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003 | What happened when I (neutered male dog) ate my owner's progesterone capsules

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Introduction: My name is "Leo" and I am a 10 years old Petit Basset Griffon. I was neutered in 2011 at the age of three years. According to my owner, I am a friendly but crazy dog because I tend to eat everything I find.

Case history: On a Saturday in November 2018, I found a blister pack with Famenita 200 mg progesterone capsules (Exeltis Germany GmbH, Ismaning, Germany) of my owner and ate 12 capsules. I cannot remember how they tasted, but I ingested in total 2400 mg progesterone. Usually my owner takes one 200 mg capsule per day against menopausal problems. Besides progesterone, the capsules contain saflor oil, gelatine, glycerol, and titandioxide.

My owner discovered the empty blister package and took me to the vets 3 days later. I was in good general health condition. The vets took a few blood samples because they wanted to know my progesterone concentration and if my organs such as the liver were able to cope with the progesterone. Progesterone concentration was measured with Immulite Progesterone Assay (LKPW), Siemens, Berlin, Germany.

Results: The first sample taken three days after progesterone ingestion revealed a progesterone concentration of 4.7 ng/mL and a slight increase of lipase and urea, and a low iron concentration. In addition, the proportions of monocytes (6%) and eosinophil granulocytes (16%) were increased. If these changes of chemical compounds and cells were a result of the progesterone ingestion, remains open. All other parameters were within the reverence levels.

Another blood sample taken seven days after progesterone ingestion showed a progesterone concentration of 1.16 ng/mL.

The vets took a third blood sample eleven days after progesterone ingestion. Progesterone concentration was below 0.2 mg/mL. Some parameters such as alkaline phosphatase, GLDH, g-GT, ALT and AST were slightly increased compