnant queen. The signs from the present case such as hypothermia, hemorrhagic vulvar discharge, dystocia and restless agree with those reported in literature. Painful and distended abdomen, dehydration, lethargy, anorexia, peritonitis, are also reported clinical signs. Severe regenerative anemia was likely due to blood uterine sequestration. Exploratory surgery was required for definitive diagnosis and treatment; no correction of the torsion before ovariohysterectomy was performed in order to prevent endotoxins and inflammatory mediators release. The timeliness of surgery allowed to achieve a good prognosis for the queen, although kittens and reproductive activity of the mother were loss. The prognosis of uterine functionality is related to the degree of torsion and the intervention time. Despite in our case it was not possible to detect specific ultrasonographic alterations, more cases are needed to investigate the role of this technique for a presumptive diagnosis of UT in order to prevent fetal and maternal losses, and preserve reproductive performance. Moreover, the hemi-hysterectomy should be considered for preserve the fertility of the queen. This last technique was not possible in our case for the vascular compromise that involved also the uterine body.

26 | Epidemiological profile of breast neoplasms in bitches attended at UENF's veterinary hospital from 2017 to 2020

Hamine Soares Gazel; Isabel Candia Nunes da Cunha
State University of the North Fluminense – UENF, Brazil

Mammary tumors (MT) are the neoplasms that most affect female dogs and are mainly associated with female hormonal disorders, thus being of rare incidence in males. They can also be highly influenced by age, spaying and use of contraceptives, among other issues. The general goal of this project is to determine the prevalence of tumors of the mammary gland in animals attended at the Veterinary Hospital (VH) of the State University of the North Fluminense (UENF), in addition to compiling and storing data to support future research in order to prevent breast neoplasms and to improve therapies applied to this pathology. A retrospective and prospective study was carried out analyzing 126 bitches with breast tumor, attended at the VH of UENF from 2017 to 2020. The owners of each animal were contacted by phone and the information on the medical records were updated. After analyzing the data, it was observed that among animals of known breed, there was a higher incidence of tumors in Poodle, Pinscher, Yorkshire and Dachshund. The most affected mammary glands were M5 (inguinal), followed by M4, M3, M2, M1 and M7, in that order. Just over 60% of the animals had multiple tumours, while about 40% had solitary tumours. In addition, almost 25% of bitches who had breast cancer ended up dying from complications related to the tumour, specially the ones that did not undergo tumour removal surgery after first medical care at the HV's reproductive clinic. There was also a higher occurrence of tumors in animals from 8 years of age, with no significant difference in the incidence between malignant and benign tumours. More than 1/3 of the animals that underwent mastectomy remained with other breast tumors, but there was no difference in occurrence of deaths from MT between this group and the one that had the tumours completely removed. A considerable amount of the analyzed bitches also presented, at some point in their life, pseudocyesis and/or uterine disorders. It was not possible to analyze the survival after the tumors' removal due to the lack of information related to the dates. The low number of animals that used contraceptives and the lack of information about the way they were administered, made it impossible to establish a correlation of this aspect with the appearance and development of breast neoplasms. The importance of diagnostic imaging to evaluate these patients must also be highlighted, since 27.5% of them presented formations suggestive of metastasis, which substantially affects the prognosis of the animals and the therapeutic conduct of the veterinarians.

27 | Chronic vaginitis in a dog induced by a migrating grass seed into the uterus: A case report

Guillaume Domain[1]; Bart Van Goethem[2]; Joke Lannoo[1]; Ann Van Soom[1]; Eline Wydooghe[1]
[1]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University*; [2]*Small Animal Teaching Hospital, Faculty of Veterinary Medicine, Ghent University Merelbeke, Belgium*

Clinical case: A ten-year-old spayed female West Highland White Terrier was referred to the teaching hospital with a history of intermittent mild mucoid vulvar discharge for the last six years. Repeated short-courses of antibiotics were given by the referring veterinarian to keep the discharge under control. However, the discharge had intensified during the last three months and was not responding to antibiotics anymore. A full blood work was performed before referral and was not remarkable. At the teaching hospital, a recessed vulva was observed at clinical examination and the excessive amount of neutrophils present in the vaginal cytology confirmed a vaginitis. Besides, abdominal ultrasound, urinalysis and urine culture revealed a multi resistant bacterial polypoid cystitis. Antibiotic therapy was started after testing for antibiotic sensitivity. Surgical removal of the polyps was planned together with an episioplasty. On the day of the surgery, urinalysis was normal and urine culture negative. Because clinical signs of vaginitis did not resolve three weeks after surgery, blood and urine tests were repeated and revealed ketoacidosis diabetes and a relapse of bacterial cystitis. After stabilization of the ketoacidosis diabetes, an abdominal ultrasound identified a hyperechoic, spindle-shaped structure in the right uterine horn. A vaginoscopy confirmed the uterine origin of the vulvar discharge and no other abnormalities were found in the urogenital tract. Based on these findings, a hysterectomy was performed and a grass seed was

identified in the cranial part of the right uterine horn after dissection. The dog is now further monitored for diabetes mellitus and the owner reported that the vulvar discharge has completely resolved since the surgery.

Discussion: In adult spayed dogs, chronic vaginitis is most commonly secondary to a condition arising from the reproductive tract, the urinary tract or a systemic disease (1). Foreign bodies in the uterus are uncommon, have been sporadically reported in the literature and can be quite challenging to detect for the veterinarian (2). In this case, concurrent diseases (diabetes mellitus and urinary tract infections) exacerbated clinical signs but were most likely not the initial cause of the vaginitis. Although it is impossible to know whether the grass seed had been present in the uterus for the last 6 years, the fact that the vulvar discharge completely resolved after surgery supports this hypothesis.

References:

[1] Kustritz MVR., Veterinary Medicine 2008; 103(10): 562–567.

[2] Benzimra C., Vlaams Diergeneeskundig Tijdschrift 2020; 90: 17–22.

28 | Ultrasound guided fetal demise in a case of incomplete medically-induced abortion in a bitch

Mathieu Giry[1]; Morgane Mantelli[1]; Vincent Leynaud[1]; Arthur Chaussende[1]; Sarah Faucher[2]; Cathy Layssol-Lamour[3]; Sylvie Chastant[4]

[1]*Internal Medicine, Toulouse National Veterinary School, Toulouse, France;* [2]*Diagnostic Imaging, Toulouse National Veterinary School, Toulouse, France;* [3]*Anesthesia, Toulouse National Veterinary School, Toulouse, France;* [4]*NeoCare, Toulouse National Veterinary School, Toulouse, France*

Clinical description: A pregnant Bleu de Gascogne bitch (aged 5 years) is diagnosed affected by an auto-immune hemolytic anemia (regenerative anemia – haematocrit 13%, 2% reticulocytes-, without any sign of haemorrhage, agglutination and direct Coombs tests positive, marked spherocytosis and hemoglobinuria +++). No primary cause of the auto-immune anemia was evidenced (namely by serology: Borrelia burgdorferi, Ehrlichia canis, Anaplasma phagocytophilum; by antigen research: Dirofilaria immitis; by PCR: Babesia sp., Erhlichia spp., Leptospira interrogans sp., no abnormality on diagnosis imaging including both abdominal US and thoracic X-rays, no recent vaccine administration).

Pregnancy was dated at ultrasonography through fetal biometry (automated calculation tool available at www.neocare.pro). Five foetuses were evidenced, one without cardiac beats. Medical induction of abortion was decided based on the potential responsibility of pregnancy into auto-immune hemolytic anemia development [1], together with the probable inability of the bitch to ensure later the proper growth and delivery of foetuses and mainly due to the administration of massive doses of teratogenic/abortive immunosuppressive molecules for the treatment of the auto-immune anemia (methylprednisolone 2 mg/kg BID IV + mycophenolate mofetil 10 mg/kg BID Po). Aglepristone (10 mg/kg SC, ALIZINE, Virbac, Carros, France) was injected at Day 3 and 4 after admission. At Day 13, one foetus was still alive with a normal cardiac frequency (>220 bpm).

Cross-talk between glucocorticoids and progesterone receptors [2] may have decreased the efficacy of aglepristone (explaining partial abortion). The limited improvement of anemia (haematocrit at Day 13 after admission: 22%) despite massive immunosuppressive therapy and the exclusion of most causes of auto-immune anemia were in favour of the responsibility of pregnancy, making even more necessary the obtention of total pregnancy termination. Since corticotherapy could not be discontinued, a failure of a repeated aglepristone treatment was expected and chemical intra-fetal injection was decided.

Based on the dam's weight and stage of pregnancy, the weight of the remaining fetus was estimated at 80 g. At Day 13, the bitch was tranquillized in dorsal recumbency and placed under cardiac monitoring. After cutaneous aseptic preparation, ultrasound-guided injection of 0.1 mg/kg (fetal weight) morphine solution was performed into the amniotic sac, followed by fetal intracardiac injection of 0.45 ml KCl, five minutes after morphine injection, The fetal cardiac arrest was noted immediately after injection. No hemodynamic nor cardiac modification was observed in the dam. The death of the fetus was sonographically confirmed 30 min and 24 h after the procedure. Thanks to a significant improvement of its medical status, the bitch left the hospital at Day 17 under immunosuppressive and antibiotic therapy. Normal uterine involution was observed at Day 25 and total recovery at Day 50.

Discussion: Intra vesicular (prostaglandins) [3] or intrafetal (KCl) [4] injection to terminate pregnancy is rarely performed in the bitch, probably due to the high efficacy of aglepristone and due to the presence of several fetuses per pregnancy. Nevertheless, in some specific instances, intracardiac administration of a low-cost KCl solution represents an efficient and safe option that can be considered.

29 | Veterinary genetic counseling influencing the clinical relationship with breeders

Cynthia O'Connor

East Bridgewater Veterinary Hospital, East Bridgewater, Massachusetts, USA

With the recent advances in molecular genetics and the growing availability of molecular technologies, we witnessed the emergency of genomics into our everyday lives. We live not only in a world where we are constantly pushing boundaries of modern medicine, but also where this technology is readily available, even to the public. This brings challenges for veterinarians and a growing need for the evolution of veterinary genetic counseling. Veterinarians need to not only understand this technology, but also to counsel clients to use it appropriately and to be an informed voice in current political and socioeconomic debates. It is important for veterinarians to assist

breeders by using available technologies to control genetic diseases while maintaining genetic diversity in the population. Veterinarians, particularly reproductive veterinarians, are in a unique position to bridge the gap between scientific advancements and clinical applications in small dog and cat breeding operations. Veterinarians can help breeders utilize tools such as mutation based genetic disease tests, linked marker tests, estimated breeding values, inbreeding or heterozygosity estimates, and phenotypic testing optimally in their breeding programs.

Clinical approach to identify genetic disease begins similarly with a thorough history and physical examination and extends to investigations of relatives, similar disease in other breeds, and similar clinical presentations in other species. With advancements in technology, the identification of a genetic cause of disease is becoming more and more accessible for breeders. Additionally, there is a current trend of consolidation of available genetic disease tests so that hundreds of genetic tests can be performed quickly on a patient at lower costs. However, it is important for breeders to understand that for most patients, only a handful of the genetic disorders out of the hundred or more tested on these panels are clinically relevant to that patient. In addition, not every genetic test is created equal and breeder clients need to understand if and how that test result should influence their breeding decisions.

Currently the vast majority of genetic tests available are for single gene disorders. Common complex disorders such as hip dysplasia leave breeders relying on phenotypic data. In this area, we can learn from production animal breeders with the use of estimated breeding values which have been utilized for decades to provide extensive databases for traits along with heritability estimates for these traits in order to improve everything from milk production, feed efficiency, and reproduction. In return, the extensive data assists researchers in developing molecular tests to increase or decrease the probability of expression of these traits leading to new methods to conquer the genetic evaluation of complex disorders.

As we live in the era of genomics, there is a growing need for veterinarians to understand the genetic technologies and to work with breeder clients to implement breeding strategies that will more effectively reach small animal breeder's goals of producing dogs and cats that not only are what is desired for appearance, temperament, and workability, but also live long, healthy lives.

30 | Ovarian follicle population and anti-Müllerian hormone in mature queens

C. Lapuente[1,2]; A. Lantermino[1]; M. Huk[1,2]; R. R. Rodríguez[1]; P. G. Blanco[1,2]; Cristina Gobello[1,2]

[1]*Center of Reproductive Physiology and Diagnostic Imaging, Faculty of Veterinary Sciences of the National University of La Plata, Argentina*; [2]*CONICET*

Introduction and aim: In mammals, follicle count has regularly been carried out either by ultrasonography or stereological ovarian biopsy, depending on the species. Although, the knowledge of follicle number contributes to: female infertility diagnosis, the follow up of the response to contraceptive or assisted reproductive treatments, it requires specific ultrasound equipment, serial examinations or laborious tissue counting. It has been shown that anti-Müllerian hormone (AMH) measurement appears as an indirect endocrine marker to evaluate ovarian follicle numbers in several species. The aims of this study were: a) To report the relative proportion and the total ovarian follicle population. b) To correlate follicle types with AMH serum concentrations in domestic queens.

Material and methods: Ten, 12–24 months old (mean±SEM 15.70 ± 1.30), short-hair female cats, which were kept under outdoors- indoors home environments, were spayed from June 2018 to March 2019 in the city of La Plata (34° South latitude and 57° West longitude), Argentina. Before surgery a single blood sample was collected. Serum was stored frozen at −70°C until AMH analysis using an electrochemiluminescence immunoassay (Elecsys®, Cobas, Roche Diagnostics International Ltd., Switzerland; Snoeck et al., 2106).

The gonads were weighed, fixed and cut every 10 µm to stain (H&E) a mean of 20 equidistant sections. Subsequently, the total number (Nt) of primordial (Pal), primary (Pry), secondary (Sry), antral (Atl) follicles were determined according to Gougeon and Chainy [1] Nt = No * St * ts / So * do, where No = number of follicles observed; St = total number of sections; ts = width of the sections (µm); So = sections observed and do = mean diameter of the nucleus of follicle type.

A Pearson correlation test was carried out between AMH concentrations and each follicle type. Differences in follicular counts between right and left ovaries were carried by the Student *t* test. Descriptive statistics was expressed as mean±SEM and the level of significance was set at $p < 0.05$.

Results: Mean±SEM AMH serum concentrations were 5.00 ± 0.60 ng/ml with a range of 2.57 to 8.45 ng/ml. Serum AMH and ovarian weight coefficient was $r = 0.20$ ($p > 0.1$). The proportion among Pal, Pry, Sry and Atl follicles were 98.87%, 0.56%, 0.32% and 0.24% respectively.

The total number and the correlation coefficient (*r*) of the same follicles with AMH were 45,240 ± 9935 $r = 0.26$ ($p > 0.1$), 227 ± 42 $r = 0.04$ ($p > 0.1$), 117 ± 16 $r = 0.15$ ($p > 0.1$) and 81 ± 14 $r = 0.85$ ($p < 0.01$). Antral follicle diameter was 122.25 ± 6.48 µm. No difference of the total number of each follicle type were found between both ovaries ($p > 0.1$).

Conclusion: Serum AMH concentrations in these female cats were in line with those reported for cats >1 year [2]. The proportions and the total number of follicles found were slightly lower than those already reported for the species [3]. No ovary seemed to be more functional than its contralateral. Similar to other species, a high correlation between antral follicles and AMH concentrations was found in these queens. AMH could be considered a practical biochemical marker of feline ovarian follicle reserve.

31 | Peripubertal ovarian and uterine ultrasonographic changes in bitches

Rosario Vercellini[1,2]; Cristina Gobello[1,2]; Paola Mendoza[1]; Silvia Olguín[1]; María Czernigow[1]; Daniel Arias[1]; Paula Blanco[1,2]
[1]*CEFIRE & MECODIAG, National University of La Plata, Argentina;* [2]*CONICET*

Introduction and objective: In female dogs, ultrasonographic characterization of the ovaries and uterus during sexual maturation has not been carried out so far. Thus, the aim of this study was to describe two-dimensional and Doppler ultrasonographic peripubertal changes of canine ovary and uterus.

Materials and methods: Eight, 3-month-old, mixed-bred, weighing 3.3 ± 0.42 (1.2–4.9) kg female dogs were included in this study. Pubertal estrus (Day 1) was diagnosed by the first appearance of more than 90% superficial vaginal epithelial cells and estrous behavior. Two-dimensional and Doppler ultrasound evaluations of the ovaries and uterus were carried out on Days -140, -90, -60, -30, 1 and 20, with a 14 MHz linear-array transducer (Toshiba Nemio XG, Japan). Experimental days were retrospectively determined. Longitudinal and transverse sections of the ovaries and uterine body diameter were measured. All anechoic spherical structures in the ovarian parenchyma were considered to be antral follicles, whilst hypoechoic or thick-walled cavitated structures (>1 mm) were recorded as corpora lutea (CLs). The mean number and size of follicles >1 mm and CLs, and the maximum diameter of the largest follicle and corpus luteum were recorded. Peak systolic velocity and end diastolic velocity of intraovarian and uterine arteries were also measured to automatically calculate the resistance index (RI). Repeated measures ANOVA followed by Tukey test was carried out to evaluate the effect of time on the two-dimensional and Doppler ultrasonographic parameters.

Results: All the bitches achieved puberty at 10.5 ± 0.5 (8–13) months of age, weighing 9.3 ± 1.8 (3.9–17.9) kg, which represented 95 ± 1.6% of their adult body weight. The mean ovarian longitudinal ($p < 0.01$) and transverse ($p < 0.01$) diameter increased gradually throughout the study, from 9.8 ± 0.4 and 5 ± 0.2 mm to 13.01 ± 0.7 and 7.8 ± 0.5 mm, respectively. Uterine body diameter also increased from 4.2 ± 0.2 mm on Day −140 to 11.47 ± 1.1 mm on Day 1, and then decreased to 7.9 ± 0.7 mm on Day 20 ($p < 0.01$). Antral follicles were first detected by ultrasound at 5.7 ± 0.6 (3.7–7.5) months of age. In the course of the study, the mean number of follicles progressively augmented from 1.1 ± 0.2 to 5.3 ± 0.5 ($p < 0.01$), while follicular size increased from 1.1 ± 0.07 to 3 ± 0.2 mm ($p < 0.01$). The largest follicles on Days -140, -90, -60, -30 and 1 were 1.2 ± 0.07 mm, 1.2 ± 0.1 mm, 1.4 ± 0.08 mm, 1.9 ± 0.1 mm and 4.1 ± 0.3 mm, respectively ($p < 0.01$). On Day 20, all the bitches presented at least one corpus luteum detected by ultrasound. The mean number and size of CLs were 3.1 ± 0.32 and 4.6 ± 0.57 mm, respectively. The largest CLs were 5.8 ± 0.72 mm. The RI of the intraovarian and uterine arteries declined from the beginning of the study to Day 1, and then increased on Day 20 ($p < 0.01$).

Conclusions: It is concluded that, in female dogs, ovarian and uterine dimensions, follicle number and size and intraovarian and uterine blood flow increased to the onset of puberty. Two-dimensional and Doppler ultrasound of the ovaries and uterus proved to be a suitable non-invasive tool for characterization of sexual maturation in the bitch.

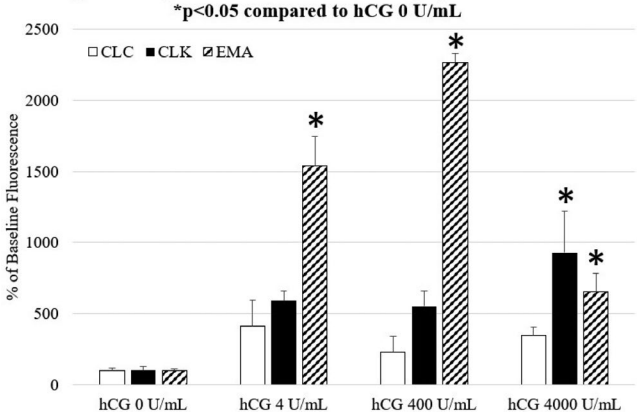
32 | Luteinizing hormone receptor activation stimulates endothelial migration of neoplastic canine T-lymphocytes

Alexa Dietz[1]; Michelle Kutzler[2]
[1]*College of Engineering, Oregon State University*; [2]*Department of Animal & Rangeland Sciences, College of Agricultural Sciences, Oregon State University, USA*

Previous research from our laboratory has demonstrated that luteinizing hormone receptors (LHR) are expressed in neoplastic lymphocytes in canine lymph nodes. Our laboratory also showed that activation of the LHR with human chorionic gonadotropin (hCG) increases LHR gene expression, cell proliferation, and adhesion to an endothelial monolayer in isolated neoplastic T-lymphocytes. The objective of the current study was to determine if hCG activation of LHR in neoplastic T-lymphocytes would increase their migration through an endothelial cell monolayer. The hypothesis was that increasing hCG concentration would induce an increase in neoplastic T-lymphocyte migration. Following the manufacturer's protocol, a commercial migration assay was used to conduct the experiment (#CBA-212, CytoSelect Leukocyte Transmigration Assay, Cell Biolabs, Inc., San Diego, CA). Canine aortic endothelial cells (#Cn304-05, Cell Applications, Inc., San Diego, CA) were cultured on 24-well plate inserts (3 µm pore size) in growth media selective for this cell type (#Cn211-500, Cell Applications) to form a monolayer, then activated with tumor necrosis factor-alpha (0.025 ng/µl) for 12 h. Immortalized neoplastic T-cell lines isolated from three dogs (CLC, EMA, CLK) with multi-centric lymphoma were cultured in RPMI media with 10% FBS for 64–69 h with increasing concentrations of hCG (0, 4, 400, 4,000 U/ml). In serum-free RPMI media, neoplastic T-lymphocytes were fluorescently labeled (CytoSelect LeukoTracker, Cell Biolabs, Inc.) and added to the inserts containing the endothelial monolayer. The inserts were added to migration chambers containing RPMI media with 10% FBS and the chemoattractant (25 ng/ml, #350-NS-010, Recombinant Human/Rhesus Macaque/Feline CXCL12/SDF-1 alpha, R&D Systems, Minneapolis, MN) except the control which contained serum-free RPMI media without the chemoattractant. After an 8-h incubation, non-migratory cells were removed and migratory cells were lysed and quantified on a fluorescence plate reader using 35% gain. The assay for each neoplastic T-cell line was repeated two times with two replicates per assay. Results (mean ± SEM) were expressed as a percentage of baseline (hCG 0 U/ml) after correcting for background fluorescence in controls. Treatments were then compared to baseline using a one-way analysis of variance (GraphPad Prism). Significance was defined as $p < 0.05$. Activation of LHR in neoplastic lymphocytes increased cell

migration at all hCG concentrations for EMA ($p < 0.0001$) and at the highest hCG concentration for CLK ($p = 0.0225$) (Figure 1).

Figure 1: Migration results for 3 canine T-lymphoma cell lines. *p<0.05 compared to hCG 0 U/mL



This is the first study to demonstrate that activation of LHR in neoplastic canine lymphocytes increases their migration through an endothelial cell monolayer. These results could explain why gonadectomized dogs with elevated circulating LH concentrations develop lymphoma at higher rates than intact dogs.

33 | Insight into canine uterine inertia – The organ bath as an ex vivo method for studying the contraction behaviour of canine pregnant myometrium responding to Oxytocin – Preliminary results

Carolin Jungmann[1]; Gemma Mazzouli-Weber[2]; Sandra Goericke-Pesch[1]
[1]*Reproductive Unit – Clinic for Small Animals, University of Veterinary Medicine Hannover, Foundation, Hannover, Germany;* [2]*Institute for Physiology and Cell Biology, University of Veterinary Medicine Hannover Foundation, Hannover, Germany*

Dystocia is a common problem in dogs. Medical treatment approaches using oxytocin might be considered, but treatment fails in about 60% of cases. Therefore, affected bitches often directly go into Caesarean (c-) section. The underlying reason for the lack of success of oxytocin is not clear and absence of adequate contractile response of the myometrium is suspected especially in case of uterine inertia, the most common cause of canine dystocia. This is, however, currently not documented. The functionality of the pregnant canine myometrium and effects of uterotonic drugs on contractions are not well understood, indicating the need for more research. We aimed to provide answers using the organ bath as an ex vivo method to study the uterokinetic activity of oxytocin in the different myometrial layers.

Interplacental site biopsies were obtained from 18 bitches undergoing medically indicated c-sections. 3–48 h after c-section, the myometrial layers were dissected microscopically from the remaining tissue using two different approaches: 1. $n = 18$: the longitudinal and circular muscle layer were separated and four strips of each layer mounted in tissue chambers according to their fibre orientation. 2. $n = 6$: the myometrium was not further dissected, but eight strips were cut. Four strips were mounted according to the orientation of the fibres of the longitudinal layer and four according to the circular muscle layer. The tissue strips were incubated with oxygenated (95% O_2 and 5% CO_2) Krebs buffer during the experiments. All mounted strips were connected to a force transducer measuring myometrial contractility. Catman DAQ software was used to record contractions visible as curves on a computer screen; data were stored on a computer for further processing. Initially, spontaneous contractility of the tissue strips was recorded. After an hour equilibration, three strips of each layer were stimulated with oxytocin (10-7 M, 10-8 M, 10-9 M), one of each layer served as untreated control. This stimulation was repeated three times with a 20 min wash out in between to ensure reproducibility of the responses. Generally, all strips responded to oxytocin with contractions. Whereas the lowest concentration induced rhythmical contractions in both layers, both higher contractions induced strong, long-lasting contractions of the longitudinal layer at the first stimulation, but no further contractions afterwards. Subjective evaluation of contraction patterns in dissected and non-dissected tissues from the same dogs ($n = 6$) did not reveal obvious differences indicating that the dissection did not impair the functionality of the myometrial layers. Although intra- and interindividual differences were visible, spontaneous contractions occurred even 36 h post-surgery demonstrating a promising viability of the tissue. We conclude that the organ bath is a suitable tool for functional studies of canine myometrial contractility and appropriate to assess concentration- response relationships to uterotonic drugs, potentially contributing to a better understanding of uterine inertia and its medical management.

34 | Insights into canine uterine inertia – The effect of PGF2α on canine pregnant myometrium in the organ bath – Preliminary results

Carolin Jungmann[1]; Gemma Mazzouli-Weber[2]; Sandra Goericke-Pesch[1]
[1]*Reproductive Unit – Clinic for Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany;* [2]*Institute for Physiology and Cell Biology, University of Veterinary Medicine Hannover Foundation, Hannover, Germany*

Uterine inertia, the failure of functional myometrial contractions, is a common cause of dystocia in the bitch. Although uterine inertia poses a high risk to negatively affect maternal and foetal health and survival, its aetiology has not been clarified yet. Options for medical treatment are limited and often not successful, making Caesarean sections the treatment of choice. PGF2α is known to trigger uterine contractions and cause dilatation of the cervix, however, its effect on the canine myometrium intra partum has not been described yet.

Hence, this study aims to investigate the uterotonic effects of natural PGF2α, dinoprost, on the canine myometrium during in labour using the organ bath, an ex vivo method that proved to be suitable to study myometrial contractility in the pregnant and non-pregnant uterus of different species, as human, cow, guinea pig, rat and dog.

Uterine interplacental site biopsies were obtained from five bitches undergoing medically indicated emergency Caesarean sections. The tissue strips were dissected microscopically to separate the myometrium from the remaining tissue. Four strips of the longitudinal and four of the circular myometrial layer each were mounted into tissue chambers and connected to a force transducer, measuring the uterine contractions. Strips were incubated with oxygenated (95% O2, 5% CO2) Krebs buffer during the whole experiment. Catman DAQ software was used to continuously record the muscle force per time. Data were recorded and stored on a computer for further processing.

Spontaneous contractility of the tissue strips was recorded over an hour. Afterwards three strips of each layer were stimulated with a natural PGF2α, dinoprost (5 × 10-5 M; 5 × 10-7 M; 5 × 10-11 M), for 20 min, one strip of each layer served as untreated control. This stimulation was repeated three times with 20 min of wash out in between. Finally, all strips were stimulated with oxytocin (10-8 M)

No response was seen following stimulation of the longitudinal and circular layer using the lower concentrations of dinoprost. A reproducible contractile effect was only visible in the circular layer after the highest concentration. The longitudinal layer did not respond in any of the five dogs with a different history of medically indicated Caesarean section. The final stimulation with oxytocin proved the viability of all strips including those that did not show a response to dinoprost.

Our observations further confirm the suitability of the uterine organ bath to study effects of uterotonic agents on myometrial contractility in the dog. Besides, our results suggest that PGF2α is not suitable to induce reproducible uterine contractions in the periparturient dog, as effects were only seen with a dosage 10,000 times above the clinically recommended dosage and above the LD50 (5.13 mg/kg). This clearly indicates that dinoprost cannot be considered an option for medical treatment of uterine inertia in dogs.

35 | The proliferation and differentiation capacity of mesenchymal stem cells isolated from different part of the feline umbilical cord

Meriem Baouche[1]; Agnieszka Krawczenko[2]; Aleksandra Klimczak[2]; Małgorzata Ochota[1]; Wojciech Niżański[1]

[1]*Department of Reproduction and Clinic of Farm Animals, Wrocław University of Environmental and Life Sciences, Wrocław, Poland;*

[2]*Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland*

Umbilical cords as a source of stem cells are of increasing interest for cell therapies. They present little ethical consideration and are reported to contain immune-privileged cells that may be suitable for allogeneic-based treatments. Mesenchymal stem cells (MSCs) have been isolated and characterized from different species and tissues; in feline, MSCs were isolated from bone marrow, amniotic membrane, fat, adipose tissue, and Wharton's jelly. Nevertheless, no one study has yet isolated them from different regions of the umbilical cord. This study aimed to investigate the possibility of obtaining the MSCs from separate regions of the umbilical cord and comparing some of their characteristics to MSCs obtained from the whole cord.

Methods: The umbilical cords (UC, *n* = 14) were collected during cesarean sections and natural delivery from queens (1.5–5 years of age) patients of the Department of Reproduction and Clinic of Farm Animals of Wroclaw. The whole umbilical cords were collected after the routine dissection about 0.5 cm far from the kittens' abdominal wall. The UCs were divided into (1) Wharton's jelly, (2) vessels, and (3) the whole cord; then, the MSCs were isolated from using collagenase type-1, and after that, MSCs were selected by plastic affinity. Cell expansion was quantified in 6 well plates; cell numbers were evaluated after different passages (P1, P2, P3), then cell doubling and doubling time were calculated. The multilineage differentiation (audiogenic, chondrogenic, and osteogenic) was confirmed in cells cultured in 6 well plates using the induction medium.

Results: The primary cell isolates from the whole UC, Wharton's jelly, and vessels exhibited a rhomboid shape after three days of culture, and after one week's culture, most of the isolated cells had a typical spindle shape, consistent with MSC morphology. Interestingly, the cells isolated from Wharton's jelly proliferated markedly faster than MSCs isolated from the other parts. In passage 3, cells from all regions of the UC displayed characteristic osteoblastic, adipocytic, and chondrocyte differentiation following culture in differentiation medium, based on histochemical staining. Compared to the cells in the control group, the induced cells exhibited morphologic changes as well as the formation of neuronal lipid vacuoles in the adipogenic cells, the accumulation of the cartilage in osteogenic cells, and the shape of the calcium in the chondrogenic cells.

Conclusion: In this study, feline MSCs from the whole cord and from the two separate regions (Wharton's jelly, vessels) were successfully isolated, cultured, and proliferated. All the obtained MSCs, regardless of the part of the UC, showed spindle-shaped morphology, plastic adherence, and potency to differentiate into chondrogenic, osteogenic, and adipogenic lineages. Interestingly, the highest cell doubling of MSCs was found in Wharton's jelly samples. As new-born kittens are relatively small when born, and in many cases obtaining the whole umbilical cord becomes challenging. The presenting results suggest that for MSCs isolation in domestic cats, the most suitable part of the UC is the Wharton's jelly. This study was co-funded by Wroclaw University of Environmental and Life Sciences, project no B030/0026/20.

36 | Comparison of cardiovascular changes in bitches suffering from cystic endometrial hyperplasia – Pyometra complex before and after ovariohysterectomy or medical treatment

Pablo Batista[1,2]; Cristina Gobello[1,2]; Analía Arizmendi[2]; Sol Arioni[1,2]; Ana Rube[1]; Mariana Tórtora[1]; Paula Blanco[1]
[1]*CEFIRE & MECODIAG, National University of La Plata, Argentina;* [2]*CONICET*

Introduction and objective: In dogs, cardiac structure and function, peripheral circulation and electrophysiology adjustments associated with uterine infection have not been reported so far. Therefore, the aim of the present study was to compare echocardiographic, hemodynamic and electrocardiographic changes of bitches suffering from cystic endometrial hyperplasia – pyometra complex (CEH-P) before and after ovariohysterectomy or medical treatment.

Materials and methods: Seventeen, 4–12 years old, intact bitches suffering from CEH-P were included in this study. In all the animals, primary cardiac diseases were ruled out. After diagnosis, the bitches were randomly allocated into two treatment groups: dogs that underwent ovariohysterectomy (OVH; n = 9), and dogs medically treated (MED; n = 8), which received cabergoline 5 µg/kg PO for 7 days and cloprostenol 1 µg/kg SC once a day for 14 days. In all the bitches, amoxicillin-clavulanate at 12.5 mg/kg PO was administrated twice a day for 14 days. The animals were echocardiographically, hemodynamically and electrocardiographically evaluated on Days -1, 3, 7, 14, 21 and 28. In OVH and MED, day 0 was defined as the day of surgery or the onset of medical treatment, respectively. To verify the comparability of the groups, comparisons with regard to body weight, age, body temperature, white blood cells count (WBC), presence of depression, anorexia, dehydration, polydipsia, polyuria, vomiting and diarrhea were performed by Student's t-test and Chi square test. Echocardiographic and electrocardiographic variables and systolic blood pressure (SBP) were transformed to percentage change ([Final value-initial value/initial value]*100) and analyzed by ANOVA for repeated measures followed by Tukey test.

Results: Body weight, age, WBC and clinical signs did not differ between groups (p > 0.1). On day -1, cardiovascular parameters were similar between OVH and MED (p > 0.1). Percentage shortening fraction (p < 0.01) and ejection fraction (p < 0.01) changes increased differently in both groups. OVH presented a remarkable rise in both parameters on day 3, while MED showed a gradual augmentation (p < 0.05). Systolic volume gradually increased (p < 0.05), showing no differences between groups (p > 0.1). Conversely, cardiac output (p < 0.01), SBP (p < 0.01), heart rate (p < 0.05), QRS amplitude (p < 0.05) and QT interval (p < 0.01) decreased progressively throughout the study, without differences between groups (p > 0.1).

Conclusion: It is concluded that bitches suffering from CEH-P presented a reversible decrease in systolic function, which was recovered more rapidly in OVH than in MED. In addition, this recovery was accompanied by hemodynamic and electrocardiographic changes. The cardiovascular system should be considered in the selection of the therapeutic option in CEH-P.

37 | Downregulation of testicular endocrine and germinative function with a 4.7 mg deslorelin implant is fully reversible, but highly individual

Sabrina Stempel, Sandra Goericke-Pesch
Reproductive Unit – Clinic for Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Interests on alternatives to surgical castration increased in the last years worldwide and deslorelin slow release implants (DSRI) are considered a suitable alternative to surgical castration in male dogs. Although meanwhile licensed for many years, some open questions regarding the reversibility of DSRI induced effects remain. Among others, the time course of restart of testicular endocrine and germinative function following treatment with DSRI is not well understood by now. To contribute to knowledge relevant for the clinical practitioner, we treated 7 healthy adult male Beagle dogs (TG; n = 6 normospermia; n = 1 pathospermia with moderate oligozoospermia and low grade teratozoospermia) with a 4.7 mg DSRI into the navel region for 5 months (D ex). At that time point, the DSRI was removed, dogs were hemicastrated under general anaesthesia, and regularly examined until d149. Testicular volumes were obtained from results of testicular measurements (TV = 0.5236*length*height*width) taken weekly. Besides, semen collections had been performed before and on a weekly base after D ex to assess semen quality [% progressive motility (PM), % living (LS), % morphologically abnormal sperm (MAS), total sperm count (TSC)]. Blood for testosterone (T) analysis was sampled on D ex and on days d7, 14, 21, 35, 56 and 149. Three healthy normospermic control dogs followed the same protocol (CG), but were treated with saline in the navel region. On D ex, 2/7 dogs had basal T (≤0.1 ng/ml), 3/7 had T < 0.3 ng/ml and 2/7 T >1.8 ng/ml. T was >0.1 ng/ml in all TG dogs from d14, and >0.5 ng/ml from d28. TV increased significantly over time in TG (p < 0.0001) and CG (p < 0.05). Considering TV on D ex as 100%, about a 4-times increase was observed in TG, a 1.58-times increase in CG. The dog with the highest T had sperm already on D ex and was normospermic from d49 after implant removal. Regarding the remaining 6 dogs, first sperm cells were obtained between d49 and 70, first assessable ejaculates between d56 and 91 and normospermia (n = 5) was reached between d84 and 113. Similar semen quality to before was reached in the dog not normospermic at inclusion. These data indicate that duration of downregulation and infertility with a 4.7 mg DSRI is not necessarily 6 months, but induced effects on testicular endocrine and germinative function are fully reversible.

38 | Dystocia in Danish and Norwegian guinea pigs – A survey among breeders

Helene Alm[1,2]; Ann-Sofi Bergqvist[1]; Maria Fura[1]
[1]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, SLU;* [2]*Regiondjursjukhuset Bagarmossen, Sweden*

The guinea pig or cavy is a large South American rodent that enjoys a steady popularity as a pet. Guinea pig patients represent approximately 7% of all veterinary consultations in the countries where this study has been conducted. Guinea pigs as a species do not suffer from many afflictions needing veterinary assistance, but one important area is reproduction problems. Diseases of the reproductive system are the third most common reason for presentation of the guinea pig for veterinary examination according to international studies. Problems associated to birthing pups are of unknown prevalence in the wild, but in captivity where man decides which animals to breed and particularly when, problems do occur. Prevalence increases in direct proportion to the sow's increasing age when primiparous.

The most common thesaurus problem is dystocia. Treatment is unsuccessful in a larger number of cases than in the more common small animal pets such as dogs and cats.

This is dependent on factors unique to the guinea pig; namely large and mature offspring at birth compared to other pet rodents and rabbits, and a pelvic girdle stretching ability disappearing, due to ossification of the pubic symphysis.

This study investigates guinea pig breeders in Norway and Denmark experience with dystocia and caesarean sections. A questionnaire regrading guinea pig dystocia was sent to breeders who were part of the local guinea pig associations in Norway and Denmark. A link to the survey was also posted on Facebook to veterinary groups and groups for guinea pig enthusiasts.

The total number of guinea pig breeders participating was 68. (Twenty from Norway and 48 from Denmark). The majority of the respondents had more than 10 adult guinea pigs and raised more than 10 litters. Of the Danish breeders, 70% have experienced dystocia among their guinea pigs while the figure among the Norwegian breeders was 88%. Fifty-nine percent of the Norwegian and 53% of the Danish breeders had sought veterinary care because of dystocia. In Norway 37.5% and from Denmark 31% of the breeders answered that Caesarean section had been performed on her or his guinea pigs at least once. Eighty-six percent of the Norwegian and 46.5% of the Danish breeders had sows that have been given other treatments than Caesarean section for dystocia. The most frequent treatment for dystocia in this survey except C-section was medical treatment and the second most common was euthanasia of the dam. All most all breeders in this study used prophylactic measures to try to avoid dystocia as genetics: not breed on large litters or big heads and previous dystocia, diet: increase vegetables, vitamin C, avoid obesity and keep sows in good condition, and most importantly not to breed too late the first time.

Norwegian and Danish breeders are well aware of that dystocia in guinea pigs is serious and often fatal, and make several preventive measures to avoid it. They often seek veterinary care for dystocia when needed.

39 | Insights into breeding management and contraception in catteries – What we learned from an online survey

Ricarda Schaper; Sandra Goericke-Pesch
Reproductive Unit of the Clinics – Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Veterinary education mainly focuses on canine reproduction. Research and education about feline reproduction is still limited, resulting in some misunderstandings between cat breeders and veterinarians. Published knowledge about cat breeders' habits regarding breeding management and contraception in catteries is limited. However, this knowledge is crucial for a good collaboration between breeders and veterinarians. We aimed to fill this existing gap by creating an online questionnaire using LimeSurvey® and distributing it via social media and emails. Questions concerned breeders' origin, breed, number of breeding queens/tomcats in the cattery, age at first estrus, use of contraceptives and ovulation-inducing drugs in queens [progestins – medroxyprogesterone acetate (MPA)/proligestone (PRO); hCG; GnRH agonists as injection (GnRH) or slow release implants (SRI); melatonin] the use of glass rods for induction of ovulation, as well as the use of contraceptives in tomcats [progestins- MPA/PRO; SRI; melatonin]. 404 breeders of in total 43 cat breeds from 24 countries participated. The majority has been breeding for >10 years (n = 195, 48.3%). The average number of animals per cattery was 3–4 intact adult queens (n = 185, 45.8%) and one intact tomcat (n = 149, 36.9%). Regarding the first estrus, breeders answered that 50.6% (n = 896) of their female cats came into heat at the age of 7–10 months. The majority of breeders (74.1%, n = 367) has used one or more of the above mentioned options for suppression of the estrus cycle or do use it on a regular base. Among those using contraceptives, progestins (n = 234, 47.3%) were most commonly administered in queens followed by SRI (n = 52, 10.5%). Many breeders did not observe side effects with the use of progestins; in the remaining changed and calmer behaviour, increased appetite, vaginal discharge possibly indicating uterine inflammation and reduced fertility after treatment were observed most frequently. Similar to this, changed (calmer) behaviour and increased appetite were observed after the use of a SRI in queens. Regarding male cats, the slight majority of breeders (n = 229, 52.6%) did not use any of the mentioned contraceptives. Among those using contraceptives in tomcats, the use of the SRI was most common (n = 141, 32.4%), normally once (n = 82, 59.0%) injected by the veterinarian into the neck (n = 101, 67.8%). Most common (side) effects observed include reduced urine spraying, calmer behaviour, reduced/no interest in estrous cats and increased appetite.

As expected and likely due to legal restrictions, long-term expertise and availability, the use of progestins for contraceptive purposes in queens is most common and frequently not associated with side effects, most frequent side effects were vaginal discharge and reduced fertility after treatment. The proportion of potential subclinical side effects such as cystic endometrial hyperplasia remains open. However, even though not registered for the use in cats, SRI are also frequently used in queens and tomcats. Despite possible limitations, as e.g. a certain bias regarding the participants' origin (German breeders being overrepresented), this is the first study with a large number of worldwide participants providing relevant insights into reproductive and contraceptive management of catteries worldwide.

40 | Reproductive loss due to Pelger-Huët anomaly in an Australian shepherd bitch

Bianca Lourdes Frehner[1]; Iris Margaret Reichler[1]; Marilisa Novacco[2]; José Suárez Sánchez-Andrade[3]; Anja Kipar[4]; Katharina Windbichler[4]; Barbara Riond[2]; Orsolya Balogh[1,5]
[1]*Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich;* [2]*Clinical Laboratory, Vetsuisse Faculty, University of Zurich;* [3]*Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich;* [4]*Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich;* [5]*Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine*

Clinical case: A nulliparous 4-year-old intact female Australian shepherd bitch was presented to our clinic for breeding management. The medical and reproductive history was unremarkable and physical examination was normal. The bitch was bred naturally, and on day 26 after ovulation (d0), 6–7 viable fetuses and two resorption sites were identified on ultrasound. The serum progesterone (P4; chemiluminescence) level was 11.9 ng/ml, serum chemistry and complete blood count (CBC) were unremarkable except for an increased C-reactive protein (CRP) level (217 mg/L) and mild anemia (Hct 39%) . The white blood cell count was within reference limits. Peripheral blood smear evaluation revealed the vast majority of neutrophils and eosinophils showing hyposegmented nuclei with a coarse, mature chromatin pattern without signs of toxicity in the cytoplasm of the neutrophils. A Pelger-Huët anomaly (PHA) was suspected. Recheck serum P4, CRP, CBC and reproductive ultrasound were performed regularly between d33 and d57. Uterine resorption sites disappeared by d35 and 6–7 live fetuses were visualized at each control. Serum P4 decreased to 4.0 ng/ml on d46, and oral progesterone supplementation (5 mg/kg body weight, PO, BID-TID) was started and continued until d58. On d51, CBC was unremarkable, CRP was within reference levels (<5 mg/L), and hyposegmentation of the vast majority of the granulocytes was still present, confirming the suspected PHA on blood smear evaluation . Other causes of inflammation were excluded based on clinical examination and reproductive ultrasound. The bitch whelped naturally three viable and four stillborn puppies on d60. In all stillborn puppies, whole-body CT scans revealed moderate to severe ex-vacuo hydrocephalus. The subsequent post mortem examination did not find any evidence of abnormal bone growth or chondrodysplasia. Gross changes were restricted to hydrancephaly and cerebellar hypoplasia. The lungs showed complete atelectasis confirming stillbirth. The histological examination of the brain of one stillborn puppy revealed severe malacia in the remaining cerebral tissue, with the presence of abundant Gitter cells; this was considered an inflammatory response secondary to hydrancephaly. Cytology of femoral bone marrow aspirates from the stillborn puppies showed severe hyposegmentation and clumped, coarse nuclear chromatin in the majority of the myeloid precursor cells, indicating PHA in the offspring . Also, the sire and the three surviving puppies were confirmed to be affected by PHA on blood smear evaluation.
Discussion: This report describes an Australian shepherd family where pairing of PHA-affected parents resulted in the birth of both healthy PHA-affected and stillborn puppies with birth defects. The latter are suspected to be homozygous for PHA. PHA is a hereditary disorder causing nuclear hyposegmentation of polymorphonuclear leukocytes (predominantly affecting neutrophils but also eosinophils and basophils). In Australian shepherds, PHA is an autosomal dominant trait with incomplete penetrance [1]. Heterozygous animals are healthy and diagnosed phenotypically by blood smear evaluation, while the homozygous form is likely lethal [1]. Skeletal deformities e.g. chondrodysplasia or polydactyly, were described in association with homozygous PHA in other species [2, 3]. Due to the potential negative reproductive consequences, our best recommendation is to avoid breedings between PHA-affected sires and bitches.

41 | How does hemicastration affect the testosterone, testicular volume and semen parameter in the dog?

Hanna Körber*, Sabrina Stempel*, Sandra Goericke-Pesch
Reproductive Unit – Clinic For Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

*Contributed equally.
Hemicastration (HEC) and the effect on the residual testis have been studied in various species, e.g. rats, sheep, pigs, cattle but only little is known about the consequences of HEC in mature dogs. Related to another project, HEC of the right testes (D (day) 0) was performed in three 2.5-year-old, healthy normospermic male Beagle dogs. Before HEC, weekly general and andrological examination including measurement of testicular dimensions (height, length, width) to calculate the testicular volume (TV) (TV = 0.5236*height*length*width), performance of semen collection and evaluation (volume, total sperm count (TSC), progressive motility (PM), morphology (MAS), sperm viability) as well as regular blood collections for testosterone analysis over a period of five months were performed. Similarly following HEC, testicular parameters were obtained and semen collected and analyzed as described above once weekly (semen from D14) until D 149. Blood for testosterone analysis was sampled on D0, weekly

until D35, then D56 and D149. Following removal of the right testis on D0 and the left testis on D149, respectively, testicular tissues were Bouin-fixed, paraffin-embedded, cut and hematoxylin-eosin stained for histological evaluation. Analysis of data was descriptive and results are presented as range [minimum–maximum]. Statistical analysis was performed using Graph Pad Prism 9.0. Values were considered to be statistically significant with $p < 0.05$. A paired *t*-test was used to identify significant differences for the TV. ANOVA (Friedman test) was performed to identify significant differences for testosterone and semen parameters over the time. TV of the left testes was 8.9–11.2 cm^3 on D0 and 13.5–17.2 cm^3 on D 149 indicating a significant increase by 152–168% over time ($p = 0.0134$). Testosterone concentrations ranged between 0.25 and 3.78 ng/ml on D0 and between 0.1 and 0.96 ng/ml and did not differ due to HEC. Semen parameters on D-7 were: semen volume 4.6–7.6 ml, TSC 428.8–1291.9 × 106, PM 80–90%, MAS 10–13.5%, viable sperms 90.5–91.5%. Following HEC, TSC decreased initially, but returned to TSCs considered as normospermic. TSC was 274.2–606.3 × 106 on D149. Independent of HEC, TSC varied obviously depending on the collection conditions (estrus teaser bitch versus teaser bitch with estrous pheromones). Semen volume, PM, MAS, viable sperms and testosterone were not affected by HEC. Subjective evaluation of the testicular histology did not reveal obvious differences in spermatogenesis between testicular tissues before and after HEC. Our results confirm that testosterone concentrations, semen volume, PM, viability, and MAS are not affected by HEC whereas TSC is. However, although TSC is reduced, still values considered to be necessary for normospermia were reached in our Beagle dogs. Different to earlier studies, HEC was associated with a significant testicular hypertrophy in our study, likely associated with the longer duration of observation. Further studies should investigate whether this hypertrophy is associated with increased Follicle Stimulating Hormone (FSH) concentrations, larger tubular diameters and increased Sertoli cell numbers as proven for the rat.

42 | Semen collection in pedigree cats

Camila L. Ackermann

[1]*Fertcat, São Paulo State University, São Paulo, Brazil*

Use of an artificial vagina (AV) to collect semen from tom cats has been described since 1970, but most articles describe a long period of training to successfully obtain semen samples. Most researchers believe that it is unpractical to collect semen from privately owned tomcats due to the time required for cat training. We aimed to collect semen from privately owned tomcats using an AV. Semen collection was attempt in twelve privately owned tomcats (nine Maine Coon, one Persian, one Ragdoll and one Siberian) from five different breeders. Each cat was trained for the use of an AV in two training sections (30–60 min each, on two consecutive days), during the training, all cats had their libido observed and scored (absent, low, medium or high) during the training. Also, aggressivity towards humans and the queens was also recorded (present or absent). We observed that most cats had high libido (58.33% of the males would immediately seek the queen to initiate courting) followed by medium (25% of the males would take at least 2–5 min to seek the queen to initiate courting) and 16.33% had low libido (when the male would take longer than 5 min or did not seek the queen to initiate courting). None of the males was aggressive towards humans or queens. We were able to obtain at least one ejaculate from 83.33% of the cats (10/12), 60% of them ejaculated in both training sections (6/10) and 40% ejaculated only once in the second training section (4/10). One male that did not ejaculate in the first section and had medium libido was treated with 150 IU of hCG to increase sexual performance and semen was retrieved 8 hs after treatment in the second section. Both males that were not able to ejaculate during training sections had low libido but were not treated for the second collection. Although for many years semen collection using an AV was considered unpractical to be performed in tomcats that did not belong to an experimental cattery, we were able to collect semen from most of our subjects within two days of training, confirming that this technique can be used successfully, even in pedigree cats.

43 | Clinical use of Fluorescence Enzyme Immunoassay (FEIA) for the determination of the day of ovulation in the canine species

Chiara Milani[1]; Elisa Lidia Boscato[1]; Tamara Badon[1]; Gianfranco Gabai[2]; Magdalena Schrank[1]; Hasan Sontas[3]; Stefano Romagnoli[1]; Antonio Mollo[1]

[1]*Department of Animal Medicine, Production and Health, University of Padova, Italy*; [2]*Department of Comparative Biomedicine and Food Science, University of Padova, Italy*; [3]*Tierambulanz Vorchdorf, Austria*

The aim of this study was to evaluate the use of FEIA for serum progesterone (P4) determination, and the accuracy of the parturition date prediction, calculated from the estimated day of ovulation.

P4 tests were performed using an Automated Immunoassay Analyzer 360 (AIA®360, TOSOH Corp., Japan) and a ST AIA-PACK PROG-III kit. Sera of two diestrous (H), two estrous (H2) and six neutered (L) healthy bitches were collected, assayed and stored at −20°C until the test of linearity, recovery, intra- and inter-assay variation were performed. For the linearity test, dilution of 1:2, 1:4, 1:8, 1:16 (H and L) and dilution of 1:2, 1:4, 1:8 (H2 and L) were used. For the recovery test, synthetic P4 (Progesterone ≥99%, SIGMA- ALDRICH Co., USA) was diluted in sera L in order to obtain dilutions of 0.5, 1, 2, 4, 8, 10, 15 ng/ml. Each dilution was analysed for linearity and recovery tests in five replicates. For both tests, Mean±SD, linearity and recovery percentages (Observed/Expected, O/E %), coefficient of variation (CV%) and linear regression function were calculated. For the intra- and inter-assay variations, pools of L, H2 and H sera were tested three times consecutively. Mean±SD and CV (%) have been calculated. In order to evaluate an association between P4 concentration on the day of ovulation and pregnancy length, serum samples were collected from 31 cases of optimal breeding time identification. Day

of mating, day of expulsion of the first pup and litter size were collected. Accuracy was calculated as the % of bitches with an interval ovulation-parturition of 63 ± 1, ±2, ±3 days.

Linearity results (O/E %) were 97.17, 91.81, 80.19 and 74.65% with an average of 88.76% from H serum sample; linearity percentage (O/E%) of 94.46, 86.40, 87.03% and an average of 91.97% from H2 serum sample. Recovery test (O/E %) results were 97.21, 103.08, 119.45, 98.80, 97.10, 88.40, 86.40% for concentrations of 15, 10, 8, 4, 2, 1 and 0.5 ng/ml. The intra–assay variation test CV (%) were 5.22%, 3.28%, 2.55% and the inter-assay variation test CV% were 3.88, 2.06 and 8.78% for concentrations of 36.16, 6.26 and 0.23 ng/ml. Mean pregnancy length from the day of ovulation was 63.3 ± 1.13 days, distributed as 63 ± 1, 63 ± 2, 63 ± 3 days of pregnancy in 85% (17/20), 95% (19/20) and 100% of cases. Average litter size was 7.19 ± 2.99 pups.

An analytic test is considered accurate when linearity and recovery test results are not exceeding the ±15% of the Expected value. This is the case of our results, with the exception of 1:8 and 1:16 dilution points of linearity test, which are probably underestimating the progesterone concentration due to cross reactivity, or due to matrix effect. Recovery test results are even more accurate. The CV% always give results lower than 9%, with highest values for the lower concentrations, that are nearest the lower limit of detection of the instrument, declared as 0.1 ng/ml. FEIA method can be considered reliable and effective in evaluating the P4 concentration for the ovulation detection in the canine species.

44 | Update on the EVSSAR Reprocases Project – How vets can contribute to learn more about rare diseases

Lisa Riege[1]; Sebastian P. Arlt[1,2]
[1]*Clinic for Animal Reproduction, Free University of Berlin, Berlin, Germany*; [2]*EVSSAR*

Introduction and objectives: With this project we aim to collect cases and information on rare diseases in small animal reproduction. Since it is difficult to perform systematic studies on rare diseases, we hope to be able to draw new conclusions regarding treatment efficacy and potential side effects from a large cases collection.

This project of the Clinic for Animal Reproduction and EVSSAR offers a platform for the collection of cases on the respective diseases as few clinical studies go beyond the few published individual case reports.

Materials and methods: For one year the project REPROCASES is now online on the EVSSAR website: www.evssar.org/reprocases.

The database is based on a RedCap application (Vanderbilt University, Nashville, USA). So far, the platform can be used to report cases of cystic ovarian disease (COD) and cryptorchidism in dogs. The database contains non-scientifically proven recommendations for medical treatment of ovarian cysts and cryptorchidism using GnRH analogues or hCG. Veterinarians from around the world can access these recommendations and are asked to fill in their cases, including treatment results. They can also enter own treatment regimes with the respective results.

If you see cases in your practice or clinic, please enter the case histories including your treatment outcomes! Forms for the documentation of other diseases in dogs and cats will be added in the next months.

Results: The first colleagues have already entered cases. So far, six COD cases were collected. Of these, four treatments were successful, and two treatments had no effect on the ovarian cysts. Furthermore, 12 cryptorchidism cases were collected. Of these, three treatments were successful and four treatments resulted in moderate movement of the non-descended testicle. Four treatment outcomes are still pending. Further details about the cases can be found on the EVSSAR website in the EVSSAR-members area. We are expecting many more case reports to be entered in the next months.

Conclusion: In general, the presented type of data collection is subject to systematic bias and robust studies offer much higher evidence. However, since these studies are currently hardly feasible, we hope that the case collection will allow gaining additional knowledge over the years. The results can help to optimize therapy recommendations and to plan future studies.

The project aims to be open for everyone. We are looking forward to your participation and would love to integrate your ideas! If you wish to add more case report forms for specific diseases or conditions or have other comments or suggestions please contact us. We hope that the collective opinions of practicing veterinarians will help us all to make better clinical decisions in future.

An expansion of the database to include further diseases and other animal species is planned.

45 | Expressions of estrogen-alpha and progesterone receptors in canine uteri of aglepristone-treated pregnant bitches for planned caesarean section

Chunsumon Limmanont[1]; Suppawiwat Ponglowhapan[2]; Sirichai Techarungchaikul[3]; Thitida Pakdeesaneha[3]; Preeda Lertwatcharasarakul[4]; Attapon Kamlangdee[4]; Siriruk Chantakru[5]; Kaitkanoke Sirinarumitr[1]
[1]*Department of Companion Animal Clinical Sciences, Faculty of Veterinary Medicine, Kasetsart University, Thailand;* [2]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Thailand;* [3]*Obstetrics Gynecology and Reproductive Unit, Small Animal Teaching Hospital, Chulalongkorn University, Thailand;* [4]*Department of Pathology, Faculty of Veterinary Medicine, Kasetsart University, Thailand;* [5]*Department of Anatomy, Faculty of Veterinary Medicine, Kasetsart University, Thailand*

Planned caesarean section (C-section) is essential for predisposing dog breed of dystocia. Aglepristone, a competitive progesterone receptor (PR), can be used for planned C-section. Molecular studies on estrogen and progesterone receptors in canine uteri following aglepristone treatment are limited in C-section bitches. This study aimed

to investigate gene and protein expressions of estrogen α receptor (ERα) and PR in uterine placental and inter placental sites following aglepristone injection for planned C-section in bitches.

The study was approved by animal welfare legislation with ID ACKU61-VET-076. Twelve bitches with normal pregnancy were enrolled into the study and divided to treatment (n = 7) and control groups (n = 5). The ovulation timing was determined by serum progesterone (P4). Serum P4, estradiol (E2) were measured before (60–61 days post ovulation) and on C-section day (61–62 days post ovulation). Aglepristone (Alizine™), 15 mg/kg, SC, was given on 60–61 days post ovulation in the treatment group, and C-sections were planned between 20–24 h later. Anesthesia and surgical procedure were followed standard protocols. The uterine placental and inter placental sites from both groups were collected during C-section. Expressions of ERα and PR mRNAs were determined using RT-qPCR. The immuno-histochemical analysis was used to evaluate protein expressions using H-score in 4 different tissue layers; surface, glandular epitheliums, stroma and myometrium at placental and inter placental sites in the uterus. Data was calculated as mean ± SD. Comparisons between two mean values. Comparisons of more than two independent groups were performed using the Kruskal-Wallis Test. A p-value of <0.05 was considered statistically significance. RStudio Version 1.0.153-© 2009–2017 RStudio, Inc. was used for statistical analysis.

There were no significant differences in age, body weight, serum E2 and P4 levels before and at C-section time between control and treatment groups. The expressions of ERα and PR mRNAs were not significant difference at placental and inter placental sites between two groups. The protein expressions of ERα were significant lower in stroma at placental site, and higher in myometrium at placental and inter placental sites in treatment group. There was no significant difference in protein expressions of PR in 4 layers of uterine placental and inter placental sites between both groups.

Increased protein expression of ERα in myometrium by aglepristone, was supported that estrogen was uptake and finally to promote myometrium contraction.

46 | Feline uterine torsion – Two case reports

Kaisa Savolainen[1]; Jaana Parek[1]; Merja Dahlbom[2]
[1]*Estonia University of Life Sciences;* [2]*University of Helsinki, Finland*

Uterine torsion is very rare condition in cats. According to a still unpublished survey of cat owners, the prevalence of uterine torsion was 0.5% of pregnancies in purebred cats. Twenty-seven percent of these torsions occurred with the first pregnancy. In cats, uterine torsion can occur at any time during gestation. Few reports have described fetal survival in a pregnant cat with uterine torsion and there are no reports of efforts to maintain pregnancy in the unaffected, contralateral horn during midgestation.

A 1.5-year-old Russian Blue cat in her second pregnancy presented with sudden lethargy, hypothermia and anemia. The cat had been bred 40 days previously. Transabdominal ultrasonography revealed several dead fetuses with only one fetus with a heartbeat. Blood work showed leukocytosis, anemia, hypoproteinemia, hypoglobulinemia, and extreme hyperglycemia. General anesthesia with an emergency laparotomy was performed. The right uterine horn had rotated 6–7 times, was very congested (purple in appearance), and contained three dead fetuses with copious hemorrhagic liquid. The left uterine horn appeared to have normal circulation (pink in appearance) with one live fetus still alive confirmed by ultrasound. Unilateral right ovariohysterectomy was performed and the queen recovered well from surgery. The queen was started on medroxyprogesterone acetate (5 mg) on the day after the surgery to assist in progestogenic support of the pregnancy. Five days after the unilateral ovariohysterectomy, the owner reported vaginal discharge from the queen and on day eight after the unilateral ovariohysterectomy, transabdominal ultrasonography revealed no fetal heartbeat. Dead fetus was removed by finishing the ovariohysterectomy.

A 4-year-old Russian Blue cat in her fourth pregnancy presented with no signs of labor on day 68 after mating with the exception of two days of moving carefully and the presence of meconium in the vagina. Transabdominal ultrasonography revealed one fetus without a heartbeat and two fetuses with heartbeats. General anesthesia with an emergency laparotomy was performed. One uterine horn had rotated twice around itself with 2 dead fetuses but the other uterine horn was normal in appearance and contained one live fetus. The kitten survived and gave birth to kittens itself one year later. The queen was able to deliver a litter of five kittens normally one year later.

47 | Deslorelin implant to induce temporary infertility in queens

Kaisa Savolainen[1]; Merja Dahlbom[2]
[1]*Estonian University of Life Sciences;* [2]*University of Helsinki, Finland*

The main reason for temporary infertility treatment in queens is excessive and disturbing estrous behavior. Deslorelin is a synthetic gonadotropin-releasing hormone agonist used off-label to temporarily suppress estrous cyclicity.

An online survey was conducted of cat owners who have used deslorelin to suppress estrous cyclicity in their queens.

A total 148 responses were received from 29 different breeds and 20 countries (91% from Europe). Most of the cats (92.6%) received an implant containing 4.7 mg of deslorelin and only 7.4% had received an implant containing 9.4 mg of deslorelin. Results between implants did not differ and were combined for purposes of analysis. The majority (70.9%) of cats had produced offspring before receiving the implant and nearly all (98.1%) of the cats were used for breeding after the implant was removed. The mean±SD duration of estrous cycle suppression lasted for 16.4 ± 6.9 months. Deslorelin significantly reduced (p < 0.0001) estrous behavior and weight loss. A change in coat quality and length was reported in 55% of the survey responses. Prolonged estrus after deslorelin implant administration

was observed in 16.9% of treated queens, which did not resolve until an ovariohysterectomy was performed in 8.1% of the queens.

The mean±SD time until estrous suppression was observed was 9 ± 21.5 weeks after implant administration. In 13% of the cases, the deslorelin implant was removed and estrous cyclicity resumed 16.2 ± 20.7 weeks later. Health problems were reported in 5.4% of the cases following implant administration and included mammary hyperplasia, transient hypertrophic cardiomyopathy, behavioral problems, pyometra and diabetes. Five females (3.4%) displayed estrous behavior following implant administration and allowed mating, that resulted in conception in 40% (2 out of 5 queens). In 9.7% of the queens, the duration of estrous cycle suppression following administration of a second implant was longer than following the first implant. However, in 22.6% of the queens, the duration of estrous cycle suppression following administration of a second implant was shorter than following the first implant. Overall, 88% of owners reported a positive experience using a deslorelin implant to suppress estrous cyclicity in their queens.

In conclusion, administration of an implant containing deslorelin is easy to use and can temporarily suppress estrous cyclicity in most queens. However, this contraceptive method is not 100% effective so queens should still be kept separated from males to prevent unwanted mating.

48 | Influence of exogenous progesterone on the results of the progesterone measurement for the determination of the bitch's mating time

Maren Sievert; Sebastian Ganz; Axel Wehrend
Clinic of Obstetrics, Gynaecology and Andrology of Small and Large Animals, University of Gießen, Germany

Introduction: Progesterone measurement is used to determine the time of ovulation and hence the optimal time for mating/insemination in the bitch. The luteinisation of the granulosa cells, which begins pre-ovulatory, characteristically leads to an analytically well detectable increase in the peripheral blood progesterone concentration of the bitch.

Progesterone measurement is inexpensive, widely available and therefore often conducted outside controlled laboratory conditions. In the past, we have repeatedly been confronted with measurements deviating from the expected results. Those in-house measurement gave false-positive values showing too high levels of progesterone which could not be reproduced by running the progesterone measurement on the same blood samples in controlled conditions.

In the following case report, we will present indications why devices can detect false-positive showing too high values in practice.

Case report: In the period from 02.10.2020 to 12.10.2020, measurements of excessively high values occurred conspicuously frequent during mating time determinations in the Clinic for Obstetrics, Gynaecology and Andrology of Large and Small Animals at the University of Giessen. The measurements were performed using mini VIDAS® (bioMérieux, Nürtingen, Germany). The results of those measurements did not correlate with the anamnesis neither with the clinic of the bitches. In several bitches, there was not any correlation between the exfoliative vaginal cytology and the progesterone level in the blood.

Initially, no cause was identified for the false-positive overly high measurements. The review of the readings in the radioimmunoassay (RIA) laboratory determined lower values as measured before. In the course of this, the batch was exchanged and the measurements repeated. These continued to show increased measurement results, but not exactly the same values as in the first measurement.

By ruling out all the possible factors, it was found that a lab technician who frequently came into contact with the device and the blood samples used a skin cream containing progestogen (bio-identical progesterone from yam root, vitamin E, taoasis base cream). After replacing all the materials with which the laboratory assistant had contact, the excessively high values no longer occurred. In the subsequent check with the RIA, a good correlation ($p < 0.05$, $r = 0.98$) was achieved.

Discussion: This case report shows that the measurement of laboratory instruments can be affected by an exogenous hormonal influence. In practice, this can lead to considerable misinterpretation. As in-house measurements become more common in modern veterinary practice, the error rate has significantly increased compared to measuring exclusively under controlled laboratory conditions. It is noteworthy that the cream application to the skin took place several hours before the laboratory equipment was touched.

49 | Increased urethral sperm number after scrotal stimulation in domestic cats

Sylwia Prochowska, Wojciech Niżański
Department of Reproduction and Clinic of Farm Animals, Wrocław University of Environmental and Life Sciences, Poland

Introduction: Urethral catheterization after medetomidine administration [1] became a method of choice for semen collection in cats. However, in our previous study we reported a huge variation in the total number of spermatozoa possible to obtain by this technique [2]. Also, in around 5% of cases this method was unsuccessful (no spermatozoa despite their presence in the epididymis). We have noticed (data not reported) that the total sperm count was higher when semen was collected from males already prepared for castration (after epilation and cleaning of scrotum). We hypothesized that manual stimulation of scrotum or testicles stimulates also sperm output. The aim of this study was to test this hypothesis that scrotal manual stimulation can improve urethral sperm collection in domestic cats.

Material and methods: The study was performed on 20 male cats of different breeds and age (8 months to 7 years), patients of Ambulatory of Department of Reproduction, Wroclaw University of Environmental and Life Sciences. Medetomidine was administered at the dose of 100 μg/kg. Five minutes after reaching the

sedation effect first semen sample was collected by urethral catheterization. Then a scrotal massage was applied for 2 min and the second sperm sample was collected right after. Both sperm samples were assessed for total sperm count and motility (CASA), viability and morphology (eosin-nigrosine staining). The results were analyzed by Wilcoxon signed-rank test (data not normally distributed).

Results: Although total sperm count showed a wide range both in first (0 to 78.0 × 106) and second sample (2.9 to 93.2 × 106), the scrotal massage allowed to obtained significantly higher number of spermatozoa ($p = 0.0015$). Viability was similar before and after the stimulation (median 92% and 90.5%, $p > 0.05$), whereas motility and the number of morphologically normal spermatozoa was higher in the second sample (median motility 60% and 70%; median morphology 17% and 30.5%, $p < 0.05$).

Conclusion: To conclude, scrotal stimulation before urethral catheterization helps to obtain higher number of spermatozoa in cats. The mechanism of this is not known and requires further research, but it may be similar to stimulation of accessory sexual glands used as a semen collection technique in several species, e.g. bulls. The explanation that the second catheterization itself was responsible for these results is unlikely – in Zambelli's study even triple catheterization did not allow to obtain significantly higher number of spermatozoa [3].

This study has been supported by the Polish National Agency for Academic Exchange under Grant No. PPI/APM/2019/1/00044/U/00001.

[1] Zambelli D, Prati F, Cunto M, et al. Quality and in vitro fertilizing ability of cryopreserved cat spermatozoa obtained by urethral catheterization after medetomidine administration. Theriogenology 2008;69:485–490.

[2] Prochowska S, Niżański W, Ochota M, et al. Characteristics of urethral and epididymal semen collected from cats – a retrospective study of 214 cases. Theriogenology 2015;84:1565–1571.

[3] Zambelli D, Raccagani R, Merlo B, et al. Sperm quality after collection with three different UrCaPI (Urethral Catheterization after Pharmacological Induction) protocols. Proceedings of XIV EVSSAR Congress Milan, 11.03.2011.

50 | Progesterone concentrations during canine pregnancy and parturition parameters

Janna Hinderer; Julia Luedeke; Lisa Riege; Peggy Haimerl; Alexander Bartel; Barbara Kohn; Corinna Weber; Elisabeth Mueller; Sebastian P. Arlt

Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Introduction and objectives: Pregnancy and lactation are amongst the most challenging phases of a bitch's life due to significant endocrinological and metabolic changes. Most studies focusing on the endocrinological aspect of pregnancy consider only a small number of animals. The aim of this study was to evaluate progesterone (P4) concentrations in a large number of bitches in all trimesters of pregnancy and to compare certain parturition parameters to previously published results.

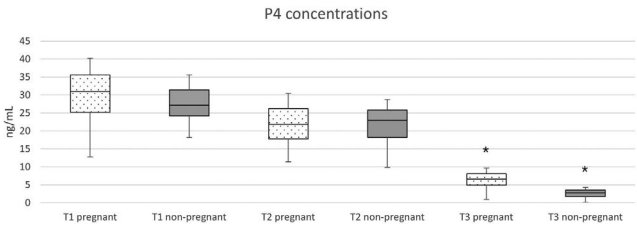
Materials and methods: For this study, 123 healthy bitches of 65 different breeds were enrolled after ovulation timing. Each bitch was examined once in every trimester of pregnancy for clinical health and blood samples were taken to determine the P4 concentration (Immulite 2000 XPi, Siemens Healthcare GmbH, Erlangen, Germany). The first appointment, T1, took place 11–19 days after ovulation, the second one, T2, which included an ultrasound, after 23–32 days. The last appointment, T3, was scheduled shortly before parturition, 52–60 days after ovulation.

Results: At the examinations during the first and second trimester, the mean serum P4 concentrations did not differ between the pregnant and the non-pregnant dogs (T1 30.95 ± 6.65 ng/ml pregnant vs. 27.20 ± 6.26 ng/ml non-pregnant; T2 21.85 ± 6.27 ng/ml pregnant vs. 22.90 ± 5.77 ng/ml non-pregnant). Only the last examination showed a significant ($p < 0.001$) difference, with the pregnant bitches exhibiting higher P4 concentrations than the non-pregnant ones (6.59 ± 2.18 ng/ml pregnant; 2.80 ± 2.26 ng/ml non-pregnant). In context of a potential hypoluteoidism, a significant number of pregnant dogs did not meet the proposed minimum P4 concentrations suggested by Becher et al. 2010. At T1 six dogs had a P4 below 20 ng/ml (lowest 12.80 ng/ml), at T2 this number increased to 33 dogs (lowest 11.40 ng/ml). In addition, at T3 19 dogs had P4 concentrations lower than 5 ng/ml (lowest 2.39 ng/ml 7 days before birth). All these dogs came to term without supplementation and without complications.

In total, 83 bitches delivered naturally on days 59–67 after ovulation (mean 63). Due to dystocia, 15 bitches delivered via C-section on days 61–68 after ovulation (mean 63). The average litter consisted of six puppies.

In this study litter size and gestation length and their influencing factors were also examined. The average litter size increased with body weight ($p < 0.001$) and was also influenced by age ($p = 0.03$; peak at 3–4 years). Gestation length was significantly shorter in larger litters ($p = 0.03$).

Conclusions: The data presented in this study confirm the accepted progress of P4 concentrations during pregnancy and metestrus. Although some of the P4 concentrations fall below previously described minimum values (Becher, Wehrend et al. 2010), there were no pre-term labours or abortions in this study that could have been ascribed to hypoluteoidism.



References:
Becher et al (2010). Luteal insufficiency in the bitch – symptoms, diagnosis, consequences and therapy. A review of the literature. Tierarztl Prax Ausg K Kleintiere Heimtiere 38(6): 389–396.

51 | Use of deslorelin implant in the treatment of persistent estrus in the bitch

Klara Jelinkova; E. Cigankova; Roman Vitasek
Department of Reproduction, Small Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno, Brno, Czech Republic

A 19-month-old bitch Staffordshire Bull Terrier was presented at the department of reproduction to have a persistent estrus. The bitch was in estrus for 47 days with serosanguinous vulvar discharge. The bitch was subjected to clinical examination focused especially on the examination of the reproductive tract.

Vaginal cytology demonstrated typically estrous smear: 100% superficial cells (10% anucleate squames) and visible erythrocytes. The progesterone level was 1.3 ng/ml. It was observed that the cervix of uterus was amplified, measured 18 mm in diameter. The uterus measured 8 mm in width, without finding any fluid and cysts. The right ovary measured 16 × 7 mm, with two hypoechogenic 6–7 mm follicular structures found. The left ovary measured 12 × 9 mm with three hypoechogenic 4–5 mm follicular structures observed. The bitch was treated by the human chorionic gonadotropin (1500 UI, Pregnyl, Organon, Netherlands) in a dose of 750 IU, administered intramuscularly. This therapy was repeated 24 h later. After 14 days in a control examination, estrous symptoms were still present. The progesterone level was 1.5 ng/ml, the vaginal cytology and the sonographic examination demonstrated the same findings as in the previous examination. The right ovary measured 15 × 7 mm, with two hypoechogenic 7 mm follicular structures found. The left ovary measured 10 × 9 mm with three hypoechogenic 4–5 mm follicular structures observed. Thus, the bitch was treated with a deslorelin implant (4.7 mg, Suprelorin, Virbac, France), subcutaneously in the post umbilical region. Twelve days after the implant insertion, the bitch did not show any clinical signs of estrus. The progesterone level was 0.6 ng/ml, the vaginal cytology revealed 50% superficial cells, 50% intermedial cells and no erythrocytes. Ultrasonographically, both ovaries were without follicular and luteal structures. The bitch owners were advised to get the implant surgically removed before the next expected estrous period.

Estrous cycle that persists longer than four weeks is considered abnormal [1]. In our case, persistent estrogenization was apparently caused by the dysfunction of hypothalamic-pituitary-ovarian axis. Furthermore, the course of the ovarian cycle could be affected by other endocrinopathies. In this case, the treatment with human chorionic gonadotropin was unsuccessful. Therefore, the treatment with an implant of deslorelin had been chosen. Initial pituitary stimulation by GnRH-agonists is followed by downregulation of the pituitary receptors [2]. In this case, the initial stimulation effect of deslorelin did not cause the follicular growth and ovulation since the progesterone level measured 12 days after the implant insertion was low. However, 12 days after the implant insertion, the estrus was suppressed. In the described case, we can consider as the benefit of the deslorelin treatment that the luteal phase of the reproductive cycle did not occur. Thus the risk of the pyometra development after the previous prolonged estrogenic stimulation has been eliminated.

[1] Grundi SA, Feldman E, Davidson A. Clinical techniques in small animal practice 2002;17:108–115.
[2] Fontaine E, Fontbonne A. Clinical use of GnRH agonists in canine and feline species. Reprod Domest Anim 2011;46:344–353.

52 | Expressions of anti-Müllerian hormone and its type 2 receptor in the ovary of pregnant and cycling domestic cats

Nilgün Gültiken[1]; Murat Yarım[2]; Hande Gürler[1]; Selim Aslan[3]; Gül Fatma Yarım[4]; Müge Tuncay[1]; Sinem İnal[2]; Sabine Schäfer-Somi[5]
[1]*Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, Turkey* [2]*Department of Pathology, Faculty of Veterinary Medicine, University of Ondokuz Mayis, Turkey* [3]*Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Turkey* [4]*Department of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuz Mayis, Turkey* [5]*Centre for Artificial Insemination and Embryo Transfer, University of Veterinary Medicine, Vienna, Austria*

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β family and exerts an inhibitory role on the development of ovarian follicles in early stages of folliculogenesis. AMH type 2 receptors (AMHRII) were identified in the human ovary and endometrium but there is no data about AMH and AMHRII expression in the cat ovary. Aim of this study was to investigate the expression of both factors in the ovary of pregnant and cyclic cats.

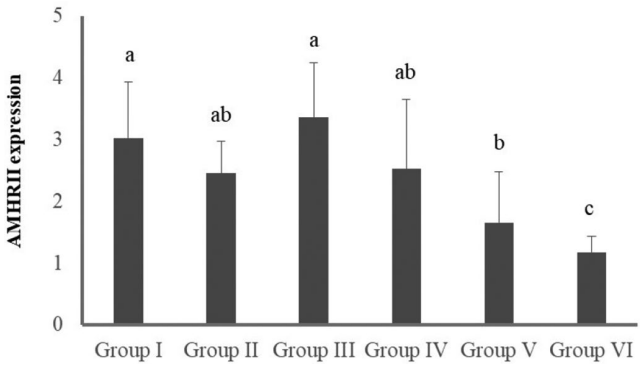
Ovaries of 33 healthy cats were investigated by immunohistochemistry. The cats were divided into groups according to their pregnancy stages: group I (n = 3, 23–30 days), group II (n = 8, 31–40 days), group III (n = 4, 41–50 days), group IV (n = 6, 51–61 days) and according to cycle stages: group V (n = 6, proestrous cats) and group VI (n = 6, oestrous cats). A blood sample was taken before ovariohysterectomy and serum AMH, plasma progesterone and oestrogen concentrations were measured by using species-specific ELISAs. Ovaries were fixed in 10% neutral formalin. A rabbit anti-AMH monoclonal antibody (GTX129593, Genetex, CA, USA) and a rabbit anti-AMHR2 polyclonal antibody (LS-B11943, LSBio, WA, USA) were used. The differences between groups were determined using Duncan test. A p-value of <0.05 was considered significant.

The serum AMH concentration was maximum between midgestation and day 50 of gestation, then decreased towards term ($p < 0.05$). The serum AMH concentration did not differ between proestrous and oestrous cats, and values of both groups were significantly lower than in groups II and III.

Plasma oestrogen concentrations of GV and GVI were significantly higher than pregnant groups ($p < 0.05$ and $p < 0.01$). Plasma progesterone concentration of GII was significantly higher than in GIII and GIV) and than in proestrous and oestrous cats ($p < 0.01$). In GV and GVI, concentration was basal and no statistical difference was determined between these groups.

In the ovaries, strong AMH immunopositivity was observed in granulosa cells of secondary, early antral and small as well as large antral follicles, and in interstitial cells of corpora lutea. No difference was observed between pregnancy stages for AMH expression (P>0.05), and no differences between pregnant and proestrous cats as well as between proestrous and oestrous cats (P>0.05). In GV, positive correlations between ovarian AMH, and antral follicles ($r = 0.931$, $p < 0.05$) and total antral follicles ($r = 0.858$, $p < 0.05$) were assessed. During midgestation, a positive correlation between the number of corpora lutea and the ovarian AMH expression ($r^2 = 0.832$, $p < 0.05$) was determined. Expression of AMHRII was assessed in close co-localization with AMH. The expression was strong in follicles that underwent atresia. No difference was observed between pregnancy stages. AMHRII expression was higher in all pregnant groups compared to oestrous cats (P<0.05), and in group I and III, the expression was higher than in proestrus cats ($p < 0.05$).

We conclude that the AMH and AMHRII co-localization points towards an autocrine effect of AMH via its receptor. The strong AMHRII expression around atretic follicles might implicate that AMH is a regulator of follicular atresia. The course of the serum AMH concentration parallels the ovarian expression and is similar to humans.



53 | Neonatal immunity: Amniotic and placental potential in canine species

Debora Groppetti; J. Filipe; F. Riva; A. Pecile; G. Pizzi; P. Dall'Ara
Università Degli Studi Di Milano, Italy

Introduction and objectives: Neonatal mortality is relatively high in dogs. The immature status of pups makes them vulnerable to pathogens such as parvovirus (CPV-2), infectious hepatitis virus (CAV-1) and distemper virus (CDV) [1]. In dogs, passive immunity which protects neonates from infectious diseases is transferred from the mother mainly by the colostrum intake and only 5–10% through the placenta [2]. Immunoglobulins G (IgGs) represent the only antibody isotype able to cross placenta and are reported also in amniotic fluid (AF) [2]. Pentraxin 3 (PTX3) is a member of the pentraxin superfamily involved in several pathological conditions including infectious, autoimmune and degenerative disorders [3].

The aim of this study was to investigate some mechanisms of passive immunity transfer through amniotic fluid and placenta in canine species at delivery.

Materials and methods: For this purpose, ten pregnant bitches undergoing elective C-section and theirs 63 pups were enrolled in this study. The titration of total IgGs (ELISA Quantitation Set®, Bethyl Laboratories, USA) and specific IgGs against CPV-2, CAV-1 and CDV (Canine VacciCheck Antibody Test Kit®, Biogal/Agrolabo, Italy) was performed both in amniotic fluid and in maternal serum collected at birth. Moreover, PTX3 mRNA expression in AF cells and placenta of each pup was investigated by Real Time PCR. Statistical analysis was performed using Graph Pad Prism 6, GraphPad Software (La Jolla, CA, USA). Normally and not-normally distributed data were analysed using Pearson and Spearman correlations, respectively.

Results: The specific antibodies against CAV-1 ($p = 0.002$) and CVD ($p = 0.046$) but not CPV or total IgGs in maternal serum and amniotic fluid were statistically correlated. Amniotic and placental PTX3 were positively linked ($p \leq 0.0001$). Neonatal mortality was associated with both low maternal total IgG levels and higher PTX3 in amniotic cells, even though without a statistical significance.

Conclusions: Amniotic fluid and placenta collection at birth may represent a valid, not-invasive method to indirectly estimate neonatal passive immunity in canine patients. Total and specific IgGs can be detected both in maternal serum and in amniotic fluid, as well as PTX3 can be found in AF cells and placenta. Although these results must be interpreted as preliminary data, an interesting potential role of IgGs and PTX3 in early detection of neonatal maturity, protection and mortality, as well as of congenital and acquired defects can be advanced.

[1] Mila H, Feugier A, Grellet A, et al. Inadequate passive immune transfer in puppies. Prev Vet Mede 2014;116:209–213.

[2] Dall'Ara P, Meloni T, Rota A, et al. Immunoglobulins G and lysozyme concentrations in canine fetal fluids at term of pregnancy. Theriogenology 2015;83:766–771.

[3] Porte R, Davoudian S, Asgari F, et al. The long Pentraxin PTX3 as a humoral innate immunity functional player and biomarker of infections and sepsis. Front Immunol 2019;10:794.

54 | Successful treatment of penile injury in a dog

Lilyana Sapitskaya
Veterinary Clinic "Astin", Moscow, Russia

Penile injury in dogs is a common urologic emergency that requires quick, decisive actions by the doctor. Treatment with urethrostomy or penis amputation may be needed. This case report aims to consider the possibility of successful treatment for penile injuries for stud dogs.

A 7-month-old intact male Dachshund presented with bleeding from the penis. The owner believed that the dog had been bitten by an estrous female. Physical examination revealed extensive damage to the soft tissues of the penis including cavernous body lacerations, penile hematoma with deviation, and tissue necrosis. There appeared to be no damage to the urethra and urethral patency was maintained. In addition, radiography did not reveal an os penis fracture. The dog was anemic (RBC: 2.9*1012/L, Hb: 65 g/L, Ht 18.7%) presumably from earlier penile hemorrhage but there was minimal bleeding evident during examination. The dog was also in depressed, anorexic, and in severe pain.

The dog was administered non-steroidal anti-inflammatories (Carprofen (Rycarfa, Krka), 2.3 mg/kg SC once and (Robenacoxib (Onsior, Elanco France) 1 mg/kg BID orally for 10 days) and antibiotics (amoxicillin/clavulanic acid (Synulox, Pfizer USA) 20 mg/kg BID orally for 21 days). The penis was cleaned with a disinfectant (chlorhexidine digluconate 0.05% aqueous solution BID until complete healing). The penis had completely healed with the preservation of all functions after 30 days.

55 | Influence of the estrous cycle stage on the feline oocyte quality and embryo culture outcome

Małgorzata Ochota; Klaudia Kołaczkiewicz; Meriem Baouche; Wojciech Niżański

Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland

Introduction: The experiment was conducted to investigate whether donors' health and phase of estrous cycle might affect the quality of feline oocytes, fertilisation outcome and feline embryo developmental competence in vitro. Up to date there is only scarce data on the sex hormonal influence i.e. the phase of estrous cycle on the suitability of feline oocytes for further in vitro maturation and subsequent embryo culture.

Materials and methods: Queens – patients of Department of Reproduction, presented for a routine spay on owners' request or castrated due to medical reasons were enrolled for this study. Full history, initial clinical examination results, vaginal cytology and serum levels of progesterone (P4) and estrogens (E2) were evaluated on the day of surgery. The collected ovaries (n = 80 pairs) were retrospectively divided into 7 Experimental Groups, based on history and clinical data, vaginal cytology and the E2 and P4 serum levels: 1. Anestrous, 2. Estrous, 3. Interestrous, 4. Diestrus, 5. MPA (queens receiving medroxyprogesterone for 30–60 days before spaying), 6. Young, 7. Old/Diseased. The Control Group consisted of randomly selected queens with unknown history and hormonal status, from which ovaries were collected and proceeded in the same way as for experimental groups.

Results: A total of 738 oocytes were obtained with the significantly lowest number collected form Old/Diseased queens comparing to control (unpaired t-test, $p < 0.001$). The oocyte quality ranged from 3 (fair) to 5 (excellent) and on average the lowest comparing to control was in Anestrous (4), Estrous (3,4) and Old/Diseased (3,5) queens (unpaired t-test, $p < 0.005$). The obtained oocytes were submitted for in vitro maturation and fertilization then the presumptive zygotes were selected for further embryo culture according to the routine protocol used in our laboratory. The worst cleave rate was noted for oocytes obtained from Estrous (28.4%), Young (27.7%) and Old/Diseased (14.3%) females, whereas in the remaining Groups it was similar to Control (47.1%). The highest blastocyst number was found in the Interestrous (n = 17) and MPA (n = 17) groups and the lowest in Estrous (n = 0) and Old/Diseased (n = 0) groups (unpaired t-test, $p < 0.005$) compared to Control. Among all the investigated groups the blastocyst rate was significantly higher in Interestrous (17.5%), Diestrus (18.5%) and MPA (15.3%) groups (one-way Anova, $p < 0.05$). However, the oocyte/blastocyst ratio was better only in Interestrous and Diestrus Groups, while the lowest in Estrous queens (Fisher exact test, $p < 0.05$) comparing to Control.

Conclusions: The obtained results shown relationship between queens' general health and estrous cycle phase and the oocyte number, quality and subsequent embryo development. The best embryo

developmental competence was noted in healthy, adult female cats with high circulating progesterone levels (self-synthetized or administered due to medical reasons – MPA) and young, healthy cycling queens being at interestrous at the moment of oocyte collection. It seems that queens in estrous and older females with reproductive tract disorders are the least suitable as the oocyte source for in vitro purposes.

56 | Cesarean section under inhalant or epidural anesthesia – Which one is better for the puppies?

Małgorzata Ochota[1]; Wojciech Niżański[1]; Zdzisław Kiełbowicz[2]; Agnieszka Antończyk[2]
[1]*Department of Reproduction, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences;* [2]*Department and Clinic of Surgery, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland*

Introduction: The objective of this study was to compare inhalant vs. inhalant with epidural anesthetic protocol for Caesarean Section on early neonatal performance and vitality.
Materials and methods: Bitches (31) undergoing elective CS were enrolled. All females received a dose of 0.2 mg/kg SC meloxicam and were randomly assigned into: Gr I (Isoflurane, *n* = 16) or Gr IE (Isoflurane + Epidural, *n* = 15). Anesthesia was induced with propofol at 4–6 mg/kg to effect, and maintained with isoflurane in oxygen. In IE group, epidural anesthesia was performed using lidocaine (3–4 mg/kg) injection into the lumbosacral intervertebral space. Directly after puppy extraction, 100 µl of mixed umbilical cord blood was collected for pH, HCO3-, pCO2, glucose and lactates analysis. The modified Apgar scoring system (AS) was used to score neonates immediately after birth (before neonatal care was instituted), at 5 and 20 min after birth. Neonates were scored: 7–10 no distress, healthy; 4–6 moderate distress, weak and 0–3 severe distress, critical (1).
Results: In Gr I = 40 and Gr IE = 44 newborns were examined. In first AS evaluation majority of puppies were critical (65% Gr I vs. 70% Gr IE). Significant differences were found in Apgar scores. Litters of IE dams improved quicker and received higher mean AS at 5 min (50% of puppies vs. only 15% in group I). Furthermore, in IE group only 11% of puppies had low (0–3 points) and 39% moderate (4–7 points) Apgar scores, whereas in I group it was 17.5% and 67.5%, respectively. Moreover, at 20 min. only 7% of puppies in IE group were considered critical and the remaining 93% were healthy, comparing to 5% critical, 37.5% weak and only 57.5% healthy puppies in Gr I. Neonatal mortality within first 48 h was similar – 10% vs 9.1% in I and IE group, respectively. Despite clear clinical differences in puppies' vitality resulting in significantly different Apgar scores, no differences were found in umbilical blood gas results among critical, weak, and normal pups in both groups. The mean pH values were 7.2 in both groups, the pCO_2 was 57.2 ± 11.5 mmHg in I group vs. 58.7 ± 15.1 mmHg in IE group, the cHCO3- and lactate levels were very similar in both groups: 22.6 ± 3.2 mmol/L vs. 22.8 ± 3.6 mmol/l and 2.6 ± 1.5 mmol/L vs. 2.5 ± 1 mmol/L, respectively. Only the mean blood glucose level was higher in IE 79.5 ± 29.8 mg/dl comparing to I group 74.5 ± 25.3 mg/dl.
Conclusions: The obtained results demonstrated that none of the two investigated anesthetic protocols had a significant influence on metabolic neonatal state and did not affect neonatal umbilical blood gas results. However, significant differences were noted in immediate postnatal puppy performance. Newborns from dams in which epidural anesthesia was performed scored significantly higher in AS at 5 and 20 min, indicating their better vitality and quicker improvement post-surgery most probably due to the reduced plane of anesthesia required.
This study was co-funded by Wroclaw University of Environmental and Life Sciences, project no B030/0026/20.
References:
[1] Veronesi M.C.et al. Theriogenology 2009, 72, 401–407.

57 | Comparative progesterone measurements using Fuji DRI-CHEM IMMUNO AU10V® and RIA

Theresa Conze; F. Schneider; Axel Wehrend
Clinic of Obstetrics, Gynecology and Andrology of Large and Small Animals with Ambulatory Service, Faculty of Veterinary Medicine, Justus-Liebig-University Giessen, Germany

To establish the progesterone measurement using the Fuji DRI-CHEM IMMUNO AU10V® (Fujifilm, Germany), progesterone measurements were performed with the devices Fuji DRI-CHEM IMMUNO AU10V® (named as IMMUNO AU10V® in the abstract) and compared with measurement using radioimmunoassay (RIA), which is regarded as the gold standard [1;2]. The Immuno AU 10V® uses the Surface Plasmon enhanced Fluorescence technology. Results are obtained in approximately 10 min, which is ideal for in-house measurements.
Frozen (28) and fresh serum samples (30) from bitches in heat and pregnant bitches were used. The blood samples were centrifuged for 5 min at 4000 rpm. Thereafter aliquots were prepared, followed by measurement by IMMUNO AU10V® and by RIA . Statistical analyses were performed.
All blood samples showed values between 0.5 ng/ml and 40 ng/ml, which is the measurement reference of the IMMUNO AU10V®. Correlation analyses were performed, which showed a significant correlation between both methods ($p < 0.001$, $r = 0.82$). Furthermore, a Bland – Altman analysis was performed. A significant trend was found in the comparison of the progesterone measurement using IMMUNO AU10V® and RIA ($r = -0.57$, $p < 0.001$). As progesterone levels in the RIA measurement increase, the difference between the methods increases. The results using the IMMUNO AU10V® are significantly lower at high progesterone levels. When calculating the concordance correlation coefficient according to Lin, a value of 0.76 was determined. Our results show a significant correlation between the results of the IMMUNO AU 10V® and those obtained by RIA. This results match the results of Fontbonne et al. (2020), who found that the progesterone values measured using the IMMUNO AU10V® were highly

correlated with those measured with CLIA [3]. However, in our study it should be noted that at high progesterone levels, measurements using IMMUNO AU10V® tend to be false low. Consistent with these results, Fontbonne et al. (2020) determined that the agreement between CLIA and IMMUNO AU10V® improved when the regression analysis was restricted to the range of values <10 ng/ml. This indicates that reference values should be established for the different phases of the estrous cycle for each essay.

Based on the results, progesterone measurement using IMMUNO AU10V® can be recommended as an accurate in-house progesterone test.

[1] Hoffmann B, Hoveler R, Hasan SH et al. Ovarian and pituitary function in dogs after hysterectomy. Journal of reproduction and fertility 1992; 96(2):837–845.

[2] Hoffmann B, Kyrein HJ, Ender ML. An efficient procedure for the determination of progesterone by radioimmunoassay applied to bovine peripheral plasma. Hormone research 1993; 4(5):302–310.

[3] Fontbonne A, Maenhoudt C, Thoumire S et al. Evaluation of surface plasmon field-enhanced fluorescence spectroscopy for rapid measurement of progesterone concentration in bitches. Am J Vet Res 2021; 82(5):417–424.

58 | Developing a canine oviduct model for in vitro oocyte maturation

Annemarie Spruijt; Jeffrey de Gier; Lotte Meeuws; Tom Stout; Karin Albers-Wolthers; Heiko Henning

[1]*Department of Clinical Sciences, Utrecht University, The Netherlands*

Introduction and objective: Assisted reproductive technologies (ART) such as in vitro fertilization (IVF) are hampered in canids because of their unique reproductive physiology [1]. The major obstacle is that canine oocytes require two to four days of oviductal maturation to become fertilizable. Despite efforts to mimic canine post-ovulatory oviduct conditions using chemically defined media, in vitro oocyte maturation (IVM) rates are variable and fertilization rates are low [2]. The aim of this study was to establish a canine oviduct epithelial cell culture that might support IVM in the future.

Materials and methods: Oviducts were obtained from bitches that underwent elective ovariectomy during anestrus. Owners gave informed consent. Three isolation methods (oviduct flushing, squeezing and enzymatic digestion) were compared (for all n = 7 dogs) to determine which resulted in the highest viable cell yield. The canine oviduct epithelial cells (COECs) were then cultured in Transwell® inserts for 1 week until monolayer formation followed by 20 days culture in an air-liquid interface, with or without basal supplementation of hormone concentration that mimicked the cyclical change from late anestrus to ovulation (progesterone, estradiol, FSH and LH; n = 4 dogs, two inserts per experimental condition). Table 1 shows specific hormone concentrations and medium changes per day.

Results: Preliminary results showed that enzymatic digestion of the epithelial lining or squeezing the oviduct between forceps yielded more viable cells per oviduct (4.64 ± 3.31 × 106 and 2.95 ± 2.30 × 106) than simply flushing the oviductal lumen (0.84 ± 0.82 × 106; p < 0.05). The epithelial origin of the harvested cells was confirmed by cytokeratin 18 immunoreactivity. Surprisingly, monolayers containing both ciliated and non-ciliated COECs were readily achieved in conditions without added hormones in 3 out of 4 samples. Mimicking the cyclical hormone changes did not improve cell morphology.

Conclusion: COEC monolayers cultured in Transwell® inserts provide a promising basis for developing a canine oviduct model to support IVM in the future.

Stage Of cycle	Day	Task	Concentrations			
			FSH	HCG	E2	P4
Anestrus	1	Switch from Liquid-liquid to air-liquid interface Medium change	13.8 U/L	1.9 µg/L	30 pmol/L	2.2 nmol/L
	2					
	3	Medium change	10 U/L	1.9 µg/L	30 pmol/L	2.2 nmol/L
	4					
	5					
	6	Medium change	10 U/L	1.9 µg/L	30 pmol/L	2.2 nmol/L
	7					
Pro-estrous	8	Medium change	10 U/L	1.9 µg/L	30 pmol/L	2.2 nmol/L
	9					
	10	Medium change	1.6 U/L	1.9 µg/L	50 pmol/L	2.2 nmol/L
	11					
	12					
	13	Medium change	1.6 U/L	1.9 µg/L	100 pmol/L	2.2 nmol/L
	14					
	15					
	16	Medium change	1.6 U/L	1.9 µg/L	353 pmol/L	2.2 nmol/L
Estrous	17	Add FSH and HCG	13.8 U/L	18.7 µg/L		
	18					
	19					
	20	Fixation and staining				

[1] Chastant-Maillard S, Chebrout M, Thoumire S, et al. Embryo biotechnology in the dog: a review. Reprod Fertil Dev 2010;22:1049–1056.

[2] No J, Zhao M, Lee S, et al. Enhanced in vitro maturation of canine oocytes by oviduct epithelial cell co-culture. Theriogenology 2018;105:66–74.

59 | Relationship between placental traits and birth weight in boerboel puppies

Amélie Mugnier[1]; Estelle Picarougne[1]; Emilie Mallauran[1]; Kurt De Cramer[2]; Sylvie Chastant[1]

[1]*Neocare, Université de Toulouse, ENVT, Toulouse, France* [2]*Rant en Dal Animal Hospital, City of Johannesburg, Gauteng, South Africa*

Introduction and objectives: Since low birth weight dramatically increases the risk for neonatal mortality in puppies [1,2], exploration of fetal growth mechanism is required to define preventive solutions. Intra-uterine growth retardation can be linked either to fetal factors (including genetics) or to placenta (with placental function limiting fetal growth) [3]. The objective of this study was to explore the relationship between puppy birth weight and placental characteristics.

Materials and methods: Non-emergency caesarean sections were performed in Boerboel dams at the time of parturition. Immediately at extraction, puppies were weighted. For each puppy, placental attachment zone was measured (length, width) and weighted. Surface (length × width) and surface to mass ratio (surface ÷ mass × 1000)

were then calculated. The ratio between placental and body weight was also calculated for each individual. Statistical analyses were performed using R software (version 4.0.4). After the bivariate analyses, a linear-mixed effects model was performed to test the influence of placental weight on puppy birth weight by taking into account other factors (sex, dam age at whelping, litter size) with litter as random effect to deal with the non-independence of puppies from the same litter.

Results: Data on 104 Boerboel puppies from 15 litters, and their placentas were included. Sex ratio was 0.8 (46 males to 58 females) and three puppies were stillborn. The mean birth weight was 579 g, with a range of 203–938 g. Placental attachment area weights and surfaces ranged from 23 to 85 g (mean = 51.3 ± 12.2 g) and 52.1 to 121.5 cm^2 (mean = 85.9 ± 12.7 cm^2), respectively. The mean surface to mass ratio of attachment area was 59 cm^2/g, with a range of 35–87 cm^2/g. The ratio between placental weight and birth weight varied from 4.1 to 15 % (mean = 9 ± 2 %). Birth weight was significantly but weakly to moderately correlated with placental weight (r = 0.57), placental surface (r = 0.42) and placental surface to mass ratio (r = 0.41). From all parameters evaluated in the multivariate model, only placental attachment area weight was significantly associated with puppy birth weight (p < 0.001). Moreover, placental weight, surface and surface to mass ratio were significantly different between puppies from the first quartile of birth weight (in the lowest 25%) and those from the last quartile (in the highest 25%; p < 0.001, p = 0.005 and p = 0.008, respectively).

Conclusions: These results suggest a relationship between birth weight and macroscopic placental traits such as weight and surface. Thereafter, comparison of microscopic placental characteristics depending on birth weight should be investigated to better understand the physiopathology of low birth weight.

References:

[1] Mugnier A et al. Birth weight as a risk factor for neonatal mortality: Breed-specific approach to identify at-risk puppies. Prev Vet Med. 2019 Nov 1;171:104746.

[2] Mila H et al. Differential impact of birth weight and early growth on neonatal mortality in puppies. J Anim Sci. 2015;93(9):4436–42.

[3] Chavatte-Palmer P, Tarrade A. Placentation in different mammalian species. Ann Endocrinol. 2016;77(2):67–74.

60 | Effects of deslorelin on cancer evolution and patient survival after radical mastectomy in queens with invasive mammary carcinoma: Preliminary results

Gogny Anne[1]; Topie Emmanuel[1]; Speiser-Fontaine Christelle[2]; Nguyen Frédérique[3]

[1]*Small Animal Reproduction Unit, Oniris, Nantes, France;* [2]*GMBO, Virbac, Carros, France;* [3]*Université de Nantes, Oniris, Inserm, CRCINA, Nantes, France*

Feline mammary carcinomas (FMCs) have been associated with short patient survival after mastectomy, and neither cytotoxic chemotherapy nor selective COX-2 inhibitors have proved to be effective. In dogs with mammary carcinoma, the GnRH agonist goserelin reduced tumor size and induced an 88% relapse-free survival at 2 years. In cats, such data are lacking. The aim of this study was to evaluate the effects of the GnRH agonist deslorelin in queens with FMCs.

This randomized, double-blind placebo-controlled study was conducted on 20 queens with invasive FMCs. The protocol was approved by an independent Ethics Committee. After radical mastectomy and randomization according to tumor stage, a 4.7 mg deslorelin implant (Suprelorin®) (N = 10 cats) or a placebo (N = 10) was injected subcutaneously every 6 months for two years. All cases were followed up for locoregional recurrence risk (LRR, local recurrence and/or lymph node metastasis), distant metastasis-free interval (DMFI), overall survival (OS, time from mastectomy to death from any cause), and cancer-specific survival (CSS, time from mastectomy to death attributable to the mammary carcinoma). Immunohistochemistry was performed to detect Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor-2 (HER2), Ki-67, and Androgen Receptor (AR). Survival analyses were performed using log-rank tests. A p value <0.05 was considered significant.

All animals combined, the mean age at the time of diagnosis was 11.3 ± 2.2 years (range: 6.8–14.6). Twelve (60%) of the queens were neutered at the time of diagnosis (n = 6 in each group), and four others (20%) after implant injection at owner request (n = 3 in the deslorelin group, n = 1 in the placebo group). The treatment group and the placebo group did not significantly differ in terms of patient age, tumor size, lymphovascular invasion, nodal metastasis, distant metastasis, histological types and grades, and ER, PR, HER2, Ki-67 and AR expression. Overall, deslorelin had no significant impact on LRR (p = 0.47), DMFI (p = 0.23), OS (p = 0.40), and CSS (p = 0.40).

Thus, conversely to the effects reported in female dogs, GnRH agonists might not be relevant as an adjuvant treatment for mammary carcinomas in queens. However, further investigations remain necessary to precisely identify deslorelin effects on the different FMC subtypes.

61 | Osaterone acetate decreases urinary cystine concentrations in dogs with type III cystinuria

Martina Reher[1]; Ulrich Merschbrock;[2]

[1]*Virbac Tierarzneimittel GmbH, Bad Oldesloe, Germany* [2]*Veterinary Clinic Dr. Ulrich Merschbrock, Germany*

Introduction and objectives: Cystinuria is a genetic metabolic disorder that results in decreased reabsorption of cystine, ornithine, lysine, and arginine from the urine. Only the accumulation of cystine leads to clinical problems such as hematuria, dysuria, recurrent urolithiasis and urinary tract infections. As a result, cystine stones may form in the urinary tract. Type III cystinuria has been described in more than 70 breeds of dogs and occurs sex-dependently only in males, which is why it is suspected that testosterone influences the development of crystals and uroliths. This is supported by the observation that affected patients benefit from castration. Based on this

consideration, the question arose whether the antiandrogen osaterone acetate is also able to reduce the urinary cystine concentration. Osaterone acetate is approved in the European Union for the treatment of benign prostatic hyperplasia in male dogs. It inhibits testosterone uptake into the prostate, acts as a competitive antagonist at the androgen receptor level, and inhibits the 5 α-reductase enzyme.

Materials and methods: To test the hypothesis that osaterone acetate may be beneficial in dogs with type III cystinuria, a total of seven privately owned, intact male dogs (5 Irish Terriers, 2 mongrels) presented to vet clinics in Germany were treated with osaterone acetate (Ypozane ND, Virbac) with a dosage of 0.25–0.5 mg/kg bodyweight daily for 7 days. Urinary cystine concentration were analysed via column chromatography before and after treatment. The data were tested for normal distribution using a Shapiro Wilk test ($p > 0.05$). Where data were normally distributed a *t*-test was used to test for significant differences ($p < 0.05$). Otherwise, a Wilcoxon signed rank test was applied ($p < 0.05$).

Results: A significant reduction in urinary cystine concentration could be observed ($p < 0.05$) from a mean of 722 µg/g creatinine (515–1697 µg/g creatinine) before treatment to 90 µg/g creatinine (12–306 µg/g creatinine) 2–5 weeks after treatment.

Conclusions: Osaterone acetate appears to reliably reduce cystine concentrations in urine, likely counteracting the occurrence of cystine crystals and stones and associated clinical problems. Further studies should follow to demonstrate the effect in a controlled trial with a larger patient population and, in particular, the long-term effect of osaterone acetate in type III cystinuria.

62 | Preliminary results on neonatal mortality and puppy loss in the first 8 weeks of life in the Bernese Mountain Dog

Magdalena Schrank, Antonio Mollo
Department of Animal Medicine, Production and Health, University of Padua, Italy

Mortality rate of puppies within the first 7–8 weeks of life is high, with stillbirth accounting for more than half of puppy losses. The Swiss Club of Bernese Mountain Dog contacted us with the request to elaborate data regarding neonatal mortality and puppy loss within their population. Data collection was performed using access to the data base DogBase (TG-Verlag Beuing GmbH) and the announcement of birth provided by the breeders at the end of the first 8 weeks of the litters' life. We included 1127 litters born between November of 2001 and December of 2020 of which 343 (30.4%) were born via c-section. Only 23 c-sections were declared as programmed. The data collected include, among others, information on date of birth, number of puppies born, number of puppies either stillborn, euthanatized or lost by spontaneous death within the first 8 weeks and the sex ratio of each category. A total of 8106 puppies were born throughout the studied period accounting for a mean litter size of 7.2 ± 3.1 puppies. Of these, 6786 puppies survived until the end of the first 8 weeks of life. The loss of 1320 puppies accounts for an overall mortality of 16.3%, of which 973 (73.7%) were stillborn, 42 (3.2%) euthanatized and 305 (23.1%) died within the first 8 weeks. Of all puppies born 50.7% were females and 49.3% were males. Evaluation of sex ratio in the groups of puppies either stillborn, euthanatized or lost without consideration of the birth has shown that the percentage of males was higher than the one of females therefore more males than females were recorded as dead at the end of the 8-week period (53.3% and 46.7% respectively). Evaluation of mortality based on type of parturition showed more males than females being stillborn or euthanatized in both eutocic parturitions and c-sections. Evaluation of spontaneous death instead shows a difference in sex ratio between c-sections (41.7% males and 58.3% females) and eutocic parturitions (54.9% males and 45.1% females). Furthermore, we can say that 13.7% of all puppies born by c-section were stillborn, compared to 11.5% of all puppies born by eutocic parturition. Litter size differed when comparing the presence or absence of stillborn puppies, with a litter size of 6.3 ± 3 puppies in litters without stillbirths and a size of 8.1 ± 2.9 puppies in litters with stillbirths. The overall mortality observed in our population is within the reported range. Although stillbirth has previously been named as the major cause for puppy loss, our results highly exceed what has been reported. Influence of c-section on mortality rate will be evaluated but may be difficult as stillborn puppies in dystocic parturitions may either be a result of dystocia as well as a possible cause for the development of dystocia. At the moment no conclusions on influencing factors may be drawn as results on statistical significance are not yet available, yet the great amount of information will permit us to investigate the mortality of puppies within this Swiss population in depth.

63 | Variations in fetal gastrointestinal motility in the last ten days of pregnancy

Giulia Siena; Chiara Milani; Stefano Romagnoli; Barbara Contiero; Francesca Di Nardo; Antonio Mollo
Department of Animal Medicine, Production and Health, University of Padova, Italy

Ultrasound (US) observation of fetal gastrointestinal motility (FGM) was suggested as a parameter to assess fetal maturity [1]. A previous study suggested a variation in the percentages of fetuses in which FGM was identified as parturition approaches [2]. The aim of the present study was to quantify the amount of FGM in relation to days before parturition (dbp), maternal size and sex ratio of pups.

Twenty-three clinically healthy pregnant bitches of 16 different breeds of 2–9-year-old and 3.5–56.8 kg bodyweight were monitored using an 8–5 MHz convex transducer connected to a US unit (Philips Affiniti 50G, Italy). US was performed in dorsal or lateral recumbency, after hair clipping and application of US gel on the abdominal region. Fetal intestine was observed for at least 30 s in a longitudinal and transversal scan on 3 of the most caudal fetuses in both uterine horns. The gestational age was counted backward from

the parturition day (day 0). The number of fetuses in which FGM was observed was recorded during two-time intervals: -11/-5 (time I) and -4/0 (time II) dbp. Mann-Whitney test was performed to analyse the variations of FGM% observed in relation to time intervals and parity of dams (primiparous vs pluriparous). Kruskal-Wallis test was performed to identify variations of FGM in relation to maternal size (small ≤10 kg, medium 11–25 kg and large ≥26 kg) and sex ratio of pups (percentage of females ≤40%, 41–60%, >60%). Significance was set as $p < 0.05$. Median and range were reported.

A total of 147 FGM observations on 50 US monitoring were performed. The FGM% was higher during time II compared to time I (33%, range 0–100% vs 100%, range 33%–100%; $p < 0.0001$). A higher FGM% was observed in small compared to large size bitches (100%, range 67%–100% vs 66%, range 0–100%; $p = 0.01$). FGM% was not affected by the parity of dams and sex ratio of pups.

The increase of FGM% in the last 5 days of pregnancy was in accordance with previous results reported in literature. In the author's opinion, maternal size may influence the easiness of FGM observation, as it could be easier to observe fetal intestinal peristalsis when the size of the bitch is small compared to large size bitches. The US equipment and positioning of the dam may influence the observations of the operator.

In conclusion, in the pregnant bitches of our study, a significant increase in the percentages of fetuses showing FGM was observed in the last 5 days before parturition. The detection of FGM may be influenced by maternal size. Further studies are needed in order to better quantify and correlate variations of this parameter with fetal maturity.

[1] E. M. U. Gil et al., "In utero development of the fetal intestine: Sonographic evaluation and correlation with gestational age and fetal maturity in dogs," Theriogenology, 84, 5, 681–686, 2015.

[2] C. Milani et al., "Ultrasonographic analysis of fetal gastrointestinal motility during the peripartum period in the dog," Anim. Reprod. Sci., 219, 2019, 106514, 2020.

64 | Preliminary studies on fetal biparietal diameter and femur development using ultrasound monitoring in British Shorthair cats

Anna Górka; Małgorzata Ochota; Wojciech Niżański
Katedra Rozrodu Z Kliniką Zwierząt Gospodarskich, Uniwersytet Przyrodniczy We Wrocławiu, Poland

The aim of this study was to investigate the development and growth rate of two fetal parameters in British Shorthair cats – biparietal diameter and femur using ultrasound imaging. In dogs there are separate formulas for estimating the date of parturition for bitches of different sizes. It was proven that using formulas that are dedicated to the animal's size influences precision of the calculations. In domestic cats' reproduction there are general formulas regardless the breed that are being used in daily practice. In early pregnancy inner chorionic cavity diameter (ICC) is being measured while during it's second half biparietal diameter (BPD) is more useful. Only for Maine Coon cats separate formulas were established [1]. In this research authors evaluated the growth rate of biparietal diameter and fetal femur length in British Shorthair breed. Due to the fact that determination of the precise time of parturition can still cause problems in cats' obstetrics, biometric measurements dedicated for separate breeds could become helpful when Cesarean sections are necessary as well as to provide medical assistance during natural delivery.

The study was performed on 21 healthy pure breed British Shorthair queens, age 1–5 years. Five queens were primiparous whereas other 16 were multiparous. Ultrasound examinations were performed weekly from the day of pregnancy confirmation (15 days after the first mating). Measurements were performed using Sonoscape s50 with a linear transducer. Biparietal diameter and femur length were measured. The images were performed on longitudinal plane. Biparietal diameter was measured when the parietal bones were parallel, in a place where the space between them is widest (at choroid plexus). Femur length was measured from it's proximal to the distal epiphysis.. Femur was first accessible 24 days before parturition, it's mean length was 5.64 mm ± 0.37. In week 7 of gestation the mean was 7.96 mm ± 1.69, in week 8: 11.44 ± 1.54 and in week 9: 15.7 mm ± 0.95. Biparietal diameter was first accessible 35 days before parturition. It's mean length in week 5 of gestation was 8.83 mm ± 0.84, in week 6: 11.29 mm ± 1.47, in week 7: 14.86 mm ± 1.18, in week 8: 17.35 mm ± 1.61, in week 9: 20.89 mm ± 1.13.

Based on the obtained measurements we can conclude that the femur growth was quicker towards the end of pregnancy (the mean 4.26 mm in week 9 comparing to mean 2.32 mm in week 6), whereas the mean skull development was noted at a higher rate in 7th and 9th week of pregnancy (respectively 3.57 and 3.54 mm) comparing to week 6th and 8th (respectively 2.46 and 2.49 mm).

It would be very useful to evaluate the pattern of fetal structures growth for different breeds of domestic cats. The accurate formulas for cats of different size and phenotype would be helpful when it comes to monitoring cat's pregnancy and estimating the time of parturition, resulting in decreased litter loss.

[1] Socha P. et al. Reprod Domest Anim 2019, 54, 622–626.

65 | Uterus masculinus: Clinical and histological findings in three dogs

Giulia Ballotta; Marco Cunto; Giuseppe Sarli; Giorgia Tura; Daniele Zambelli
University of Bologna, Italy

Uterus masculinus (UM) in dogs is rarely described in literature. Its seems to be a rare condition, often its presence is asymptomatic and occasionally detected as incidental finding during routine ultrasonography or surgery, rarely it could be identified consequently to related symptoms [1]. The aim of this case description is to describe clinical and histological findings in three dogs with diagnosis of UM.

A miniature Schnauzer (case 1), a mongrel (case 2) and an important sire of Basset hound (case 3) were referred to the University Veterinary Hospital "Giuseppe Gentile" respectively for abdominal pain, abdominal swelling and diagnosis of orchitis. All dogs underwent complete clinical visit and ultrasound examination. Neutrophilic leukocytosis was observed in case 2 and 3. All dogs presented a testicular neoplasia, Sertoli cell tumour was diagnosed in case 3, case 1 and 2 were referred after surgical removal of the testis without histopathological examination. Ultrasonographically a tubular fluid-filled structure with a thin irregular wall located cranially to the prostate was detected. It appeared to be in continuity with the cranial part of the gland. All dogs underwent an explorative laparotomy where a bi-horned structure connected by a pedicle to the dorso-cranial portion of the prostate was recognised, resected and subjected to histology. Orchiectomy was performed in case 3. Macroscopically, these structures resembled a fluid-filled female uterus. Histology revealed the presence of a uterine structure, morphologically similar to the female counterpart. Various types of epithelial cell lining were found, including simple columnar, simple stratified and squamous epithelium. On immunohistochemistry, anti-Müllerian hormone (MIS) labelled canine endometrial glands but only multifocal positivity of endometrium was detected.

Diagnosis of UM is challenging due to unspecific clinical sign, ultrasound description and lack of clear histological criteria. Ultrasonographically it is commonly misdiagnosed as paraprostatic cyst because of its anatomical position and cystic appearance [2]. But the anatomical origin of those two structures seems to be quite different, in fact despite Müllerian ducts in male dog degenerate during foetal life, a small portion remains as a vestigial UM and opens into the prostatic urethra at the seminal hillock and this site of attachment is undocumented for any paraprostatic cysts. Moreover its pathogenesis has still to be elucidated, different factors seems to be involved: is often reported in association with Sertoli cell tumour, the absence of MIS or not-expressed MIS receptors are thought to be the cause and in Miniature schnauzer a congenital anomaly (Persistent Müllerian Duct Syndrome) has been described. Authors suggest the importance of an accurate ultrasound evaluation of the male reproductive tract in order to better recognised and described those cases. In addition, histological examination could be a good tool in order to confirm the suspicion of UM or to understand the mechanisms underlying the disease.

References:

[1] Vegter, A.R., et al., Persistent Mullerian duct syndrome in a Miniature Schnauzer dog with signs of feminization and a Sertoli cell tumour. Reprod Domest Anim, 2010. 45(3): p. 447–52.

[2] Atilola, M.A.O. and P.W. Pennock, Cystic uterus masculinus in the dog. Veterinary Radiology & Ultrasound, 1986. 27(1): p. 8–14.

66 | Impact of intra-uterine position on placental and fetal development in the canine species

Sylvie Chastant[1]; Emilie Mallauran[1]; Estelle Picarougne[1]; Kurt De Cramer[2]; Amélie Mugnier[1]

[1]*Neocare, ENVT, France* [2]*Rant En Dal Animal Hospital, City of Johannesburg, Gauteng, South Africa*

Introduction and objectives: In the canine species, birth weight exhibits a large variability amongst individuals of the same litter [1]. Since arterial blood flow mainly enters the uterus through the ovarian side, nutrients might be provided through a decreasing gradient to fetoplacental units from the apex to the corpus uteri [2]. Conversely, foetuses positioned at the apex of the uterine horn might benefit from a limited space for their development due to limitations on the ovarian side. The objective of this study was to evaluate the impact of intra-uterine position on puppy birth weight and placental characteristics.

Materials and methods: Nonemergency cesarean sections were performed in Boerboel dams (n = 12 ; 5 below 2 years of age; 3 aged 3 or 4 years; 3 aged 5 years or more) at the time of parturition. At extraction, the position of each puppy was noted (horn side, position within the horn). Position was standardized (from 0 to 1) in order to neutralize the effect of the number of puppies within a horn as [1/(number of puppies within the horn − 1)] × (rank within the horn − 1), with the rank of the puppy at the apex numbered as 1. For each puppy, the placental attachment zone was weighted and its area calculated (length × width). Statistical analyses (bivariate analyses, linear-mixed effects model) were performed using R software (version 4.0.4).

Results: Data on 12 litters (86 puppies with the corresponding placenta; 1–14 puppies per litter with a median of 6) were analysed. Eleven puppies were positioned within the corpus uteri (12.8 %), 29 in the left uterine horn (33.7 %) and 46 in the right one (53.5 %), with a maximum of 8 puppies per horn and a minimum of zero (mean 4.5 fetoplacental units/horn). Mean difference between the number of puppies within right and left horn is 1.3 (95% CI: 0.02–2.6). The mean birth weight was 577 ± 138 g (range 200–938 g), with an intra-litter coefficient of variation of 16.1 ± 9.9%. No difference neither of birth weight nor of placental characteristics was evidenced according to the horn's side ($p < 0.05$). Intra-uterine position (standardized within the horn) had no impact on birth weight and placental characteristics ($p < 0.05$). Nevertheless, the puppy of maximal weight was found at the apex in 10/12 litters.

Conclusions: From these preliminary results, intra-uterine position does not seem to affect neither placental gross development, nor fetal growth whereas the opposite has been observed in rabbits and swine [3,4]. Observations have to be extended on a larger set of data, allowing to evaluate not only the impact of the anatomical position but also the influence of the sexes of the flanking fetuses [5].

References:

[1] Mugnier A et al 2019. Prev Vet Med 171:104746.

[2] Even MD et al 1994 J Reprod Fertil 102(1):245–52.

[3] Jang YD et al 2014 J Anim Sci ;92(10):4400–6.

[4] Bautista A et al 2015. Physiol Behav. 138:101–6.
[5] Lents CA, Freking BA. 2019. Anim Reprod Sci 209:106139.

67 | Reversibility of long-term use of deslorelin implants for fertility control in male dogs

Bruna Mendes[1]; Cristelle Navarro[1]; Cristelle Fontaine[1]; Tim E. Trigg[2]
[1]*GMBO, Virbac, Carros, France;* [2]*PT Consulting, Balmain, NSW, Australia*

Introduction: Few studies show the reversibility after one or two deslorelin implants, however little is known about reversibility after its repeated use over time. This study aimed to determine the return to semen production after repeated administrations of 4.7 mg deslorelin implants given to male dogs over periods greater than 700 days.
Materials and methods: Ten sexually mature, healthy male dogs, aged between 1.5 and 8 years, were given a single 4.7 mg implant (Suprelorin™, Virbac) subcutaneously four times at six-month intervals. Plasma testosterone (T) and testicular size measurements were made before the first implant, then on day (D) 2, D4, D7 and D14 after each implant, and then every 2 weeks until the next implant. T level was considered as negligible below 1 ng/ml. Semen collection was attempted before at the time of each implantation and after the last implantation. Adverse events were recorded.
Results: All dogs, but one, developed negligible T levels by D14 after the first implant [range: 7–28 days]. This suppression was maintained for a period over 720 days from the initial suppression, except in one dog that raised T levels during a temporary period [approximately 40 days] just before the second implant. Semen could not be collected from this dog during that period, suggesting the dog remained functionally infertile. Successful semen collections were made before D0 and then only 8 to 15 months after the last implant [D784 to D1009]. All dogs showed a trend of reduced testicular size during the study period. In one dog, at one examination during the study period, only one testicle was palpated. Two dogs died during the trial (one due to a snakebite and the other one was euthanized). No adverse events were recorded in the 8 dogs which completed the study.
Conclusions: This study supports the safe reversibility of semen production after the use of Suprelorin™ 4.7 mg every 6 months for a 2-year period for fertility suppression in male dogs. Additional data on semen quality are required to confirm the effective return to fertility.

68 | Efficacy of repeated administrations of deslorelin implants for long term suppression (3 years) of fertility in male dogs

B. Mendes[1]; C. Navarro[1]; C. Fontaine[1]; T. E. Trigg[2]
[1]*GMBO, Virbac, Carros, France;* [2]*PT Consulting, Balmain, NSW, Australia*

Introduction and objectives: For over 10 years, deslorelin implants have been used for fertility control in male dogs. However, little is known about repeated uses of these implants in a dog. The aim of this trial was to determine the efficacy and safety of repeated, single administrations of 4.7 mg deslorelin implants.
Materials and methods: Ten healthy mature male dogs (17.8 ± 2.2 kg bodyweight) were administered subcutaneously a total of six 4.7 mg deslorelin implants (Suprelorin™, Virbac) at six-month intervals. Plasma testosterone concentration ([T]) and testicular measurements were taken in day (D) 0, D2, D7, and D14 after each implantation, and then every 2 weeks until the next implant. [T] was considered as negligible with values <1 ng/ml. Any adverse reactions were recorded.
Results: Most dogs (90%) developed negligible [T] within 36 days after the first implant (range: 7–49 days), which was maintained following all subsequent implant administrations. Three dogs had [T] >1 ng/ml during a mean period of 33 days (range: 17–63 days) that reduced to negligible values following administration of the second implant. However, semen collection attempts on day 180 were unsuccessful in all dogs, indicating that they remained functionally infertile. The duration of testosterone suppression was at least 1151 days (up to >1325 days). Although more variable, mean testicle size at D0 (13.02 ± 0.96 cm) was reduced by D180 (10.09 ± 0.78 cm) ($p < 0.001$, Paired *t*-test). One dog developed considerably larger testicles for a period of approximately 80 days, which coincided with an increase in [T]. No other adverse reactions were observed.
Conclusions: This study showed that the use of Suprelorin™ 4.7 mg every 6 months resulted in safe, long-term reversible infertility for a period of 3 years.

69 | Evaluation of breed, age, parity and pre-treatment in bitches with dystocia – A retrospective analysis of 280 cases

Judith Krohn; V. Fux; Axel Wehrend
Klinik für Geburtshilfe, Gynäkologie und Andrologie der Justus-Liebig Universität Giessen, Germany

Dystocia is a very common problem in the bitch. Rapid and systematic examination and purposeful therapy are essential for the prognosis of bitch and puppies. Over the last decades predisposing factors for the incidence of dystocia have been investigated. For breeders and owners knowledge of these factors is valuable in terms of prevention and preparation. In this investigation data of bitches presented with a dystocia in the clinic for obstetrics, gynecology and andrology of the university of Giessen between 2000–2011 was included. The breed, age, number of parities and pre-treatment before presentation in the clinic were recorded to illustrate their influence on a prolonged or complicated progression of parturition. In contradiction to other retrospective evaluations a standardized examination protocol was applied throughout the whole period.
For statistical analysis the absolute and relative incidence of the investigated factors was calculated.
In total dogs of 82 breeds were presented for cesarean section. Breeds were subdivided into five groups: giant, large, medium, small

and toy-breeds. Bitches belonging to the large-breed group represented 42% of included patients, followed by toy-breeds (23%) and small breeds (12%). Most frequently German shepherd bitches were treated (9.4%), followed by dachshund (6.5%) and chihuahua (4.3%). The age of the bitches ranged from 1 to 16.5 years with the majority (90.3%) being 2–8 years old. The average age was 4.9 (±2.24) years. A cesarean section was more often performed in primiparous bitches (45.3%) than in pluriparous ones. A total number of 79 bitches (28.2%) received pre-treatment before surgery was performed. In 69.3% of investigated cases pre-treatment consisted of oxytocin-administration. Breed is meant to have an influence on the incidence of dystocia in the bitch. Large breeds, toy breeds and small breeds also were found to represent the majority of cases in other studies. In contradiction to our results several other authors found toy breeds to have the highest incidence of dystocia. A predisposition in large breeds is controversial in the existing literature. The German shepherd is not found to have specific risk factors for dystocia, the high number of patients treated in this investigation could be due to an outstanding popularity in Germany. Dachshund and chihuahua were also found to be predisposed for dystocia in other studies. The age range found in this study is identical with findings by other authors and reflects the typical age bitches are used for breeding. The fact that primiparous bitches are more often found to show signs of prolonged parturition was confirmed by the evaluated data.

Treatment with oxytocin is a very common procedure to support uterine contractions and thereby facilitate birth via the natural birth canal. A close monitoring of fetal stress and appropriate therapy are essential.

In conclusion most predisposing factors for a higher incidence of dystocia found in literature could be confirmed in this study.

70 | NET formation of polymorphonuclear neutrophils isolated from canine colostrum

Lukas Demattio[1]; D. Grob[2]; I. Conejeros[2]; A. Taubert[2]; C. Hermosilla[2]; Axel Wehrend[1]

[1]*Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals of the Justus Liebig University Giessen;* [2]*Institute of Veterinary Parasitology of the Justus Liebig University Giessen*

Introduction: Colostral leukocytes have a special importance for the transmission of maternal immunity to the neonate. Maternal leukocytes and particularly polymorphonuclear neutrophils (PMN) are known to migrate into the colostrum, where they are found in higher numbers than in mature milk (Gonzalez and Santos, 2017). Several studies have shown that it seems pivotal for immunological development of neonates that they ingest either cell-free or cell-containing colostrum (Reber et al., 2008). These vital immune cells, including PMN, are absorbed in small intestine of neonates and subsequently distributed throughout the organism with a predisposition to lymphoid tissue (Liebler-Tenorio et al., 2002). Our study focuses on a specific group of colostral leukocytes: polymorphonuclear neutrophils (PMN) and early innate effector mechanisms. PMN have been shown to be capable of forming so called neutrophil extracellular traps (NETs) in other localizations against invasive pathogens (Jeffery et al., 2015, Wei et al., 2016).

Materials and methods: In our study, PMN were isolated from canine colostrum by density gradient centrifugation and this was performed using Biocoll-Separation® solution. Afterwards canine PMN were incubated with Neospora caninum tachyzoites and the calcium ionophore A23187 (Merck, Munich) as positive control. The N. caninum tachyzoites were added in a concentration of 1: 2 compared to PMN. For negative controls, non-stimulated PMN were processed equally. NETs were detected by immunofluorescence microscopy analysis. Here, DAPI was used to stain DNA and specific antibodies were used to detect neutrophil elastase (NE) and histones to demonstrate the characteristic co-localization of these molecules in NETs structures.

Results: The results show that canine colostral PMN indeed respond to stimulation with A23187 or N. caninum tachyzoites by forming NETs.

Conclusion: This is the first evidence that canine colostral PMN are capable of casting NETs. Further knowledge on the immunological meaning of colostral leukocytes is an important component to improve the current understanding the neonatal immune system. These data might lead to the better understanding of newborn-related diseases.

Literature:

Gonzalez, D. D. and Santos, M. J. D. (2017): Bovine colostral cells-the often forgotten component of colostrum. JAVMA 250: 9.

Jeffery, U., Kimura K., Gray R., Lueth P., Bellaire B., LeVine D.. (2015): Dogs cast NETs too: canine neutrophil extracellular traps in health and immune-mediated hemolytic anemia. Vet Immunol Immunopathol 168(3–4): 262–268.

Liebler-Tenorio, E. M., Riedel-Caspari, G. and Pohlenz, J. F. (2002): Uptake of colostral leukocytes in the intestinal tract of newborn calves. Vet Immunol Immunopathol 85(1–2): 33–40.

Reber, A. J., Donovan, D. C., Gabbard, J., Galland, K., Aceves-Avila, M., Holbert, K. A. (2008): Transfer of maternal colostral leukocytes promotes development of the neonatal immune system I. Effects on monocyte lineage cells. Vet Immunol Immunopathol 123(3–4): 305–313.

Wei Z., Hermosilla C., Taubert A., He X., Wang X., Gong P., Li J., Yang Z. and Zhang X. (2016). Canine Neutrophil Extracellular Traps Release Induced by the Apicomplexan Parasite Neospora caninum In Vitro. Front. Immunol. 7:436.

71 | Influence on fertility of the motile sperm subpopulations present in cryopreserved and chilled dog semen samples

Ana I. Peña Martínez; U. Ramil; J. J. Becerra; P. G. Herradón; L. A. Quintela

Unit of Reproduction and Obstetrics, Department of Animal Pathology, Faculty of Veterinary Medicine, University of Santiago de Compostela, Lugo, Spain

The aim of this study was to investigate the relationship between the relative proportion of motile sperm subpopulations present

in chilled and cryopreserved dog semen samples and field fertility. Motile sperm subpopulations were analysed in 55 frozen-thawed and 27 chilled semen samples that were to be immediately used for AI.

Just after thawing if frozen, or after warming if chilled, a 5 µl-aliquot of each sample was used to evaluate sperm motility and concentration by means of a CASA system (SCA 6.0, Microptic, Barcelona, Spain). Depending on the sperm concentration of each sample, another 5 µl-aliquot was diluted at ratio 1:2 to 1:8 with the same thaw medium used for AI or with Tris buffer, so that sperm concentration were adjusted to 30–50 × 10^6 sperm/ml. Five microscopic fields were recorded from each sample and saved for future analysis. Only samples with clean extracellular medium and clearly drawn sperm trajectories were included in the study.

Artificial insemination of bitches was always performed by endoscopic TCI; 63 bitches received 1 AI and 19 two. The ovulation day was considered when serum progesterone concentration increased twice or more from an initial rise of 1.5–3 ng/ml, and AI was scheduled for 2–4 days post-ovulation. Bitches older than 5 years, or younger with history of infertility, were excluded from the study.

CASA-derived kinematic parameters from all the motile spermatozoa captured from the 82 samples were imported into a single data set and submitted to multivariate K-means cluster analysis; all the motile sperm were classified into 4 subpopulations with different motility patterns: 1) a sperm subpopulation with hyperactivated-like movement, showing high velocity and low progressiveness (sp1); 2) one with low velocity but high progressiveness (sp2); 3) one with poorly motile sperm, showing the lowest velocity and linearity (sp3); and 4) a subpopulation with the highest velocity and progressiveness (sp4).

Pregnancy rates and litter sizes were, respectively, 76.4% (42/55) and 6.29 ± 2.76 for AIs with frozen semen and 59.3% (16/27) and 5.25 ± 3.07 for AIs with chilled semen.

Chi-square analysis indicated that fertility was significantly influenced by the following variables: total number of motile sperm ($p = 0.028$), percentage of spermatozoa in sp4 ($p < 0.000$) and number of sperm in sp4 ($p < 0.000$). Total motility % or sp1, sp2 and sp3 percentages had no effects on fertility. The influencing variables were included as categorical covariables in a regression analysis model to evaluate the probability of pregnancy depending on category. Binary logistic regression by the backsteps (conditional) method showed that only the number of sperm included in sp4 had significant effect on fertility: frozen-thawed semen samples with a sp4 containing 35–60 × 10^6 and >60 × 10^6 sperm, or chilled semen samples with a sp4 containing 50–150 × 10^6 and >150 × 10^6 sperm were, respectively, 18 and 200 times more likely of producing pregnancy than frozen-thawed samples with sp4 <35 × 10^6 sperm or chilled samples with sp4 <50 × 10^6. In conclusion, the number of sperm assigned to sp4 seemed to be the most relevant motility parameter to predict semen fertility.

72 | Cystic endometrial hyperplasia in adult minipigs – Report of 19 cases in a Kune Kune herd

Johannes Herbel[1]; Sophie Dürlinger[2]; Vera Martin[2]; Rene Brunthaler[3]; Christian Knecht[2]; Sabine Schäfer-Somi[4]

[1]*Obstetrics And Reproduction, Department For Small Animals And Horses, Vetmeduni Vienna;* [2]*University Clinic for Swine, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna;* [3]*Institute for Pathology, Department for Pathobiology, Vetmeduni Vienna;* [4]*Artificial Insemination and Embryo Transfer, Department for Small Animals and Horses, Vetmeduni Vienna*

The interest in keeping minipigs as pets is increasing, however, the knowledge about their physiology and requirements is not. The occurrence of cystic endometrial hyperplasia (CEH) in aging minipigs has been described repeatedly (1,2); this degenerative disease is the consequence of reproductive rest, since the mucus membranes are not renewed. In the current case series, we describe the occurrence and treatment of CEH in 19 Kune Kunes from one herd of experimental animals in Austria. The age at presentation averaged 5.5 years ± 9.2 months and they were kept under the same conditions. One sow had two litters before, all others never had litters. First signs of disease were mucoid vaginal discharge and/or decreased appetite. After detection of the first cases, the whole herd was clinically and sonographically examined and a complete blood count was taken.

Only one sow had fever and clinical signs of intoxication/sepsis. She was euthanized after ovariohysterectomy during the recovery phase due to hypoxemia. Pathohistology revealed severe CEH/pyometra and high grade *E. coli* was detected bacteriologically.

All other animals were clinically healthy, however, variable grades of CEH and amounts of intrauterine fluids were found during sonographical examination. The blood pictures mostly revealed leucocytosis with left shift and a variable increase in liver enzymes and c-reactive protein (CRP).

All sows underwent ovariohysterectomy in general anesthesia. The operation was performed by using a sealing device (LigaSure®). The special anatomy/operation situ, the big vessels and fatty tissues required careful and thorough handling of the sealing device. All sows except one survived and recovered well; one sow died because of severe stump bleeding after surgery.

Pathohistology revealed the characteristic image of a CEH in all cases (high grade $n = 12$, medium grade $n = 4$, low grade $n = 3$), with variable uterine diameters (up to 15 cm) and multiple endometrial cysts (diameter 0.5–9 cm). In some cases, the uterine cavity was filled with cysts, the thickness and tension of the uterine wall varied. In four sows, endometritis was detected. All ovaries had corpora lutea and follicles, only in one sow ovarian cysts were detected.

We conclude that adult minipigs show first signs of CEH at early ages, however, in most cases without clinical signs. The prognosis is good when ovariohysterectomy is performed in time, before infection causes endometritis/pyometra. Minipigs, which are not used for breeding, should be surgically or chemically castrated at the age of

6 months, allowing an uncomplicated operation (ovariectomy) and quick recovery.

References:

[1] Ilha MR, Newman SJ, van Amstel S, Fecteau KA, Rohrbach BW. Uterine lesions in 32 female miniature pet pigs. Vet Pathol. 2010;47(6):1071–5.

[2] Harmon BG, Munday JS, Crane MM. Diffuse cystic endometrial hyperplasia and metastatic endometrial adenocarcinoma in a Vietnamese pot-bellied pig (Sus scrofa). J Vet Diagn Invest. 2004;16(6):587–9.

73 | Placental ingestion is associated with postparturient diarrhea

Karolina Brunius Enlund[1]; Elin Lundgren[1]; Anna Edner[2]; Ann Pettersson[1]; Carl Brunius[3]; Ragnvi Hagman[1]

[1]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Anicura Animal Hospital, Falun, Sweden;* [3]*Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, Gothenburg, Sweden*

Introduction and objectives: Dystocia is a common problem in bitches, and the majority is surgically treated [1]. It is not known how frequently gastrointestinal disturbances occur after normal whelping, and they may be a side-effect of some drugs used postoperatively for pain-relief. The aim of the study was to investigate the occurrence of gastrointestinal disturbances in bitches during the periparturient period, and to identify possible associations with Caesarean section and placental intake.

Materials and methods: A survey was constructed [2] and sent by email to breeders in Sweden (Swedish Kennel Club owner registry). The survey included 36 questions related to the bitch, her general gastrointestinal health, the parturition, and presence of possible gastrointestinal disturbances before, during or after the parturition. Bitches with reported sensitive stomach were excluded from all analyses. Frequency of feces with high water content was compared between bitches that were surgically treated and bitches that had a normal whelping, and between bitches with or without placental intake. The results were analyzed using Chi-squared test, with a level of significance $p < 0.05$.

Results: The survey was sent to 14,831 breeders, with 1,584 surveys completed (11% response rate). Of the respondents, 17% noted that veterinary care was needed during the whelping and about 9% noted that the bitch received a Caesarean section. Of 253 bitches with dystocia that needed veterinary care, 142 (56%) were treated by Caesarean section, which is comparable to earlier reports [1].

Conclusions: During the first three days following parturition, diarrhea or feces with high water content was present in one third of the bitches. A significant association was detected between intake of placenta and diarrhea or feces with high water content the first day after parturition, but there was no association with Caesarean section. The occurrence of vomiting before and after whelping did not differ significantly. Vomiting was only reported in a few bitches.

References:

[1] Bergström A, Nödtvedt A, Lagerstedt A-S, et al. Incidence and breed predilection for dystocia and risk factors for caesarean section in a Swedish population of insured dogs. Vet Surg 2006;35:786–791.

[2] Ejlertsson G, 2014. Survey studies in practice. Student literature, Lund, Sweden.

74 | A retrospective study of 92 female cats with pyometra

Emelie Thorén, Bodil Ström Holst, Ragnvi Hagman

Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Introduction and objectives: Uterine diseases such as pyometra are common in unspayed queens1,2. The safest and most effective treatment is ovariohysterectomy1,2.

Materials and methods: A retrospective study was performed. No ethical permission was needed according to Swedish regulations. All queens (103) diagnosed with pyometra at the University Animal Hospital (UDS), Swedish University of Agricultural Sciences, Uppsala, Sweden during the time period January 1st 2008 to June 1st 2018 were included. Eleven cats were excluded because of other diagnosis (cystic endometrial hyperplasia ($n = 1$), mucometra ($n = 1$), metritis postpartum ($n = 2$)), or antimicrobial or ciclosporin/cortisone therapy several months prior to admittance ($n = 2$). Data from case history, results of clinical and diagnostic imaging examinations and laboratory analyses, postoperative hospitalization, presence of peritonitis and mortality were included. Prolonged postoperative hospitalization was determined as ≥2 days.

Results: Ninety-two queens were included where of 67 were surgically treated, 14 purely medically treated, 4 euthanized instead of treated and 7 were dismissed without treatment. The age range was 6 months to 16 years (mean ±SD age 4.9 ± 3.8 years). The mean weight ±SD was 3.5 ± 0.7 kg, range 2.1–5.5 kg. The mean ±SD diameter of the uterus was 1.7 ± 1.1 cm, range 0.5–7.0 cm. The most common clinical sign was vaginal discharge, as noted in 88% of the 92 queens. Other frequently reported signs of illness, present in over 20%, were lethargy, depressed general condition, dehydration and licking of the vulva, gastrointestinal disturbances, fever and distended abdomen. In approximately 51% of the cats, abdominal pain was noted on palpation. Of the hematological abnormalities, leukocytosis, monocytosis and neutrophilia and presence of band neutrophils were common. In the surgically treated queens (67), peritonitis was the most common complication. Moreover, in 27% the postoperative hospitalization was increased.

Conclusions: In this study, case history, clinical- and laboratory findings in queens with pyometra are reported, which may facilitate early recognition and diagnosis of the disease.

[1] Hagman R. Pyometra in Small Animals. Vet Clin North Am Small Anim Pract. 2018;48:639–661.
[2] Hollinshead F, Krekeler N. Pyometra in the queen: To spay or not to spay? J Feline Med Surg 2016;18:21–33.

75 | Influence of days before parturition on fetal kidney measurements in the canine pregnancy

Giulia Siena; Francesca Di Nardo; Stefano Romagnoli; Antonio Mollo; Barbara Contiero; Chiara Milani
Department of Animal Medicine, Production and Health, University of Padova, Italy

Ultrasound (US) monitoring of canine fetal kidney from its first appearance to parturition is easy to perform and could be useful to assess fetal development [1]. The aim of this study was to evaluate the measurements of different canine fetal kidney structures as tools for determining their variations in the last ten days of pregnancy.
Ten clinically healthy pregnant bitches of 10 different breeds, of 2–8-year-old and 8.8–40.3 kg bodyweight were monitored at least twice during time intervals from −10 to 0 days before parturition (dbp) using an 8–5 MHz convex transducer connected to a US unit (Philips Affiniti 50G, Italy). US was performed in dorsal or lateral recumbency. Two renal sonograms were collected in a longitudinal scan on three most caudal fetuses in both uterine horns. Fetal kidney length (L), cortex (CT) and medullary thickness (MT), and their ratio (CT/MT) were measured. Gestational age was counted backward from the parturition day (day 0). A statistical analysis was performed using a linear mixed model considering maternal size (small ≤10 kg, medium 11–25 kg and large 26–40 kg) as fixed effect, dbp (−10 to 0) and litter size as covariate, and the bitch as random and repeated effect. Least-square means and standard errors were calculated. Post-hoc pairwise comparisons among levels were performed with Bonferroni correction. Significance was set as $p < 0.05$.
A total of 123 measurements of L and 119 of CT and MT were obtained from 29 examined fetuses. In one bitch, only two fetuses were examined. Dbp significantly influenced L, CT, MT and CT/MT ($p < 0.01$). Estimated regression coefficients were directly correlated with dbp for L, CT and MT (−0.68 ± 0.14, −0.04 ± 0.01 and −0.12 ± 0.02 mm, respectively). Instead, CT/MT decreased as parturition approached (0.01 ± 0.004 mm). MT has a higher increase compared to CT, therefore CT/MT decreased as parturition approached, whereas MT and CT increased. A statistically significant difference in L between small and large size bitches was evident (17 ± 1 vs 24 ± 2 mm, $p = 0.02$). In CT, a significant difference was evident between maternal sizes, with the lowest values in small and higher values in large size bitches (1.57 ± 0.04 vs 1.77 ± 0.04 vs 1.99 ± 0.05 mm, $p < 0.001$). Litter size did not affect any of the parameters considered.
In dogs, the ultrasonographic measurement of fetal kidney length resulted to be related to the period of pregnancy [1]. Renal cortex and medullary thickness and their ratio could be used to monitor the development of human fetal kidneys [2]. Our results are in accordance with what is reported in human medicine.
Canine kidney L, CT, MT and CT/MT are related to dbp and they could be used to monitor kidney structures development. Further studies are needed to use these parameters for the prediction of parturition day.

[1] E. M. U. Gil, et al., "Early results on canine fetal kidney development: Ultrasonographic evaluation and value in prediction of delivery time," Theriogenology, 107, 180–187, 2018.
[2] A. Devriendt, et al., "Fetal kidneys: Additional sonographic criteria of normal development," Prenat. Diagn., 33, 13, 1248–1252, 2013.

76 | Intact versus neutered male cats: Preliminary results on the usefulness of a single coat testosterone concentrations measurement

Jasmine Fusi[1]; Tanja Peric[2]; Massimo Faustini[1]; Francesco Castelli[3]; Maria Cristina Veronesi[1]
[1]*Università Degli Studi Di Milano – Department of Veterinary Medicine, Italy* [2]*Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Italy* [3]*Faculty of Veterinary Medicine, Veterinary Teaching Hospital, University of Teramo, Italy*

To recognize if a male cat is neutered or cryptorchid, the gold standard method is the exploratory laparotomy: invasive, costly and potentially risky (Morrow et al., 2019). Alternatively, the neuter status of the cat can be assessed by blood testosterone (T) or LH analysis (Morrow et al., 2019), but also this method can be stressful for the cats, so that alternatives for hormone investigations are desirable. In males, search for T-dependent penile spines to discern between intact and neutered cats is useful but sometimes misleading. Therefore, the availability of a simple, non-invasive tool to distinguish between intact and neutered male cats could be useful. Many studies proved the reliability of coat for the retrospective evaluation of long-term hormonal accumulation, thanks to the ability to incorporate hormones that remain stable until analysis. Beside the advantage of retrospective accumulation, that limits the frequency of samplings, the coat is collectable without invasiveness, respecting the animal welfare. The aims of the present study were to: 1) assess usefulness of coat for the measurement of long-term accumulation of T in domestic male cats; 2) assess the usefulness of a single coat T measurement to discern between intact and neutered male cats. This preliminary study was performed on 25 male cats, belonging to private owners, of different breeds, healthy, in good general conditions and classified as follows: intact (aged >6 months, with evidence of puberty such as sexual behaviour observed at least two months before entering the study and penile spines presence at the time of entering the study) ($N = 19$); neutered (record of bilateral orchidectomy by at least 6 months, with no further signs of sexual behaviour) ($N = 6$). To collect the coat, in all cases an area of 5 cm^2 was shaved from the dorsal surface of the forearm; the mean ± SD amount of

coat collected for each sample was 90.6 ± 24.4 mg (range: 17.3–126.8 mg). The coat was stored in paper envelopes at room temperature until the analysis of T concentrations by RIA. Although the results showed higher T concentrations in intact than neutered male cats (median, min-max: 1.70, 1–3.01 pg/mg vs 0.99, 0.72–1.45 pg/mg, respectively), the statistical analysis, performed with a Robust ANOVA followed by post Hoc test, failed to detect significant differences. The results agree with a previous study (Terwissen et al., 2014), in which no significant differences were found for T concentrations in coat between intact and castrated males. In conclusion, although T is quantifiable in coat of male cats, the utility of a single measurement of coat T to discern between intact and neutered male cats deserves further investigations.

Terwissen CV, Mastromonaco GF, Murray DL. Influence of adrenocorticotrophin hormone challenge and external factors (age, sex, and body region) on hair cortisol concentration in Canada lynx (Lynx canadensis). Gen Comp Endocrinol. 2014;194:162–7.

Morrow LD, Gruffydd-Jones TJ, Skillings E, et al. Field study assessing the performance of a patient-side blood test to determine neuter status in female cats based on detection of luteinising hormone. J Feline Med Surg. 2019;21:553–8.

77 | Follicular cysts in a female dog after administration of a deslorelin implant

I. Alves[1]; L. Sales[2]; A. T. Reisinho[2]; Luisa Mateus[3]

[1]*Instituto Superior de Agronomia, Universidade de Lisboa, Portugal;* [2]*Hospital Escolar, Faculdade de Medicina Veterinária, Universidade de Lisboa, Portugal;* [3]*CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal*

Clinical case: A 10-year-old female Old English Sheepdog was presented for treatment to prevent estrus. The dog weighed 30 kg, and had no history of abnormalities, having whelped twice (last litter in 2016) and with an average of 180 ± 20 days between cycles. The bitch had a normal proestrus and estrus and was on day 40 of diestrus. Concentrations of progesterone were not determined. No ultrasonographic exam was done previously to the treatment. One 4.7-mg deslorelin implant was injected S/C between the shoulder blades. Ten days later, vulvar sanguinolent discharge started, together with edema of the vulva. Five weeks later (Day 51 after implantation), the dog was presented because of prolonged signs of proestrus. Cytological examination of stained vaginal smears indicated the presence of intermediate cells (80%) and superficial cells (20%). Ultrasonographic findings indicated cystic endometrial hyperplasia (CEH) and multiple thin-walled cystic structures on both ovaries, with a diameter ranging from 5 to 8 mm. Haematological and biochemistry blood analysis were performed and all results were within normal range. No hormonal measurements were done but the bitch presented thinning of the coat, most probably due to prolonged elevated estrogen levels. Due to the age of the dog and the presence of CEH, no medical treatment was tried and ovariohysterectomy was performed. Samples of the uterus and ovaries were fixed in 4% formalin and prepared for histopathological examination. That revealed moderate multifocal, cystic endometrial hyperplasia. On both ovaries, multiple anovulatory cysts and 5 small corpora lutea were found. Following the ovariohysterectomy, the dog recovered quickly, including hair regrowth.

Discussion: The use of deslorelin implants in bitches is not completely free of negative side effects, with the risk of side reactions being higher in older female dogs and females with ovarian or uterine abnormalities (1). In this case the preexistence of ovarian and uterine alterations cannot be excluded due to the age of the dog. Induction of estrus is expected within 4–8 days after implant insertion. To avoid this effect, implant insertion should be done during diestrus, when progesterone (P4) concentration is ≥5 ng/ml (2). However, this strategy is not always successful and estrus induction has been observed even with P4 concentrations as high as 60 ng/ml (3).

It is suggested that a complete gynecological examination is to be performed before implanting deslorelin, together with a risk/benefit assessment. The possibility of estrus induction within two weeks after implantation is always to be taken into account. Also, these induced heat cycles may not progress normally, with prolonged estrus/development of ovarian cysts being one of the possible side effects in implanted bitches. The implant should therefore be administered SC cranially and close to the umbilicus for easy excision if deemed necessary.

Funding: UIDB/00276/2020.

References:

[1] Arlt SP et al., N Z Vet J 2011 59; 87–91.

[2] Trigg T et al., J Reprod Fertil 2001. Suppl 57, 255–261.

[3] Fontaine E & Fontbonne A. Evssar Congress, Louvain-la-neuve, Belgium, 14th–15th May 2020, pp 15–17.

78 | Successful ICSI with vitrified epididymal cat spermatozoa

Martina Colombo, Maria Giorgia Morselli, Gaia Cecilia Luvoni

Dip. Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare "Carlo Cantoni", Università degli Studi di Milano, Italy

Introduction and objectives: Albeit more common for oocytes and embryos, vitrification has more recently been applied to mammalian spermatozoa, but it is far from standardization. In the domestic cat, model of choice for the development of gamete preservation protocols for endangered felids, few conflicting reports were published. The aims of this study were to evaluate the morphofunctional integrity of cat epididymal spermatozoa vitrified in pellets or straws with two different extenders and to assess their fertilizing ability by intracytoplasmic sperm injection (ICSI).

Material and methods: Epididymal spermatozoa (10 cats) were analyzed as fresh (FS) or vitrified (VS). For vitrification they were diluted 1:1 with Extender 1 (E1: TRIS + 20% egg yolk + 0.25 M sucrose) or Extender 2 (E2: Ham's F-10 + 1% BSA + 0.4 M sucrose) and,

after 5 min equilibration, 10 µl were dropped into liquid nitrogen or loaded in straws and then immersed into liquid nitrogen. Warming was performed at 37°C in TRIS (E1) or HF-10 (E2). In all groups [FS; VS at warming (T0) and after 6 h (T6)], motility, morphology (Bengal Rose/Victoria Blue B), membrane (hypo-osmotic swelling test) and acrosome integrity (fluorescein isothiocyanate/propidium iodide staining) were assessed. The best extender (E1/E2) and packaging (pellet/straw) were chosen for ICSI. In vitro matured oocytes were microinjected with VS (n = 20), or frozen spermatozoa (positive control, n = 26), or just pierced without sperm injection (negative control, SHAM-ICSI, n = 21). Presumptive embryos were cultured for up to 7 days and embryo development was recorded. The number of nuclei was confirmed by Hoechst staining. Data were analyzed by Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner test (morpho-functional parameters) or Fisher's exact test (ICSI) with significance set at $p \leq 0.05$.

Results: FS had better motility (mean % ± SD: 61 ± 15.2), normal morphology (41.4 ± 24.7), membrane (75.3 ± 13.9) and acrosome (78.2 ± 9.66) integrity than VS at warming (T0), irrespective of the extender used (E1/E2). In VS no differences were found for morphology, membrane and acrosome integrity, regardless of extender (E1/E2), packaging (pellet/straw) or time (T0/T6), although these parameters tended to worsen along time. Motility was similar between extenders and packagings at T0 (E1 pellet 21.7 ± 7.4; E2 pellet 12.7 ± 8; E1 straw 17.7 ± 10.2; E2 straw 11.4 ± 8.3) and decreased at T6 (p = 0.05; E1 pellet 3.6 ± 2.9; E2 pellet 0 ± 0; E1 straw 2.7 ± 3; E2 straw 0.1 ± 0.3). E1 pellet was chosen for ICSI since its motility remained higher over time (p = 0.03 vs E2 at T6). These VS were able to support embryo formation, with 25% cleavage and 5% morulae rate, which were similar to those obtained with frozen spermatozoa (46.2% cleavage, 11.5% morulae, p = 0.21). No cleavage was observed with SHAM-ICSI (p = 0.02 vs sperm-injected groups).

Conclusions: Feline epididymal spermatozoa maintained better morphofunctional parameters after vitrification with E1. Such VS were able to successfully fertilize mature cat oocytes by ICSI and produce embryos until advanced stages of in vitro development. This study demonstrates that the ultimate goal of having a fast and easy technique for cryopreservation of male germplasm under field conditions is becoming more than achievable.

This study was supported by the Polish National Agency for Academic Exchange, Grant PPI/APM/2019/1/00044/U/00001.

79 | Use of a bipolar vessel sealing device in canine orchiectomy

Halit Kanca; Ceren Yaman
Faculty of Veterinary Medicine, Ankara University, Turkey

Introduction and objectives: The efficacy and safety of bipolar vessel sealing (BVS) devices have been described in several surgical procedures in dogs, especially during laparoscopic procedures. The objective was to compare the BVS method with routine ligation in prescrotal open orchiectomy of dogs in terms of surgical time, surgical site complications and postoperative pain scores.

Materials and methods: Fifty healthy adult dogs admitted for elective castration were randomly assigned to either ligation or bipolar BVS groups. The dogs received 0.2 mg/kg meloxicam sc as preemptive analgesic and a single injection of 15 mg/kg amoxicillin trihydrate im was used for prophylaxis. Dogs were premedicated with 0.01 mg/kg medetomidine im before induction of anaesthesia with propofol 6 mg/kg iv. Anaesthesia was maintained with isoflurane. Orchiectomy was performed by a single veterinary surgeon using the standard open technique. In the ligation group, the vaginal tunic and the spermatic cord were separately ligated by using 0, 2-0 or 3-0 Polyglactin 910. Bipolar vessel sealing instrument (LigaSure™ 5 mm blunt tip 37 cm sealer; Medtronic) was used both for sealing and dissection of the vaginal tunic and the spermatic cord in the BVS group. Valleylab™ LS10 Generator energy platform (Medtronic) was used as the energy source. The skin incision was closed with a 2-0 or 3-0 Poly lactic-co-glycolic acid. Postoperative pain was evaluated and scored at 15 min, 1, 2 and 24 h. Swelling and bruising of the surgical sites were scored at 24 h. Student t-test and Mann-Whitney U-test were used in the comparison of continuous variables between the study groups. The Friedman test was used to analyse the difference between times for pain scores and a Wilcoxon matched-pairs test with Bonferroni correction was used for pairwise comparisons. Correlations between surgery duration, age, body weight, the severity of surgical site swelling and severity of surgical site bruising were assessed using Spearman's correlation coefficient.

Results: There were no significant differences between the age (p = 0.168) and the bodyweight (p = 0.678) of dogs. The median duration of surgery was 10.18 (range 7.47–12.33) and 8.30 (range 7.03–10.17) minutes in the ligation group and BVS group, respectively (P<0.001). There was a significant effect of age (r = 0.458, p = 0.021) and body weight (r = 0.432, p = 0.031) of the animal on the duration of surgery in the BVS group. Pain scores were significantly reduced by time in both groups (p = 0.001). Lower pain scores were observed in the BVS group at postoperative 15 min (p = 0.001) and 1 h (p = 0.045). The mean swelling score was lower in the BVS group compared to the ligation group (p = 0.034). The surgical site bruising score at 24 h was significantly lower (p = 0.015) in the BVS group. Surgical site bruising and swelling were not associated with the duration of surgery, age or bodyweight of the animal in either group (P>0.05).

Conclusions: The use of an electrothermal BVS device was associated with significantly shorter surgery times and lower postoperative pain and surgical site complications compared with ligation in canine open orchiectomy, possibly because of better haemostasis and less tissue handling and damage.

80 | Cytotoxicity assay for the assessment of anaesthetic toxicity in puppies born by C-section: Preliminary results

Renatha Almeida De Araújo[1]; D. Armani[1]; Samara Beretta[1]; Samara Almeida-Junior[2]; J. Rocha-Junior[1]; M. Bianchini[1]; J. Bonavina[1]; A. Milare[1]; R. Furtado[2]; Maricy Apparicio[1]
[1]*São Paulo State University- Unesp- FCAV, Jaboticabal São Paulo, Brasil;* [2]*University of Franca, Franca São Paulo, Brasil*

Despite of the well-established protocols for veterinary obstetric care, little is known about the pharmacological safety of these drugs, specifically in pregnant bitches and their litters, since most data available in veterinary medicine are extrapolated from studies in rodents and humans.

In vivo mitotic indices assay in rodent, recommended by regulatory agencies, are capable to assess the safety of pharmaceutical products 24–48 h after the administration of the substance to be tested, by the collection of bone marrow or peripheral blood samples, in order to prepare hematological slides. Cytotoxicity is assessed from the ratio of immature erythrocytes (E-IM) to total erythrocytes. Depression of the E-IM ratio highlights the cytotoxic potential of the substance tested. In this preliminary study, we aimed to adapt the methodology for application in canine species.

Four litters were obtained from healthy English bulldog bitches, with 2–3 years old (all ASA1) submitted to elective c-sections; a total of 21 puppies were evaluated (ethical approval 011302/19). The anesthetic protocols consisted of induction with propofol 3 mg/kg, maintenance with isoflurane vaporization and epidural anesthesia with 2 mg/kg 2% lidocaine. C-sections were performed and APGAR score was obtained; all puppies scored ≥8. Forty-eight hours after parturition blood samples were collected from puppies' hind paws and blood smear slides were prepared. Staining and slide evaluation were performed following the Peripheral Blood Micronucleus Assay Protocol adopted by MacGregor [1]. Data were analyzed by ANOVA followed by Tuckey Test.

The means and SD of the relationship between E-IM and the total of erythrocytes obtained for each litter were 0.0296 ± 0.02; 0.0260 ± 0.008; 0.028 ± 0.0101; 0.0336 ± 0.0143. No significant difference was observed between puppies born from different litters nor within the same litter, indicating that different puppies responded similarly to anesthetic exposure. The technique proved to be suitable for canine species; compared to rodents (0.09 ± 0.03) [2], these results might indicate some cytotoxicity, however additional studies with a wide range of animals are necessary to drawn a reliable conclusion.

[1] MacGregor JT, Wehr CM, Gould DH. Clastogen-induced micronuclei in peripheral blood erythrocytes: the basis of an improved micronucleus test. Environ Mutag 1980; 2: 509–514.
[2] Furtado MA, Almeida LCF, Furtado RA, et al. Antimutagenicity of rosmarinic acid in Swiss mice evaluated by the micronucleus assay. Mutation Res 2008; 657: 150–154.

81 | Cytotoxicity assessment and comparison of Lidocaine and Ropivacaine in puppies born by C-section: Preliminary results

Renatha Almeida De Araújo[1]; D. Armani[1]; Samara Beretta[1]; Samara Almeida-Junior[2]; J. Rocha-Junior[1]; M. Bianchini; J. Bonavina[1]; R. A. Furtado[2]; Samara Milaré[1]; Maricy Apparicio[1,2]
[1]*São Paulo State University (UNESP), FCAV, Jaboticabal-SP, Brasil;* [2]*University of Franca, Franca, SP, Brasil*

No substantial data are available concerning veterinary perinatal pharmacology and therapeutics. Even human medical professionals suffer from the lack of information in this field, and nearly 90% of the drugs used in neonatal and pediatric patients are off-label.

Clinical trials are an important step to establish safe drug protocols that may attend to these vulnerable populations. In vivo, mitotic indices assay in rodents enables the assessment of the safety of pharmaceutical products by analyzing hematological slides prepared using samples of peripheral blood or bone marrow collected 24–48 h after the drug administration. Cytotoxicity is determinate by the ratio of immature erythrocytes (E-IM) to total erythrocytes. In this preliminary study, we aimed to evaluate how two different anesthetics protocols affect newborn puppies delivered by elective C-section by assessing the cytotoxic potential.

Eight litters born from bitches of different breeds, between 1.5 and 3 years old (all ASA1) submitted to elective c-sections were evaluated (ethical approval 011302/19). Bitches were randomly located in two groups (L = lidocaine and R = ropivacaine). In both groups, parturients received anesthetic induction with propofol 3 mg/kg, maintenance with isoflurane vaporization. In group L (n = 4) bitches received 2 mg/kg 2% lidocaine by epidural anesthesia, while in group R (n = 4) epidural anesthesia was performed with 1.5 mg/kg 1% ropivacaine. Immediately and 48 h after parturition blood samples were collected from the puppies' hind paws and blood smear slides were prepared. Staining and slide evaluation were performed following the Peripheral Blood Micronucleus Assay Protocol adopted by MacGregor [1]. Cytotoxic effect of lidocaine and ropivacaine in puppies was evaluated 48 h after treatment, and analyzed by ANOVA test of variance followed by Tukey post-test with significance at 0.05 (95% confidence).

The means and SD of the relationship between E-IM and the total of erythrocytes immediately after birth were 0.01760 ± 0.005832 (n = 42). After 48 h of the birth were 0.02288 ± 0.009156 (n = 12) for lidocaine (p = 0.0619), and 0.02240 ± 0.008540 (n = 10) for ropivacaine (p = 0.1320). No significant difference was observed between puppies born from bitches submitted to different anesthetic protocols. The technique proved to be suitable for canine species compared to rodents (0.09 ± 0.03) [2]. These results suggest some cytotoxicity, however additional studies using a larger group of animals are necessary to assess a more solid conclusion.

[1] MacGregor JT, Wehr CM, Gould DH. Clastogen-induced micronuclei in peripheral blood erythrocytes: the basis of an improved micronucleus test. Environ Mutag 1980; 2: 509–514.

[2] Furtado MA, Almeida LCF, Furtado RA, et al. Antimutagenicity of rosmarinic acid in Swiss mice evaluated by the micronucleus assay. Mutation Res 2008; 657: 150–154.

82 | Colonization of canine intestinal microbiota and the effects of route of delivery and vaginal seeding: Preliminary results

Samara Beretta Gomes Da Silva[1]; Maricy Apparicio[1]; Marita Vedovelli Cardozo[2]; Renatha Almeida de Araújo[1]; Daniela Jorge Coutinho Armani[1]; Melissa Oliveira Bianchini[1]; Jaqueline Tamara Bonavina[1]; João Domingos Rocha Junior[1]
[1]*Department of Pathology, Theriogenology and One Health, School of Agricultural and Veterinarian Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal – SP, Brazil*, [2]*Department of Microbiology, School of Agricultural and Veterinarian Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal – SP, Brazil*

Dysbiosis is an alteration of the intestinal microbiota that has been observed in children who were born by C-section and, therefore, who were deprived of contact with the vaginal microbiota during the passage through the birth canal. Several studies in humans have shown that the increased incidence of many diseases in populations may be closely related to dysbiosis and research using the seeding technique have shown promising results [1]. In veterinary medicine, there is scarce information about the microbiota of canine neonates born by vaginal delivery and C-section and no description on the use of vaginal seeding in animals. This study aimed to analyze the composition of the intestinal microbiota of canine neonates born by vaginal delivery and elective C-section, as well as to investigate for the first time the influence of vaginal seeding. One adult pregnant bitch was submitted to elective C-section and another one was monitored during vaginal birth. To determine the correct time to perform the C-section, it was considered: drop in the dam's rectal temperature, P4 ≤2 ng/ml, decrease in amniotic fluid, presence of intestinal peristaltism as well as decrease in fetal HR. Samples from the mouth, vagina, anus, and breast region of the dams were collected before epidural anesthesia (C-section) and at the first signs of prodrome (vaginal delivery). Swab samples from the anus of the newborn were collected immediately after removal from the uterus and then vaginal seeding (rub the gauze removed from the mother's vagina in the mouth, face, and body of the newborn) was performed. So, the experimental groups were: G1 (n = 4) – newborns born by C-section + vaginal seeding; G2 (n = 4) – newborns born by c-section without seeding; G3 (n = 1) – neonate born by vaginal delivery. In all groups, stool samples were collected from the neonates on days 1, 2, 3, 6, 9, and 15. Bacteria counting was performed through surface plating and the groups of enterobacteria, Staphylococcus, and Streptococcus were quantified. Results show that newborns already have intestinal microbial colonization on the first day of life, regardless of the route of birth (Table 1). There was a decrease in the population of Staphylococcus sp over the days, in all groups, while in bacterial colonization by Streptococcus sp and enterobacteria, there was population growth up to day 6 in G1 and G2 and up to day 9 in G3. There was bacterial growth in the samples collected from the vagina of the bitch submitted to C-section, while curiously no bacteria growth was observed in the samples obtained from G3. No enterobacteria grew in the breast and mouth samples from any of the groups. Although these preliminary results point out that microbiota of the newborns might not be influenced by the route of delivery, it does not allow us to draw a proper conclusion and so additional research is being conducted in our laboratory to clarify this finding.

[1] Dominguez-bello et al., Nature Medicine 2016; 22:3–250.

Collection days	*Staphylococcus* (UFC/g)			*Streptococcus* (UFC/g)			Enterobacteria (UFC/g)		
	G2[1]	G1[2]	G3[3]	G2	G1	G3	G2	G1	G3
1	1,0 x 10^7	9,28 x 10^6	3,4 x 10^4	4,16 x 10^7	1,28 x 10^7	3,9 x 10^4	7,2 x 10^5	4,7 x 10^6	0
2	2,7 x 10^5	1,3 x 10^7	3,4 x 10^5	5,32 x 10^7	1,14 x 10^8	2,09 x 10^6	2,97 x 10^8	3,5 x 10^7	6 x 10^4
3	1,01 x 10^5	6,9 x 10^5	3 x 10^5	6,88 x 10^6	3,5 x 10^7	3,51 x 10^6	4,3 x 10^8	3,56 x 10^8	4 x 10^3
6	1,26 x 10^6	1,5 x 10^4	6,7 x 10^5	2,92 x 10^8	3,06 x 10^8	5,87 x 10^7	4,82 x 10^8	4,0 x 10^8	1,1 x 10^8
9	1,57 x 10^5	2,3 x 10^5	5,64 x 10^6	1,2 x 10^8	1,62 x 10^8	7,36 x 10^8	4,0 x 10^8	2,1 x 10^8	8,63 x 10^8
15	2,7 x 10^3	1,71 x 10^4	2 x 10^2	6,2 x 10^7	6,3 x 10^8	1,15 x 10^9	1,62 x 10^8	1,23 x 10^8	2,8 x 10^7

Material collection	*Staphylococcus* (UFC/g)		*Streptococcus* (UFC/g)		Enterobacteria (UFC/g)	
	C[4]	VB[5]	C	VB	C	VB
Breast	1,2 x 10^3	0	0	3 x 10^4	0	0
Vagina	3,3 x 10^4	0	1,8 x 10^5	0	5,96 x 10^5	0
Mouth	0	1 x 10^3	1,4 x 10^5	3 x 10^3	0	0
Anus	0	0	1,8 x 10^5	1,6 x 10^5	2,8 x 10^4	1,8 x 10^4

[1] Cesarean section with vaginal seeding
[2] Control Cesarean Group
[3] Normal Delivery Group
[4] Bitch undergoing cesarean section
[5] vaginal birth

83 | True vaginal prolapse associated with retroflection of the urinary bladder in a bitch: A case report

Samara Beretta Gomes da Silva[1]; Maricy Apparicio[1]; Paola de Castro Moraes[1]; Camila Vasconcelos Ribeiro[1]; Marina Vilela Estevam[1]; Nathalia Franzoni Smargiassi[2]; Marjury Cristina Maronezi[2]; Yuri da Silva Bonacin[2]; Pamela Oliveira de Freitas[2]; Gilson Hélio Toniollo[1]; Lindsay UnnoGimenes[1]
[1]*São Paulo State University (UNESP), Jaboticabal, SP, Brazil, Brasil*
[2]*Autonomous Veterinarian, Brasil*

A two-year-old Brazilian Mastiff bitch was admitted to the Obstetrics and Animal Reproduction sector of the Veterinary Hospital "Governador Laudo Natel" at UNESP Jaboticabal with a history of a bloody vaginal discharge for approximately a week, a swollen vulva, and male attraction, which indicates that the animal could be in proestrus. Physical examination revealed that the animal was prostrate, presented a low body score and the perineal region was circumscribed by adherent feces, characterizing diarrhea. In the perivulvar region, there was a mass measuring 14.64 × 12.46 × 10.07 cm (length × width × height) projected through the vulva. Transabdominal ultrasound evaluation (S2000 Siemens, 9 MhZ linear transducer) was performed and the ovaries and uterus were not observed in the abdominal cavity. Ultrasound of the prolapsed structure revealed a retroflected bladder, which presented a usual shape, thickened echogenic walls, regular internal margins, with moderate liquid repletion, and lumen filled with anechoic content with discrete cellularity. Uterus with slightly heterogeneous

parenchyma was observed surrounding the urinary bladder. Urethral catheterization was performed and continuous retrograde urethrocystography was conducted to visualize the integrity of the urethra that was preserved and an oval-shaped structure, compatible with the urinary bladder was observed. For treatment, manual reduction of prolapse and urinary bladder followed by ovariohysterectomy was performed, without complications during the procedure The patient was followed up for seven days after the procedure, with no hematological changes or prolapse recurrence, However, it was not possible to follow the animal in the long term due to the incommunicability of the owner. Urinary bladder catheterization followed by retrograde urethrocystography to assess the integrity of the urethra to avoid trans and postoperative complications had not yet been reported in cases of true vaginal prolapse associated with urinary bladder retroflection, being a crucial tool for prognosis and surgical planning. We believe that the early diagnosis associated with the imaging examinations, such as retrograde urethrocystography and ultrasound of the protruding mass, was crucial to prevent any possible trans and postoperative complications associated with the manipulation of these organs.

84 | Extra-skeletal osteosarcoma in the vulva: An extremely rare case in the bitch

A. R. Tartarelli, G. H. Toniollo, Maricy Apparício[1];
S. Beretta, G. P. Lima; R. O. Vasconcelos
São Paulo State University (UNESP), FCAV, Jaboticabal-SP, Brasil

Vaginal and vulvar tumors are the most frequently found in the reproductive tract of bitches and generally have a histological character of benignancy. On the other hand, osteosarcomas are the most common bone tumor, representing 5–7% of all malignancies described in dogs [1], but rarely found in extra-skeletal sites. Few cases have been reported in Veterinary Medicine and none in the reproductive tract of domestic bitch. This report aims to describe a case of extra-skeletal osteosarcoma in a vulva of a 12-year-old mixed breed bitch. The owner reported that the tumor was noticed more than a month ago and has increased significantly its dimensions since then. Upon palpation, the nodule was approximately 6 cm in diameter, with high stiffness, adherence, and without flotation or ulceration points. The patient presented bloody vaginal discharge, dysuria, lymphadenomegaly in popliteus, dehydration, anorexia, and progressive weight loss. She has been castrated 4 years ago and she had been submitted to bilateral mastectomy in a private clinic, with no histopathological examination of the excised tumor. A blood test revealed regenerative anemia, thrombocytosis, hyperphosphatemia, and azotemia. Biochemistry profile within normal limits. Urinalysis indicated the presence of two protein crosses, transitional, squamous cells, granular cylinders, and bilirubin crystals. Abdominal ultrasound examination revealed alterations in kidneys, liver, adrenal, and stomach. Thoracic radiographic examination of the patient showed alterations suggestive of heart disease and in the left caudal lobe, a circular, radiopaque area of approximately 0.55 cm in diameter, suggestive of neoplasia. No radiographic alteration was found in the cervical, thoracic and lumbar spine. To alleviate clinical symptoms, a vulvoplasty was carried out. Excision of the tumor was extremely difficult due to adhesions on the perineal and mammary regions and reconstruction of the vulva was carried out by using a skin flap obtained from the inguinal area. Based on the histopathological result, chemotherapy was indicated but the owner never returned for re-evaluation. Five months after the surgery, we were able to contact the owner who reported patient death due to severe lung metastases diagnosed via radiographic exams performed by another veterinarian. Chemotherapy was never performed.

[1] Langenbach A, Anderson MA, Dambach DM, Sorenmo KU, Shofer FD. Extraskeletal osteosarcomas in dogs: a retrospective study of

169 cases (1986–1996). Journal of the American Animal Hospital Association 1998; 34: 113–120.

85 | Expression of estrogen (ER) and progesterone (PR) receptors in oviducts of bitches in the pre and post-ovulatory period

C. B. M. L. Felippe[1]; M. R. Tavares[1]; R. A. Furtado[2]; C. E. Fonseca-Alves[3]; D. G. Borges[2]; Maricy Apparício[1]
[1]*São Paulo State University (UNESP), FCAV, Jaboticabal-SP, Brazil;* [2]*University of Franca, Franca-SP, Brazil;* [3]*São Paulo State University (UNESP), FMVZ, Botucatu, SP, Brazil*

Several ovulatory aspects differentiate canids from other domestic species, but one in special: oocyte maturation occurs in the oviduct under the influence of high concentrations of progesterone. The difficulty to mimic this milieu in vitro hinders the application of reproductive techniques, limits the results of embryo production, and prompts investigations focusing on reproductive physiology and endocrinology. Thus, this study investigates the expression of estrogen and progesterone receptors in the infundibulum, ampulla, and isthmus of canine oviducts in the pre and post-ovulatory periods. The bitches were selected and separated according to the reproductive status: in the pre-ovulatory period (up to 48 h before ovulation; $n = 5$), and post-ovulatory (up to 72 h after ovulation; $n = 4$). Reproductive status was determined by a combination of vaginal cytology, serum progesterone concentration, and macroscopic aspect of the ovary. After elective ovariohysterectomy, the oviducts were dissected and properly prepared for immunohistochemical analysis (evaluated for the presence and absence of estrogen and progesterone receptors, intensity, and quantity of receptors). The results revealed that the mucosa of the oviduct presented a higher score for progesterone receptors in both periods, although the score was lower for the post-ovulatory period. The expression of estrogen receptors was higher in the submucosal layer in the three segments, but it was greater in the infundibulum ($p < 0.05$). These results are in accordance with the possible changes that occur in the oviductal environment as a result of hormonal influence: from cell differentiation to receive immature oocytes to oocyte transport performed by muscle contraction and ciliary movement towards the uterus. Therefore, both progesterone and estrogen play important roles in bitch oviducts in different periods, oviduct segments, and layers.

86 | Anencephaly and Palatoschisis in two canine neonates

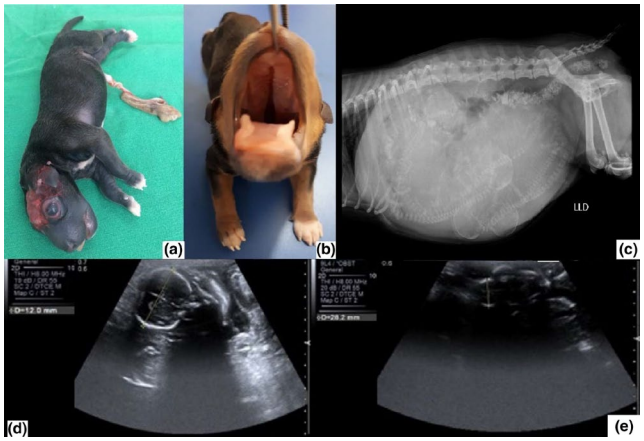
M. V. Estevam[1]; N. F. Smargiassi[2]; S. Beretta[1]; P. E. B. Martinelli[1]; R. O. Vasconcelos[1]; Maricy Apparício[1]; G. H. Toniollo[1]
[1]*São Paulo State University (UNESP), FCAV, Jaboticabal, SP, Brazil;* [2]*São Paulo University (USP), Ribeirão Preto, SP, Brazil*

The objective of this report is to describe two cases of anencephalic newborns presented at the Veterinary Hospital, Unesp, Jaboticabal. In case 1, a 4-year-old mixed breed bitch was brought for consultation due to dystocia after giving birth to one healthy puppy. A series of congenital defects (Fig 1A, B) was noticed in one of the puppies: anencephaly, acrania, palatoschisis, eyelid aplasia, and macroglossia. The owner didn't have any prenatal data such as fetal biparietal diameter (BPD) and HR. In case 2, a Lhasa Apso, 2 years old, was presented for gestational evaluation at approximately 52–54 days after mating. According to the owner, she was accidentally mated to an American Pit Bull Terrier, of unknown age and history. Radiographic examination revealed 5 fetal skulls and 6 columns (Fig 1C). Five days later, the bitch presented prodrome behavior and was brought for consultation. Ultrasound revealed 5 fetuses with HR close to 180 bpm and BPD of approximately 28.2 mm (Fig 1D). A sixth fetus presented a HR of 220–230 bpm and BPD of 12.0 mm (Fig 1E). A significant drop in fetal HR prompted us to do a c-section, followed by OHE. The litter consisted of 5 healthy puppies and one anencephalic, presenting the same malformations reported in case 1. Both bitches were primiparous, fed with commercial food without any vitamin supplementation and as far as the owners known, they were not exposed to teratogens during pregnancy. The dams and the other neonates were clinically healthy and were released hours after birth. The two anencephalic showed motor reflexes and dyspnea at birth and due to their condition, were euthanized minutes after delivery; the bodies were sent to necropsy and no other malformation was found. Some breeds are more susceptible to these conditions, such as pit bulls, however, due to financial constraints, a genetic testing was not carried out. It is known that the etiology of anencephaly is multifactorial and may involve genetic factors and exposure to teratogens [1], however, the lack of the parent's data does not allow to draw a proper conclusion. Most cases of neural tubal defects (NTD) are isolated but may appear associated with other malformations such as cleft palate when there is a mutation in the SHROOM3 gene [1]. The same was described in mice, but until now, there is no report in dogs. To the authors knowledge there is no epidemiological survey of anencephaly in this species, although the increasing number of reports on this condition in dogs and the fact that two newborns in different litters were presented within a year in our institution, might indicate that anencephaly is not as uncommon as it is believed and prompts the need for genetic evaluation to clarify the genes involved, especially when it is associated with other malformations, as in the cases presented in this report.

Reference:

[1] Deshwar AR, Martin N, Shannon P et al. A homozygous pathogenic variant in SHROOM3 associated with anencephaly and cleft lip and palate. Clin. Genet. 2020; 98; 299–302.

Figure 1.



87 | Field-friendly techniques for sperm banking from wildlife species

Gabriela Mastromonaco; Paula Mackie
Toronto Zoo, Canada

Biomaterials banking has long been promoted as a valuable tool for the preservation of biodiversity. Storage of gametes and embryos can provide insurance against a sudden loss of genetic diversity due to man-made or natural events. While thousands of genetic samples (e.g. blood, tissue, bone) have been collected across all taxa, viable reproductive cells capable of producing offspring have been banked primarily from zoo-housed animals. Despite >30 years of research on wildlife gamete and embryo cryopreservation, acquisition of samples from free-ranging animals has been impeded by the need for specialized equipment and technical expertise, lack of standardized protocols, and most importantly, lack of prioritization, or even acceptance, by conservation biologists and wildlife veterinarians. Our current research focuses on establishing protocols for semen collection and cryopreservation in carnivores that are easily transferrable to field conditions and don't depend on time- and labor-intensive techniques. Implementation of urethral catheterization following medetomidine-based anaesthesia has enabled us to successfully collect sperm from a variety of species, including tigers, lions, hyenas, wolves, polar bears, and more, precluding the need for electroejaculation equipment and expertise. Sperm characteristics are similar to, and in some cases better than, those from electroejaculated samples (Mackie et al., 2020). While sperm have been successfully cryopreserved and used for artificial insemination or in vitro fertilization in more than 100 species (Mastromonaco and Songsasen, 2020), wild felids can be challenging to cryopreserve successfully due to inherent differences in cryosensitivity resulting from high percentages of morphological abnormalities (Pukazhenthi et al., 1999). Our objective was to develop a field-friendly rapid freezing technique that supports similar, if not improved, post-thaw parameters in felids using the domestic cat as a model. Testes obtained from local veterinary clinics immediately post-castration were used for epididymal sperm collection. Sperm samples were divided equally among Optixcell (O) and TEST (T) extenders and frozen in straws either by immersing into liquid nitrogen vapour in a dry shipper overnight (D) or directly into liquid nitrogen (N) as follows: OD, ON, TD and TN. Sperm assessments (motility, viability, morphology, acrosome status, zona pellucida binding) were carried out on raw, pre-cool, post-cool and post-thaw sperm. Compared to average raw, pre-cool and post-cool sperm characteristics, significant changes were observed in all treatment groups post-thaw (motility: 68% vs 46%, viability: 75% vs 41%, normal morphology: 63% vs 22%, intact acrosome: 92% vs 61%, for raw sperm and OD groups, respectively). Both extenders performed better using dry shipper freezing compared to liquid nitrogen freezing (OD = TD > ON > TN) with OD showing the greatest zona pellucida binding rates. The Optixcell dry shipper freezing protocol (OD) has now been tested in a variety of wild species to date, including cheetah, snow leopard, cougar, lion, Sumatran tiger, and spotted hyena, with some success. These data provide support for the applicability of a rapid freezing technique for felids. The protocol could be readily implemented in field conditions without access to liquid nitrogen, which would enhance opportunities for banking from free-ranging animals, an important step towards long-term genetic preservation in threatened felids.

88 | Endometrial hyperplasia in rabbits (*Oryctolagus cuniculus*): Is it similar to the bitches?

Danaê F. A. Cação[1]; M. Apparício[1]; G. Marietto-Gonçalves[2]; F. Oliveira[3]; B. F. Firmo[1]; R. Gargano[4]; J. Takaki[5]; A. B. De Nardi[1]
[1]*São Paulo State University (UNESP), FCAV, Jaboticabal, SP, Brazil;* [2]*Doc Bird – Consultancy in Avian Medicine, Exotic and Wild Animals, Botucatu, SP, Brazil;* [3]*Self-Employed Veterinarian, Hortolândia, SP, Brazil;* [4]*Paulista University (UNIP), Campinas, SP, Brazil;* [5]*Vet Célula Laboratory, Itatiba, SP, Brazil*

Uterine disorders are the most common condition of the female rabbit. Adenocarcinoma is the most frequently condition followed by endometrial hyperplasia (EH). There are a few retrospective studies about these pathologies and up to now the knowledge about the histopathological presentation of EH is limited. The present study aimed to verify if the classification of Dow [1] employed in bitches for EH could be applied for female rabbits and if so, if there is a correlation with the age of the animal. For that purpose, we performed uterine ultrasound examination in 34 pet rabbits which were referred for elective ovariohysterectomy. Only those presenting uterine alteration compatible with endometrial hyperplasia were selected for this study (n = 18), which included: increased uterine diameter, hypoechoic, and swollen walls, and/or irregular walls with no intraluminal fluid. The rabbits had ages ranging from 6 months to 9 years old and were clinically healthy and asymptomatic at the time of the surgical procedure. A complete blood count and biochemical tests (ALT, AST, urea, and creatinine) were also performed to assess their general condition. The excised uterine horns and cervix were

identified and sent for histopathological evaluation. The degree of EH was determined based on the classification proposed by Dow [1] for bitches, from grade I to IV. Kendall's tau was applied to correlate the EH degree and age of the animals, $p < 0.05$. Histopathological evaluation revealed that six (33.3%) had uterine alterations compatible with type I, three (16.6%) with Type III and nine (50%) with Type IV. There was a strong correlation between the age of the animals and the degree of EH: female rabbits older than 2.5 years were more prone to develop grade III and IV; grade I was only detected in does with less than 2.5 years. None of the animals present grade II. There was no significant difference in the hematological and biochemistry parameters among the animals. According to the results, it was concluded that Dow's classification can be successfully applied to classify EH in female rabbits. Moreover, it seems that as the animals get older, uterine endometrium is more prone to change into more advanced stages of hyperplasia. Whether or not these alterations can lead to the development of clinical signs, if not diagnosed at a proper time, must be further elucidated.

[1] Dow C. The cystic hyperplasia-pyometra complex in the bitch. Journal of Comparative Pathology, 1959;69:237–250.

89 | Delayed ovulation and abortion due to a transcervical endometrial polyp in a German Shepherd bitch

M. Andrea Pasquale Del Carro
Iunovet, *Aix en Provence*, *France*

History: A five years old German Shepherd bitch was presented in consultation for abortion at day 51 post ovulation (p.Ov). The bitch had two previous litters with normal delivery. Bacterial culture performed after abortion revealed no pathogen bacteria in the genital tract. The next oestrus cycles were monitored.

First cycle: Bacterial culture showed absence of vaginal pathogens. Ovulation occurred at day 31 after the onset of the oestrus. The bitch was mated naturally. One alive foetus and 4 resorption sites were detected by ultrasound at day 26 p.Ov. Progesterone was evaluated weekly: no signs of luteal insufficiency were detected. The foetus was aborted at day 56 p.Ov.

Second cycle: Bacterial culture at the beginning of oestrus showed the presence of Kocuria kristinae. Antibiotic treatment has been performed for 2 weeks (Amoxicillin and Clavulanic acid). Ovulation occurred at day 34 after the onset of the oestrus. The bitch was mated naturally. No foetus was detected during the diagnosis of pregnancy at day 28 p.Ov. The only abnormality observed by ultrasound was an enlarged and heterogenous cervix. A vaginoscopy was performed during anoestrus. It revealed the presence of a 3 cm long, pediculate, transcervical, haemorrhagic polyp. The polyp has been resected endoscopically. Histology confirmed a fibro-epithelial polyp modified by haemorrhage.

Third cycle: Bacterial culture at the beginning of oestrus showed absence of vaginal pathogens. Ovulation occurred at day 14 after the onset of the oestrus. The bitch has been mated naturally. Four foeti were observed at the ultrasound. No progesterone monitoring or supplementation was performed. Natural delivery of 4 alive puppies occurred at day 62 p.Ov.

Discussion: Endometrial polyps are focal tumour-like growths structures protruding into the uterine lumen [1]. This condition affects usually eldering bitches and is associated with pyometra in 50% of the cases [2]. In this bitch the diagnosis by abdominal palpation or ultrasound was not possible. No other uterine lesions were associated with the polyp. The clinical signs observed were abortion and delayed ovulation maybe because of the transcervical localization of the polyp. Maternal oestradiol and oestrone increase consistently during pregnancy. These changes could increase the polyp size and induce the opening of the cervix and abortion. The relationship between delayed ovulation and endometrial polyp is unclear. The ovarian function of the bitch was not altered after the ablation of the polyp: ovulation occurred naturally, no luteal insufficiency was observed . Delivery occurred at term. May the endometrial polyp was a source of chronic uterine inflammation and consequent prostaglandins production. These could perturbate the ovarian activity. Endometrial polyps' development are considered as an abnormal non tumoral uterine response to endogenous steroids stimulation [2]. Ovario-hysterectomy is the treatment of choice considering the risk of pyometra associated. Endoscopic ablation is possible, but the risk of relapse is high because it doesn't allow to have free margins.

References:

[1] Gelberg HB et al. Hyperplastic endometrial polyps in the dog and cat. Vet. Pathol. 21:570–573(1984).

[2] Marino G et al. Endometrial polyps in the bitch: a retrospective study of 21 cases. J Comp Pathol. 2013;149(4):410–6.

90 | Early detection of bacterial infection in the newborn dog – Evaluation of six blood biomarkers

Hanna Mila[1]; Mickael Winkler[1]; Jean-Pierre Braun[2]; Catherine Trumel[3]
[1]*NeoCare*, *Université de Toulouse*, *ENVT*, *UPS*, *Toulouse*, *France*;
[2]*Animal and Comparative Clinical Pathology*, *Toulouse*, *France*;
[3]*CREFRE*, *Université de Toulouse*, *INSERM*, *ENVT*, *UPS*, *Toulouse*, *France*

Introduction and objectives: Bacterial infections, and in particular sepsis, are pathologies frequently observed in puppies during the neonatal period, being identified as cause of death in majority of neonatal mortality cases. The clinical signs of generalized bacterial infection are not very specific and mortality very rapid after their appearance: on average 1 day after first signs. The objective of this study was to evaluated several potential early blood biomarkers of bacterial infection in the newborn dog (between birth and three weeks of life) and to provide their reference intervals in healthy puppies.

Materials and methods: Within one breeding kennel, puppies were followed since birth until 21 days of age and their blood was

collected at Day 2, 7, 14 and 21. Among all followed puppies (50% of small, 15% of medium and 35% of large breeds puppies), were included in the following study: 56 puppies in a good health until D21 and 44 puppies dying between D2 and D21 with signs of bacterial infection according to the post-mortem examination (including positive bacterial culture on the spleen tissue). The following blood biomarkers were analyzed on weekly collected samples and stored at −20°C during seven years before the assay: canine procalcitonin (PCT), C-reactive protein (CRP), total protein (PT), albumin (Alb), albumin/globulin ratio (Alb/Glob) and immunoglobulin G (IgG) at D2 only. The reference intervals of studied biomarkers were determined with the Reference value advisor freeware. The effect of the breed size, sex, age and health status were evaluated on the different blood biomarkers using general linear models. The ROC curves were drawn and areas under the curves (AUC) were calculated to estimate the clinical value of the different biomarkers to differentiate puppies at increased risk of death.

Results: The references intervals of studied biomarkers are provided in the Table 1. In this population of healthy puppies, breed size, age and health status had influence on all tested biomarkers ($p < 0.05$ for all tests). TP and Alb concentrations were increased at D2; PCT and Alb/Glob were increased at D7 and CRP was increased at D21 comparing with other time points. TP, Alb and IgG were increased in small, and PCT and Alb/Glob in medium sized breeds comparing with other breed sizes. All biomarkers, expect TP, were significantly different in dying vs healthy puppies (Table 1). PCT and Alb/Glob have the highest AUC from all tested biomarkers (respectively, 0.69 and 0.67) with lower PCT and higher Alb/Gl values in dying vs surviving puppies ($p = 0.013$ and 0.006).

Conclusions: The following study provides the reference values of several blood biomarkers of immune status in the newborn dog. According to these preliminary results, two biomarkers, PCT and Alb/Glob, need further investigation to evaluate their clinical relevance in distinguishing puppies at a higher risk of bacterial infection. Moreover, both tests require to perform blood sampling and external laboratory tests. Thus, those methods require further validation, i.e. on specimens easily obtained on newborns and in a non-invasive way such as urines or saliva.

Table 1. Median values of blood markers of bacterial infection evaluated at two days of age depending on the health status until 21 days of 100 included puppies.

Blood parameter at D2	Health status	n	Median (min-max)	*P* value
Procalcitonin (ng/ml)	ALIVE	56	0.61 (0.02-3.53)	0.013
	DEAD	42	0.31 (0.06-1.58)	
C-reactive protein (mg/L)	ALIVE	56	<5 (5-268)	0.014
	DEAD	38	<5 (5-62)	
IgG (g/L)	ALIVE	56	3.58 (0.35-25.78)	0.047
	DEAD	35	1.69 (0.29-10.38)	
Total protein (g/L)	ALIVE	56	41.2 (25.6-60.2)	0.474
	DEAD	38	43.1 (29.6-58.2)	
Albumin (g/L)	ALIVE	56	23.0 (10.0-34.0)	0.044
	DEAD	36	24.5 (15.0-35.0)	
Albumin/Globulin	ALIVE	56	1.25 (0.33-2.15)	0.006
	DEAD	36	1.51 (0.33-2.20)	

91 | Immunohistochemical evaluation as a complement in the diagnosis of disorders of sexual development in a French bulldog dog

Natalia R. da Silva, Luana M. S. Amaral, Vinícius W. Silva, Julia R. Greghi, Ariel de Aguiar, Thalita E. S.de Oliveira, Guilherme S. Cardoso, Maria Isabel Mello Martins
Universidade Estadual De Londrina, Brasil

A 7-month-old French Bulldog canine, phenotypically female, with historic of progressive clitoral hypertrophy for two months ago. Animal never showed signs of heat or urination problems, but the exteriorized structure caused a lot of discomfort. In the gynecological examination, a firm structure of 3 cm was observed in clitoral region (micropenis) visualized externally to the vulva. Intact vagina and urethra at vaginoscopy, with normal morphology and location. Vaginal cytology compatible with anestrus. Radiography confirmed penile bone in the clitoral fossa. Ultrasonography showed a structure on the left side similar to a gonad, with its respective uterus. In view of the findings and suspicion disorders of sex development (DSD), the animal was sent to exploratory celiotomy where the two uterine horns and their respective gonads were removed, followed by amputation of the micropenis. Macroscopically, the structures in the topography of the ovary presented a morphology compatible with testicles, sexual reversion confirmed by microscopy, where seminiferous tubules and bilateral hypoplastic epididymis were observed. In addition, penile bone, horn tissue and uterine body were within physiological standards. Immunohistochemistry in gonadal tissue showed positivity in reactions with antibodies for progesterone (hPRa2) and estrogen receptors (EP1) and negative for polyclonal androgen receptors and polyclonal prostate-specific antigen. There are reports of dogs with DSD in different species and breeds for at least 100 years, however, it is possible to infer an increase in French Bulldog dogs in the last 10 years, which can be explained by the high degree of consanguinity. Usually, animals with clinical suspicion of DSD seed care during puberty, between 4 and 12 months of age, with a history of externalized structure in the vulva, as in this report. During surgery it was possible to observe that the micropenis morphology was very similar to a real penis, with swelling of the false bulb and glans-like tissue around the bone tissue. Male pseudo-hermaphrodite animals are commonly uni- or bilateral cryptorchids, in this case and in other reports the testicles were in the abdominal cavity. The removal of the gonads and amputation of the micropenis is the most indicated to avoid complications during the estrus season. This animal presents characteristics of male pseudohermaphroditism owing to presence of testes, uterus and external genitalia predominantly feminine with vulva and micropenis, however, has female hormone receptors observed in gonadal tissue by immunohistochemistry.

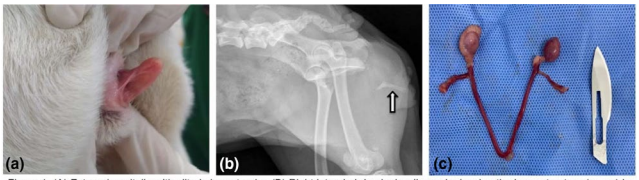


Figure 1. (A) External genitalia with clitoris hypertrophy. (B) Right lateral abdominal radiograph showing the bone structure (arrow) in the perineal region. (C) Macroscopic aspect of the internal genitals.

92 | Symmetric dimethylarginine (SDMA) measurement and Doppler ultrasonography of the renal and uterine artery in bitches with complex cystic endometrial hyperplasia-pyometra

Letícia A. S. Correia, Luiz G. C. Trautwein, Josiana F. Schnitzer, Myrian Hidalgo, Ana Beatriz Almeida, Karina K. Flaiban, Lucienne G. P. Giordano, Maria Isabel Mello Martins
Universidade Estadual de Londrina, Brasil

Cystic Endometrial Hyperplasia (CHC)-Pyometra Complex is an urgent condition of the reproductive tract of bitches and acute kidney injury is a common consequence in patients. This study aimed to perform biochemical tests, urinalysis, urinary protein-creatinine ratio (UPC), measurement of SDMA, Doppler velocimetric assessment of uterine and renal arteries of a patient with HEC-pyometra complex at diagnosis and after an average of 6 months for renal evaluation. Bitches were evaluated in M1 (n = 36), moment of diagnosis and M2 (n = 16), mean of 6 months after diagnosis. The control group (n = 6) were healthy bitches submitted to Doppler ultrasonographic evaluation of the uterine artery and renal arteries. Patients at moment M1 were classified as azotemic (AZO) (creatinine >1.5 mg/dl) (n = 12/36) and non-azotemic (NAZO) (creatinine <=1.5 mg/dl) (n = 24/36). Comparisons were made between M1 and M2 with the animals present at both times. For the evaluation of statistical significance, $p < 0.05$ was considered. At M1 there was no difference between AZO and NAZO patients for SDMA (p = 0.059), but SDMA means were above the reference limit in both groups. A difference in SDMA (p = 0.009) was obtained, with higher values in patients with a closed cervix compared to the open one. In M2, the mean values of serum creatinine, urea and SDMA were 0.92 mg/dl, 33.8 mg/dl, and 11.8 µg/dl, respectively, and there was no azotemia in any bitch, but there was an increase in the limits SDMA reference in two bitches. The comparison between M1 and M2 was significant for SDMA and urea (p = 0.008 and p = 0.037) and not significant for creatinine (p = 0.058). The mean UPC of the animals in M1 was 0.82 ± 0.35, with 71.5% proteinuric and 28.5% proteinuric borderlines, and in M2 the mean was 0.25 ± 0.17 with only one patient considered proteinuric. For ultrasonographic evaluation, there was statistical difference between control animals and animals with pyometra in the parameters of peak systolic velocity (PSV) uterine artery (p = 0.001), end diastolic velocity (EDV) of uterine artery ($p < 0.001$), pulsatility index (PI)) of uterine artery (p = 0.003), resistivity index (RI) of uterine artery (p = 0.001). There was a significant difference between the AZO and NAZO groups in PI of the left and right renal artery and RI of the right renal artery (p = 0.008), but when comparing moments M1 and M2 there was no statistical difference. The presence of strong and moderate correlations in M1 were observed between SDMA with creatinine (r = 0.721), as well as with urea (r = 0.808), uterine artery EDV (r = 0.722), PI right kidney (r = 0.789), PSV kidney left (r = 0.840) and left kidney EDV (r = 0.660). This work allows us to affirm that there is the presence of acute kidney injury due to the increase of SDMA in NAZO animals, and they are higher in patients with closed cervix pyometra; Uterine artery Doppler velocimetric alterations are significantly altered compared to the control group and the renal arteries Doppler velocimetric alterations are altered at the time of diagnosis and remain after 6 months. This work shows the clinical importance of performing complementary tests and specific renal care in patients treated for pyometra.

93 | Factors influencing the day of canine parturition estimated from serum progesterone concentration measured by Fluorescence Enzyme Immunoassay (FEIA)

Hasan Sontas[1]; Chiara Milani[2]; Barbara Contiero[2]; Charlotte Sontas[1]; Stefano Romagnoli[2]
[1]*Tierambulanz Vorchdorf;* [2]*Department of Animal Medicine, Production and Health, University of Padova, Italy*

Introduction and objectives: Pregnancy length in the bitch is 61.0–65.2 days after the day of ovulation determined by serum progesterone (P4) concentration using enzyme immunoassay or chemiluminescence methods [1, 2]. Therefore, the objectives of the present study were a) to determine the length of pregnancy after the first breeding based on ovulation timing determined by serum P4 concentration measurement using Fluorescence Enzyme Immunoassay (FEIA) method and b) to investigate factors that may influence duration of canine pregnancy.
Materials and methods: In this study, the optimal breeding day/s were recommended according to the combined findings of vaginoscopy, vaginal cytology and serum P4 concentrations determined by FEIA assay (AIA 360, TOSOH Corp., Japan). A total of 131 privately owned female dogs of various breeds (n = 48) and body weights (3.1–57 kg) presented for determination of ovulation timing from April 2016 to November 2019, were included. Breeding was recommended as soon as the P4 concentration was above 6 ng/ml [1]. The day(s) of breeding(s) were obtained from the breeders by a telephone call. Bitches that delivered naturally (n = 1102) or those that underwent a C-section (n = 29) after giving birth to at least one puppy were included in this study. Factors included in the current study were age, breed, body weight (≤10, 11–25, 26–40 and ≥41 kg [3]), date of progesterone measurement, type of breeding (mating vs artificial insemination) and size of the litter (≤4 puppies; 5–9 puppies; ≥10 puppies). The effect of these variables on the length of pregnancy was determined using a linear ANOVA model. $p < 0.05$ was statistically significant.
Results: The mean length of gestation from the estimated day of ovulation and from first mating were 63.8 ± 0.3 and 64.5 ± 4 days, respectively. Dogs weighing ≤10 kg had a significantly shorter pregnancy

length compared to those with ≥41 kg (64.9 ± 0.4 vs. 66.4 ± 0.5 days, p = 0.041). The duration of pregnancy was significantly longer in 1 and 8 years-old bitches (68.3 ± 0.9 and 66.2 ± 0.5 days; p = 0.001) compared to dogs between 2 and 7 years of age (64.9 ± 0.3 days). Ten bitches delivered either earlier or later than estimated day for parturition which was calculated from the day of ovulation. Neither number of puppies nor type of breeding had statistical influence on duration of pregnancy.

Conclusions: This study shows that duration of gestation from the day of ovulation and breeding determined by the measurement of serum P4 concentration using FEIA method was significantly influenced by the age and the body weight of the bitch and serum P4 concentration.

94 | The challenges of diagnosing canine ovarian remnant syndrome with laboratory tests: A clinical case

Besim Hasan Sontaş[1]; C. Sontaş[1]; K. Sick[2]
[1]*Tierambulanz Vorchdorf, Austria*; [2]*Laboklin GmbH, Germany*

Clinical case: A 4-year-old, spayed, female American Staffordshire Terrier dog weighing 29 kg was presented with a 7-week history of vaginal bloody discharge. The bitch was ovariohysterectomized about 3 years before the presentation. Upon inspection, the vulva was slightly swollen and vaginoscopy showed slightly edematous vaginal folds and the presence of a small amount of dark red discharge. A vaginal smear contained approximately 50% cornified epithelial cells. Upon abdominal ultrasonography, the cervix (transversal image; 18.7 mm × 23.7 mm; sagittal image with uterine body: 15.3 × 58.7 mm) and the uterine body (transversal image; 9.3 mm × 10.4 mm) was without fluid accumulation or cystic structures. Two hypoechoic ellipsoid structures measuring 18.5 mm × 9.8 mm and 18.6 mm × 10.8 mm were identified behind both kidneys, respectively. A venous catheter was placed, and blood sample was collected for the measurement of serum progesterone (P4) (AIA 360, Tosoh, Japan) and anti-Müllerian hormone (AMH) concentrations (Table 1). Measurement of AMH concentration was performed at a commercial laboratory (Laboklin GmbH & Co. KG, Bad Kissingen, Germany). A GnRH stimulation test was performed according to a published previously protocol (Table 1) [1]. A tentative diagnosis of ovarian remnant syndrome (ORS) was made, and surgical removal was performed. A large, cystic and solid mass located in the right ovary; small retained left ovary encapsulated in the ovarian bursa and the uterine remnant were excised surgically. Histopathological examination revealed a right ovary with multiple cysts and hyperplastic granulosa cell strains in immediate neighborhood of the cysts; a left ovary with multiple primordial, primary, and secondary follicles and multiple regressing corpora lutea; and uterine tissue with cystic endometrial hyperplasia and onset of adenomyosis. Five months after the surgery, the bitch was clinically normal with no vaginal bleeding.

Discussion: Anti-Müllerian hormone is solely secreted by the granulosa cells in female animals; therefore, measurement of AMH concentration is a useful marker to identify dogs with ovaries or ovarian remnants [2]. In the case presented here, GnRH stimulation test resulted in a significant increase in E2 concentration. However, both post-GnRH E2 concentrations were lower than the threshold concentration (6 pg/ml) reported previously in dogs with confirmed ovarian remnant tissues [1]. Therefore, measurement of AMH concentration should be considered in dogs with presumed diagnoses of ORS when serum E2 concentration is less than 6 pg/ml following a GnRH stimulation test. Further studies comparing the specificity and sensitivity of different laboratory tests including AMH, E2, P4 and luteinizing hormone in diagnosing canine ORS are necessary.

P4 (FEIA)		E2 (CLIA)			AMH (CLIA)
On admission	*Two weeks after the 1st measurement*	*Pre-GnRH*	*Post-GnRH; 60 minutes*	*Post-GnRH; 90 minutes*	*On admission*
0.2 ng/ml	0.2 ng/ml	0.8 pg/ml	1.6 pg/ml	1.8 pg/ml	0.1 ng/ml

References:

[1] Günzel-Apel AR1, Buschhaus J, Urhausen C, Masal C, Wolf K, Meyer-Lindenberg A, Piechotta M, Beyerbach M, Schoon HA. Clinical signs, diagnostic approach and therapy for the so-called ovarian remnant syndrome in the bitch. Tierärztl Prax 2012;40(K): 35–42.
[2] Place NJ, Cheraskin JL, Hansen BS. Evaluation of combined assessments of serum anti-Müllerian hormone and progesterone concentrations for the diagnosis of ovarian remnant syndrome in dogs. J Am Vet Med Assoc 2019;254:1067–1072.

95 | Evaluation of uterine/placental echotexture at different pregnancy stages in dogs

Feride Zabitler[1]; Selim Aslan[1]; Isfendiyar Darbaz[1]; Osman Ergene[1]; Sabine Schäfer-Somi[2]
[1]*Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Near East University, Turkey* [2]*Clinic for Obstetrics, Gynecology and Andrology, University of Veterinary Sciences Vienna, Veterinärplatz, Austria*

B-Mode ultrasonography (USG) is the most commonly used method to diagnose pregnancy. B-Mode USG images can be supported by computer-assisted echotexture programs. Parameters such as the gray level, homogeneity, correlation and entropy in the region of interest (ROI) fields selected on the ultrasound images obtained in dogs. Echotexture analysis can provide information about early pregnancy and the course of pregnancy (Scully et al.,2015).

The aim of this study was to to assess normal echotexture features of the healthy canine uterus and placenta in the different gestational periods.

During this study, 151 USG images were obtained from 37 pregnant dogs between day 17 and 62 of gestation and 755 ROI within these images were evaluated. While choosing the ROIs, care was taken to choose regions in the uterus/placenta (early stages) or the placenta (later stages), but not in fetal fluids or external tissues. Selected ROIs were fixed at 100 pixels ("count"), which is 10 × 10 (w*h).

The data were grouped as GI (d 17–25/early implantation), GII (d 26–40/mid-gestation), GIII (d 41–50/post mid-gestation) and GIV (d 51–62/late pregnancy). The mean gray value (MGV), standard deviation for grayscale value (StdDGV), minimum and maximum gray value (MinGV, MaxGV), Modal/Mode gray value (ModGV), median gray value (MedGV), homogeneity (Angular Second Moment, ASM), contrast (CONT), correlation (COR), regional homogeneity (Inverse Difference Moment, IDM) and the entropy (ENT) parameters in the Region of Interest were evaluated.

The difference between groups was determined by applying the Kruskal-Wallis test (non-homogeneous data; Shapiro-Wilk Test), and Mann–Whitney *U* test (SPSS® Statistics 26.0). In case of homogeneous distribution, the One-Way ANOVA (Tukey) test was used. The Pearson's Correlation Test was used for the correlation assessment.

In terms of MGV, there was a significant increase in GII and GIII compared to GI ($p < 0.0001$) and there was a significant decrease towards GIV ($p < 0.0001$). The course of MGV was paralleled by ModGV, MinGV, MaxGV and MedGV ($p < 0.0001$); there was a significant positive correlation between MGV values and ModGV, MinGV and MaxGV, resp. (0.999;0.975;0.990; $p < 0.001$, $p < 0.025$ and $p < 0.01$, resp.); the highest correlation was obtained with MedGV (1.000, $p < 0.0001$).

On the other hand there was a significant increase in CONT in GIII and GIV, compared to GI and GII ($p < 0.05$; $p < 0.001$). There was no difference between groups in terms of ASM, COR and ENT ($p > 0.05$). However, positive correlations between ASM-COR and COR-IDM were assessed (0.997; 0.997, respectively, $p < 0.005$). The highest correlation value was obtained between IDM and ASM (1,000; $p < 0.0001$, respectively). CONT revealed the change between early-middle and late pregnancies.

Conclusion: ROI measurements of the MGV, StdDGV, MinGv, MaxGV and the MedGV parameters reflect the changings caused by placental growth and differentiation, and the increase in vascularization towards mid gestation. The high level of positive correlation, particularly with other sub-MGV parameters (ModGV, MinGV, MaxGV and MedGV) reveals that all grey parameters reflect the changes in these gestational periods. The increasing CONT reflects the changes between postimplantation and late pregnancy. Further investigations are necessary for the establishment of standard values that may be helpful for the early diagnosis of placental alterations.

References:

Scully, S., et al. (2015): Theriogenology 83.3 449–458.

96 | Suppression of oestrus with human oral testosterone supplementation in a bitch: A case report

Cindy Maenhoudt[1,2]; F. Ruiz[1]; Alain Fontbonne[1,2]

[1]*Centre d'Etudes en Reproduction des Carnivores (CERCA);* [2]*l'Unité de Médecine de l'Elevage et du Sport (UMES) de l'Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Paris, France*

Androgens have been used for oestrus suppression in the bitch, but there are few reports about their use for treatment of pathological conditions in the genital tract. Androgens act through negative feedback on the pituitary gland, which prolong anoestrus [1]. Androgen treatment should be started at least 30 days before the onset of the next oestrus and it may be administered for up to two years, with a return to oestrus ranging from 7–200 days after end of treatment. The main androgen used in the USA is mibolerone and this is not commercially available for dogs in France [2].

A 3-year-old multiparous Chow-Chow was presented for medical contraception consultation. The reproductive history included a single puppy pregnancy and the development of pyometra treated successfully medically with aglepristone (Alizine®, Virbac, France) by the referring veterinarian. Transabdominal ultrasonographic examination revealed cystic endometrial hyperplasia (CEH) with intrauterine fluid accumulation and ovarian cysts. A deslorelin implant (Suprelorin® 4.7 mg, Virbac, France) was placed subcutaneously during oestrus and aglepristone treatment was initiated after ovulation. However, three months after the implant administration, the bitch returned to oestrus and the intrauterine fluid had persisted despite being treated with weekly aglepristone injections. The following month, testosterone undecanoate (Pantestone®, MSD, France; 40 mg/day orally until onset of oestrus) was started to prolong anoestrus. Side effects of treatment included weight gain and slight dominant behaviour. Transabdominal ultrasonography revealed no intrauterine fluid accumulation and inactive ovaries. The bitch remained in anoestrus (progesterone <1 ng/ml and anoestrous vaginal cytology) for 10 months before the return of oestrus with an anovulatory cycle.

This case report describes successful oestrus suppression in a bitch with human oral testosterone supplementation. More research is needed to determine if long-term therapy with oral testosterone is safe and effective in dogs.

[1] Romagnoli S, Sontas H. Prevention of breeding in the female. In: BSAVA Manual of Canine and Feline Reproduction and Neonatology, 2010:23–33.

[2] Root Kustritz M.V. Managing the reproductive cycle in the bitch. Vet Clin North Am Small Anim Pract 2012;42(3):423–37.

97 | Oestrous follow-up and pregnancy results after ovulation detection using the FUJI DRI-CHEM IMMUNO AU10V

Cindy Maenhoudt[1,2]; S. Thoumire[1]; Z. Niewiadomska[1]; J. Roos[1]; F. Ruiz[1]; G. Robiteau[1]; Alain Fontbonne[1,2]
[1]*Centre d'Etudes en Reproduction des Carnivores (CERCA)*; [2]*l'Unité de Médecine de l'Elevage et du Sport (UMES) de l'Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, Paris, France*

Timing the day of ovulation is considered as one of the most important factors for optimal breeding time in bitches. Different machines and assays have been introduced in the dog breeding industry. The aim of this clinical study was to evaluate the practical use of progesterone FUJI DRI-CHEM IMMUNO AU10V (FUJIFILM Corporation, Japan) to detect ovulation in bitches from different breeds and to analyze the pregnancy results after artificial insemination.

Sixty-four bitches from 29 different breeds were presented at our consultation for oestrous follow-up and subsequent artificial insemination. The average age was 3.8 ± 1.6 years (2–7.5). In the represented breeds, the schipperke (±4 kg) was the smallest and English mastiff (±80 kg) the largest. Repeated blood samples, in average every 48 h around time of ovulation, vaginal smears and ovarian ultrasound examinations were performed at each follow-up. Progesterone levels were assayed on serum samples (dry test tubes without gelosis) using AU10V. The bitches were bred once or twice by transcervical insemination (TCI) using fresh semen (n = 44-FS) or frozen-thawed semen (n = 20-FTS). Mean progressive motility was 79.8 ± 7.6% and 49.7 ± 13.0% respectively for FS and FTS. Pregnancy diagnosis was performed between 22–25 days post-ovulation. Information regarding parturition date and litter size were obtained either directly from the clients or through the French Kennel Club (https://www.centrale-canine.fr/lofselect). The ethical committee for clinical studies of the ENVA approved the trial. Progesterone levels were in average 2.60 ± 0.83 ng/ml (n = 28) two days before ovulation, 3.83 ± 1.13 ng/ml (n = 31) one day prior, 5.84 ± 1.46 ng/ml (n = 56) at ovulation; 10.37 ± 3.05 ng/ml (n = 39) on the following day and 15.66 ± 3.69 ng/ml (n = 25) two days post-ovulation. TCI was done in average 1.7 ± 0.6 and 3 ± 0.4 days after ovulation respectively for FS and FTS group. In 63.7% (28/44) and 15% (3/20) of the bitches for FS and FTS group respectively, two inseminations were done at 24 or 48 h apart; otherwise only one TCI was performed. Pregnancy rate by ultrasound examination was 97.7% (43/44) in the FS group and 65% (13/20) for FTS. From the 56 pregnant bitches, 52 bitches have already whelped. Natural whelping occurred in 32 bitches at 62.53 ± 1.29 days post-ovulation in average and elective C-section was performed in 20 bitches at least 61 days post-ovulation after the prepartum drop in progesterone. For the FS group, average litter size was 6.9 ± 2.3 puppies (3–12) and for FTS, 6.2 ± 2.9 puppies (3–12).

Progesterone assayed by AU10V is a reliable method to estimate ovulation, since pregnancy length from ovulation to whelping was in accordance with the literature [1]. The overall whelping rate and litter size after TCI was high as has been published, for fresh and frozen semen [2, 3].

[1] Mir F, Billault C, Fontaine E, et al. Estimated pregnancy length from ovulation to parturition in the bitch and its influencing factors : a retrospective study in 162 pregnancies. Reprod. Domest. Anim,2011;46:994-998.
[2] Hollinshead FK, Hanlon DW. Factors affecting the reproductive performance of bitches: a prospective cohort study involving 1203 inseminations with fresh and frozen semen. Theriogenology,2017;101:62–72.
[3] Mason SJ. A retrospective clinical study of endoscopic-assisted transcervical insemination in the bitch with frozen-thawed dog semen. Reprod Domest Anim,2017;52(2):275–280.

98 | Key aspects of domestic cat spermatogenesis

Gobello Cristina
Center of Reproductive Physiology, Faculty of Veterinary Sciences, National University of La Plata & CONICET, Argentina

The knowledge of domestic cat *(Felis silvestris catus)* spermatogenic function is not only useful for breeding and contraceptive purposes buy also for biomedical and wildlife research. At present, most wild felines are listed as threatened or endangered species and domestic cat proved to be a useful model. The aim of this lecture is to provide a detailed and comprehensive review of the normal and abnormal spermatogenic process in domestic cats.

Spermatogenesis is a complex and cyclic process in which a spermatogonia differentiates into a highly differentiated cell: the spermatozoa. This process includes different cell associations in the testicular seminiferous tubules called stages. Thus, the seminiferous epithelium cycle is the sequence of events that occurs from the disappearance of a given stage to its reappearance in a given area of the tubule. In mammals the whole duration of spermatogenesis (i.e. from A1 spermatogonia to spermiation) takes approximately 4.5 cycles (França & Godinho, 2003).

Mean adult domestic cat testis weight is approximately 1.2 g, providing a gonadosomatic index (percentage of testes of the body weight) of approximately 0.08% which is quite low in relation to other mammalian species. Furthermore, a non- significant low- medium correlation (r = 0.36) was observed between testes and body weights in cats (França & Godinho, 2003).

The volumetric rate of testicular parenchyma components also varies among species, mainly the proportion values taken by seminiferous tubules. This parameter is one of the major factors responsible for the efficiency in sperm production in a given species (França & Godinho, 2003). Seminiferous tubules are the main compartment of feline testis occupying 88% of organ parenchyma. This proportion is in the upper level of most mammals and leaves only 12% to the intertubular compartment. Leydig cells occupy 50% of this intertubular compartment, being responsible of the androgens production. The mean tubular diameter and epithelium height in a mature cat are 223 and 81 µm, respectively. The total length of seminiferous tubules

per testis (23 m) and the Leydig cell volume density correlate with testis weight (r = 0.99 and r = 0.80, respectively).

In the domestic cat, eight stages (I-VIII) were described for the seminiferous cycle according to acrosomal development and specific composition and topography of cell generations in a tubule cross-section. These stages were grouped into three spermatogenic phases: premeiotic (stages I-III), meiotic (stage IV), and postmeiotic (stages V-VIII) which concludes the whole process with spermiation of mature spermatozoa into the tubular lumen during stage the VIII. The frequencies of these three phases are 45.5%, 17.6%, and 36.9%, respectively. In the domestic cat, the mean duration of the seminiferous epithelium cycle is 10.4 6 ± 0.3 days, thus the total length of spermatogenesis takes 46.8 days (França & Godinho, 2003).

Normal spermatogenesis is a fine equilibrium between spermatogonia proliferation and germ cell apoptosis. In cats, as well as in other mammals, cell degeneration and loss normally occur during the different steps of spermatogenesis, being its highest level at meiosis. The cat meiotic index (round spermatids produced per pachytene primary spermatocyte) is 2.8 ± 0.3. This result reveals that 30% of cell loss occurs during the two meiotic divisions, which is a mean value among species (França & Godinho, 2003).

Sertoli cells are key elements for normal spermatogenesis. The number of these cells (32.0 ± 3.9 × 106) per gram of testis found for cats is also in the middle range of most mammalian species investigated. Importantly, each Sertoli cell supports a limited number of germ cells in a species-specific manner. Spermatogenic efficiency correlates with the number of germ cells supported by each Sertoli cell. Sertoli cell efficiency, defined as the total number of germ cells or the number of round spermatids per Sertoli cell is 9.8 ± 0.8 and 5.1 ± 0.6, respectively which are one of the lowest among studied mammals (França & Godinho, 2003).

In the feline prepubertal period, the seminiferous tubules are separated by abundant interstitium and the tubular diameter is small (< 100 μm) with no lumen. These tubules show undifferentiated Sertoli cells and one or two spermatogonia (Sanchez et al., 1993). Histologically, puberty could be defined as the occurrence of the first meiotic processes. An increase of seminiferous tubule diameter and lumen as well as of the epithelial height, due to the appearance of more mature spermatogenic cell types, occurs with sexual maturation. In a recent study, feline puberty could be evidenced by computer-assisted image analysis of testicular B mode ultrasonogram. Both testicular parenchyma echogenicity and heterogeneity significantly augmented at sexual maturation. Furthermore, seminiferous tubular diameter showed a strong correlation with testicular echotexture (D'Francisco et al., 2020). Similar findings have been described in other domestic animals.

Photoperiod is the most important physiological environmental cue that may influence feline spermatogenesis. Although increased apoptosis is the routine mechanism involved in feline testicular regression during reproductive quiescence (Prochowska et al., 2021), germ cell desquamation has been recently described in other species. This latter mechanism might also be relevant in cats but it has not been studied so far.

Thus, Kirkpatrick (1985) found higher testicular weights and plasma testosterone in June with the presence of seminiferous sperm throughout the seasons of the year. Spindler & Wildt (1999) did not also find variations in epididymal sperm quality. Axnér & Linde Forsberg (2007) described higher normal spermatozoa from February to July. Blottner & Jewgenow (2007) found a moderate increase of testicular testosterone and seminal quantity and quality in Spring. Siemieniuch (2008) did not find significant apoptotic changes in the germinal epithelium of cats castrated in summer versus winter. Nuñez et al. (2012) obtained better epididymal sperm morphology during winter and spring without annual variation in serum testosterone.

Although these studies were carried out at latitudes in which seasonality should have been expressed, they were carried out in castrated client-owned cats in which the effect of natural photoperiod might have been doubtful. Only one study has been carried out in five cats kept under natural photoperiod (Tsutsui et al., 2009) in which luteinizing hormone and testosterone concentrations were higher during the breeding season in three of the cats with a non - significant superior overall semen quality in two of them.

Several congenital (e.g. cryptorchidism, hypoplasia) and acquired etiologies can alter normal feline spermatogenesis. Thus, testicular inflammation may be caused by trauma or local and systemic infections. Toxins, and certain medications e.g. glucocorticoids, ketoconazol, antineoplasic agents, and sexual hormones can severely affect spermatogenesis. Any testicular immune mediated, nutritional (deficits of vitamin A, riboflavin, linoleate acid), thermal, endocrine or metabolic insult may also cause spermatogenic disruption (Gobello in press).

Evidence of defective spermatogenesis is manifested by seminal teratospermia (> 60% abnormal spermatozoa in an ejaculate e.g. coiled flagellum, microcephalic, macrocephalic, etc) which is considered as a major drawback to fertility. A permanent form of feline teratospermia is attributed to a reduced heterozygosity and it is usually seen in highly inbred wild felids as well as in inbred pedigree or colony domestic cats (Axnér & Linde Forsberg, 2007). This kind of teratospermia presents a higher number of germ cells per Sertoli cell along with a loss of sperm quality. An increased proportion of elongated spermatids at the spermiation stage, a higher frequency of this stage as well as a high number of sperm with cytoplasmic droplets suggest an impaired spermiation process in this teratospermic males (Pukazhenthi et al., 2006).

Recently, abnormalities in feline spermatogenesis could be detected by computer-assisted image analysis of testicular B mode ultrasonogram. Thus, parenchyma echogenicity but not heterogeneity decreased along with the histological reduction in epithelium height and tubular diameter (D'Francisco et al., 2020). Although, fine needle aspiration using small diameter needles (22G) was reported to be safe and reliable, testicular biopsy is the gold standard for spermatogenesis assessment and testicular lesions diagnosis. In practice, biopsy is rarely carried out because of concerns about complications i.e. inducing immune intolerance by disruption of the blood-testis barrier. Thus, there is scare histological knowledge about the typical feline abnormal spermatogenic patterns.

Mild androgen deprivation during spermatogenesis is often manifested by the retention of mature spermatids without spermiation. Conversely, severe androgen deprivation experimentally induced by the administration of a potent GnRH antagonist caused diminution of the germinal epithelium height at expense of spermatocytes, spermatids and spermatozoa scarcity. An increase in tubular cellular debris was also found in the tubular lumen. Thus, germ cell development is apparently arrested at the spermatogonia level, which is the major site of disruption of spermatogenesis when gonadotrophin suppression is carried out in humans.

When the Sertoli cell toxicant, RTI-4587-073(I), was administered to tom cats the seminiferous epithelium rapidly vacuolized and sloughed into the tubular lumen causing severe tubular disorganization. Furthermore, multinucleated giant cells were found both in the germinal epithelium and semen. These cells arose from fusion of exfoliated spermatids with abnormal intercellular bridges (D'Francisco et al., 2019). A similar disruption of Sertoli - germ cell adhesion might also occur during feline seasonal regression.

It is concluded that a) In spite of the low gonadosomatic index and Sertoli cell efficiency, due to the high seminiferous tubule volume density and the relatively short spermatic cycle length, domestic cat spermatogenic efficiency can be considered moderate among mammals b) According to the existing reports it seems that, in the domestic male cat, seasonality is moderate to null. c) Microscopic interpretation of infertility requires a deep understanding of the spermatogenic process as well as the species response to disruptors. Changes in the composition of the spermatogenic cell population affect testicular echotexture. Testicular ultrasonography combined with computer assisted image analysis of testicular parenchyma is a potential alternative to more invasive or indirect methods of assessing testicular function, such as biopsy or semen evaluation.

References:

Axnér, E., & Linde Forsberg, C. (2007). Sperm morphology in the domestic cat, and its relation with fertility: a retrospective study. Reproduction in Domestic Animals. 42(3),282-291

Blottner, S., & Jewgenow, K. (2007). Moderate seasonality in testis function of domestic cat. Reproduction in Domestic Animals, 42(5),536-540.

D Francisco, F., Lapuente, C., Merlo, M.L, Barbeito, C., & Gobello, C. (2020). Feline testicular ultrasonogram differentiates pre vs. postpubertal and normal vs. disrupted spermatogenesis. Theriogenology. 157,503-507. doi:10.1016/j.theriogenology.2020.08.012.

D'Francisco, F., Merlo, M.L., Vercellini, R., Blanco, P., Barbeito, C., & Gobello, C. (2019). Effect of the indenopyridine RTI-4587-073 (I) on feline testicle. Animal Reproduction Science, 205,10-17. doi: 10.1016/j.anireprosci.2019.03.014.

Gobello, C. (2022). Causes of infertility in the tom. In: A Johnson & M Kutzler (Eds) Feline Theriogenolgy CAB International Wallingford United Kingdom (in press)

Kirkpatrick, J.K. (1985). Seasonal testosterone levels, testosterone clearance, and testicular weights in male domestic cats. Canadian Journal of Zoology, 1285-1287

Prochowska S, Partyka A, & Niżański W. (2021). Expression of Apoptosis-Related Genes in Cat Testicular Tissue in Relation to Sperm Morphology and Seasonality-A Preliminary Study. Animals (Basel), 12,11(2),489

Pukazhenthi, B. S., Neubauer, K., Jewgenow, K., Howard, J., & Wildt, D. E. (2006). The impact and potential etiology of teratospermia in the domestic cat and its wild relatives. Theriogenology, 66(1), 111-121. doi: 10.1016/j.theriogenology.2006.03.02

Sanchez, B., Pizzaro, M., Garcia, P., & Flores, J.M. 1993. Postnatal development of seminiferous tubules in the cat. Journal of Reproduction and Fertility, Suppl 47,343-348.

Siemieniuch, M.J. (2008). Apoptotic changes in the epithelium germinativum of the cat (Felis catus domestica, L. 1758) at different ages and breeding seasons. Reproduction in Domestic Animals, 43(4),473-476.

Tsutsui, T., Onodera, F., Oba, H., Mizutani, T., & Hori, T. (2009). Plasma hormone levels and semen quality in male cats during non&breeding and breeding seasons. Reproduction in Domestic Animals, 44 Suppl 2,291&293. doi: 10.1111/j.1439&0531.2009.01449.x.

99 | Evaluation of short-term safety of ultrasound guided foetal fluid sampling in the dog (*Canis lupus familiaris*)

Smadar Tal[1]; Sebastian Patrick Arlt[2]

[1]*Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel* [2]*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany*

Introduction: In humans, analysis of amniotic fluid is widely used for diagnostic and prognostic purposes. Amniocentesis has scarcely been used in veterinary medicine to date, yet may have tremendous potential for clinical and research applications in dogs. The aim of our study was to establish a method of foetal fluid sampling in the bitch, as well as to assess safety aspects of the methodology. We hypothesized that as a result of foetal fluid sampling, signs of injury will be detected macroscopically on the uterine surface, allantoic and amniotic sacs, or conceptus's skin, in less than 10% of puncture sites in the uterus.

Approach: Foetal fluid collection procedures were performed on a cohort of 21 healthy intact pregnant bitches. Gestational age was assessed by measuring biparietal head diameters (BPD) of fetuses using ultrasonography. Two transabdominal ultrasound guided methods were assessed: the "free hand" and the needle-guided bracket sampling. Both techniques were used on most bitches. In addition, through a subsequent routinely scheduled ovariohysterectomy, fluid was directly collected. Samples from 98 conceptuses were collected at day 46.7 ± 7.5 of pregnancy.

Results: The mean number of conceptuses sampled per bitch (n = 21) was 4.7 ± 2.1 (range 1–8 conceptuses). Fetal fluid from 40 fetuses was collected using the free hand technique, and from 58 fetuses it was collected using the needle guided method. Fetuses sampled were located on both the left (n = 37) and right (n = 46) uterine horns,

and in the uterine body (n = 11). The amount of fluid retrieved varied between 0.5 and 5.0 milliliters per collection. Macroscopic examination of the uterus and conceptuses identified 53% of the puncture sites. Neither fluid leakage nor foetal injury was detected, and six hematomas (5.8%) were visible.

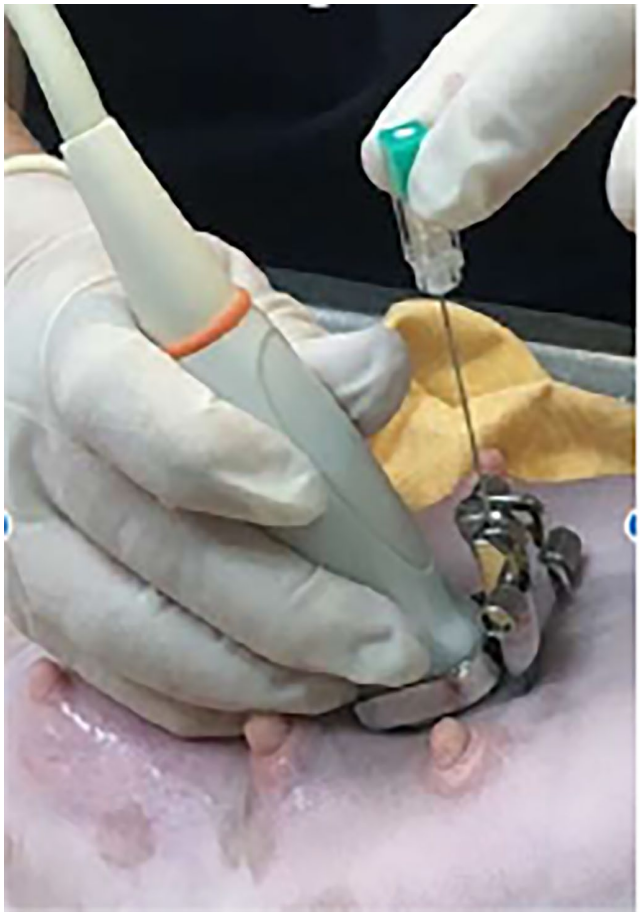
Ultrasound guided foetal fluid collection was found to be potentially safe, and can be performed by using either transabdominal method.

Interpretation: The main conclusion from this study is that percutaneous foetal fluid collection between days 35 and 62 from ovulation via centesis causes only little short-term damage to the uterus, the extraembryonic membranes and the fetus. The same outcomes were gained for amounts of foetal fluid retrieval, and the incidence of injuries was low for both ultrasound- guided procedures.

In accordance with our hypothesis, we can confirm that in less than 10% of sampling punctures in the uteri and conceptuses, clinically relevant signs of injury were detected.

It is possible that microscopic injuries occurred as a result of the centesis, and were not identified. For these it can be assumed that they would not have been of clinical relevance.

Further studies, with a larger cohort and a follow-up to an examination of newborn puppies postpartum are required in order to evaluate the possible long-term effects of foetal fluid centesis procedure on the canine pregnancy, and on the sampled offspring. Pending future studies on long term safety, the procedure may be considered as a routine method, with minimal injury.



100 | Infectious diseases update: Canine brucellosis

Tayse Domingues de Souza

Pontifical Catholic University of Chile, Santiago, Chile. Email: taysedomingues@gmail.com

Canine brucellosis is an insidious reproductive disease related mostly to infertility and perinatal mortality. Widespread and underdiagnosed in many countries, in most continents, the Gram-negative intracellular coccobacillus *Brucella canis* is the species adapted to dogs, although *B. suis*, *B. melitensis*, and *B. abortus* can also infect canines exposed to hunting or cattle contact [1,2].

B. canis infection remains a diagnostic challenge despite the advances in available tests, mostly due to its immunopathogenesis in dogs [3,4]. The increasing awareness about its economic impact in dog breeding [1,5], its zoonotic relevance [1,6,7], and the availability of different diagnostic methods and protocols [3,4] has shed more light on canine brucellosis in the recent years.

In Europe, epidemiological evidence indicates circulation of *B. canis* in canine population for many years [1]. A retrospective study analysed the database of a European veterinary laboratory, between 2011 and 2016, and identified *Brucella*-positive dog samples from 14 European countries, with 3.7% (61/1,657) and 5.4% (150/2,764) positivity in PCR and serology, respectively [4]. Outbreaks in Europe have been documented due to international traveling of dogs [8], while appropriated regulations are yet to be implemented in most countries.

Brucellosis is a highly transmissible disease. Infection occurs vertically and horizontally, through the placenta, mucosal contact with or ingestion of secretions (vaginal, semen, milk, urine) or blood from bacteraemic dogs [1]. In many cases, dogs are congenitally infected, the birth of infected and healthy puppies in the same litter is not uncommon, maintaining the infection in the breeding stock [9]. After an initial period of bacteraemia, that can last weeks to months, *B. canis* may establish in distinct organs and induce lymphadenopathy, prostatitis, scrotal dermatitis, epididymitis, metritis, placentitis, abortion, myocarditis, and, mostly in neutered dogs, discospondylitis, ophthalmitis, and meningitis [1,2,4,8,9,10].

In chronic infections, antibody levels increase, what correlates with a decrease in bacteraemia. As a stealth pathogen, *B. canis* can persist in dogs, inside macrophages, for months or years, with intermittent bacteraemia and shedding of the agent through secretions. In many cases, the infection with *B. canis* may go unnoticed. As documented in experimental infection, dogs do not even present fever, what contributes to lack of suspicion, underdiagnose, and the persistence of the bacteria in the dog population. *B. canis* evades immune system in many ways, what has direct implication in diagnosis, with frequent false negative results in different serology tests, while the nonbacteraemic animals will present false-negative results in PCR and blood culture [1,3].

Infected dogs usually develop lymphadenopathy, with most of the clinical signs being highly unspecific, as a dull coat, exercise intolerance, backpain, uveitis, and splenomegaly. Stud dogs may develop

sperm cells defects as a consequence of *B. canis* infection and sperm-autoantibody production, with agglutination of sperm heads in cytologic smears and fresh semen preparations. Chronic prostatitis, scrotal dermatitis, epididymitis, are also common, with or without orchitis, which tend to develop into testicular atrophy [1,10].

In bitches, infection may lead to variable vaginal secretion, embryo resorption, abortion, late fetal death, stillbirth, and birth of infected neonates, which may seem healthy or not. *B. canis* is usually present in large amounts in placenta and may be absent from foetal tissues, when abortion/foetal death happens due to placenta insufficiency, before foetal infection occurs. Although recognized as the most important aetiology in canine abortion, most *B. canis*-positive pregnancies do not develop into abortion, instead, they will progress to whelping, with the delivery of live puppies amongst stillborn, usually in different degrees of autolysis. Asymptomatic infections do occur [1,3].

In canine stillborn and neonates, varying amounts of *Brucella* organisms were revealed by immunohistochemistry (IHC) in many tissues, mainly along intestinal mucosa, suggesting that oral infection through ingestion of amnion or milking possibly plays an important role in canine brucellosis. Surprisingly, infection with *B. canis* was also immunodetected in neonatal testicles, epididymis, and uterus. In many cases, *B. canis*-positive canine foetuses and neonates were coinfected with opportunistic bacterial agents [9], possibly a consequence of *Brucella* interference with the host immune mechanisms [1].

Diagnostic approach requires the association of history, clinical signs, and a combination of laboratory tests to be accurate. Bacterial isolation is the "gold standard" proof of infection, but *B. canis* may take a few weeks to grow in selective culture media and poses serious biohazard to laboratory workers, requiring high biosafety level facilities

101 | Cell-cell interaction in the parturient canine uterus is altered in uterine inertia compared to obstructive dystocia

Lea Magdalena Rempel[1]; Iris M. Reichler[2]; Orsolya Balogh[2,3]; Sandra Goericke-Pesch[1]

[1]*Reproductive Unit – Clinic for Small Animals, University of Veterinary Medicine Hannover Foundation, Hannover, Germany, Germany* [2]*Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Switzerland* [3]*Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, USA*

Uterine inertia, the lack of functional uterine contractions, is the most common cause of dystocia in the parturient bitch and negatively affects maternal and foetal health. Gap and tight junction proteins play an important role in cell-cell communication and are involved in myometrial contractions. We hypothesized that bitches diagnosed with primary uterine inertia (PUI) have altered uterine expressions of Connexin 26 (Cx26), Connexin 43 (Cx43), Claudin 3 (CLDN3), Claudin 11 (CLDN11), Zona occludens-1 (ZO-1), Junctional Adhesion Molecule A (JAM-A) and Occludin (OCLN) compared to bitches suffering from obstructive dystocia (OD). Uterine tissue samples from interplacental (IP, n = 19) and uteroplacental sites (UP, n = 8) were obtained from bitches during medically indicated Cesarean sections. Grouping of dogs was as described earlier (Rempel et al. 2021, Cell Tissue Res. 385, 251). Mean age of bitches presented with PUI and OD was 4.7 ± 1.9 and 3.5 ± 2.0 years, respectively, and mean current pregnancy number was 2.9 ± 1.2 (PUI) and 1.8 ± 0.8 (OD). RT-qPCR was done using whole tissue homogenates and primers specific for canine Cx26, Cx43, CLDN3, CLDN11, ZO-1, JAM-A and OCLN. GAPDH and ß-actin were used as housekeepers. Immunohistochemistry (IHC) was performed for Cx26, Cx43, CLDN11, ZO-1 and JAM-A. Statistical analysis using GraphPad Prism v. 7.02 (GraphPad Software. Inc., San Diego, CA, USA) was performed to compare mRNA expression between PUI (n = 11) and OD (n = 8) in IP, and localizations (IP versus UP, PUI/OD summarized), and $p < 0.05$ was considered statistically significant. Additionally, PUI was subdivided according to litter size, i.e. small (PUI-S, n = 4, qPCR/5, IHC), normal (PUI-N, n = 4) and large (PUI-L, n = 3), with normal being defined as within ± 1 standard deviation (SD) of breed average. Gene expression among PUI subgroups and between PUI-N and OD were compared. Expression of Cx26 ($p < 0.01$), Cx43 ($p < 0.001$), CLDN11 ($p < 0.01$), ZO-1 ($p < 0.01$), JAM-A and OCLDN ($p < 0.0001$ each), but not CLDN3 differed between PUI and OD. Comparing PUI-S/N/L, a significant difference was found for ZO-1 ($p < 0.05$). Comparing PUI-N and OD, expressions of Cx26, CLDN11, JAM-A and OCLDN ($p < 0.05$ each) differed. Comparing IP and UP, only JAM-A expression ($p < 0.05$) was different. IHC revealed specific immunopositive signals for all parameters studied. Among other cell types, the myometrial smooth muscle cells of the longitudinal and circular layers stained immunopositive. Our results indicate that the investigated gap and tight junction proteins are critically involved in cell-cell communication and their altered expression in PUI points to a role of all except CLDN3 in the etiology of PUI in our samples, likely by altered expression in the myometrium.

This study was supported by Agria and SKK Research Foundation for companion animals (grant no. N2014-0002).

102 | Perils and pitfalls of research of effects of neutering of dogs

Sebastian P. Arlt

Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany

Frequently, dog owners ask their vets for counseling regarding pros and cons and optimal time for neutering of female dogs. This task is complex and should include recent and valid research data (1).

In the past decades, several case reports, observations from different countries such as the United States or Sweden with different habits or legal regulations – i. e. very frequent early neutering vs. almost no neutering – and cohort studies have been published. Cohort

studies are prospective or retrospective and usually compare an exposed with an unexposed group (cohorts). The outcome is measured after a specific time. For example, one could assess the incidence of a disease in a cohort of neutered dogs compared with a cohort of intact dogs.

An advantage of neutering a female dog is that ovarian diseases and metropathy do not occur anymore. In addition, some studies have shown that early neutering reduces the risk of mammary neoplasia to a certain extent, even if the scientific basis for this observation is weak. The effect might be smaller than some older publications suggest (1).

Disadvantages of neutering female dogs include urinary incontinence, a higher risk of different types of neoplasia and musculoskeletal disorders. Some of these phenomena might be based on hormonal changes. It has been shown that GnRH and FSH and LH are upregulated in neutered dogs because of the missing negative feedback from the gonadal steroids.

Overall, the scientific evidence needs to be improved. In many studies small numbers of animals are used which impedes the generalizability of the findings. Often incomplete data about the dogs is presented. A lot of research findings are derived from necropsies during which it can be defined if a dog is neutered or not but often nothing is known about the age at neutering or other aspects of the reproductive and medical history.

In many studies comparability of the animals of a neutered and an intact cohort may not be given. For example, a data analysis of 2.2 million dogs has been published, showing that neutered dogs become in average more than two years older (2). Sadly, the authors did not take potential confounders into account. Therefore, the presented differences in the life span may more rely on the living conditions of the dogs rather than on the neuter status. It can be assumed that the neutered dogs were living with a family since their puppy age under good medical care including vaccinations, deworming, neutering and health surveillance. The intact dogs may be kennel or stray dogs, which have never been taken to a vet and maybe even do not get food of sufficient quantity and quality.

In some cases it may be difficult to judge retrospectively, which was cause and which was effect. For example neutering might lead to a higher risk of cruciate ligament ruptures (CLR). On the other hand, breeders might let their vets neuter a dog after a diagnosis of CLR because this dog is not suitable for breeding anymore.

In research also breed specific aspects need to be considered, especially when it comes to incontinence or other diseases with a well know breed specific incidence. Finally, potential confounders may influence each other. For example, if neutered dogs become obese, this may increase the risk for musculoskeletal disorders or other diseases.

Conclusion: Interpretation of research data on the effects of neutering dogs is difficult. If we consider specific questions and read new publication, we need to assess if the data and conclusions given are really objective and robust.

References

1. Arlt et al. (2017): Theriogenology 79:9. 1278-1283

2. Banfield Group (2013): https://www.banfield.com/Banfield/media/PDF/Downloads/soph/Banfield-State-of-Pet-Health-Report_2013.pdf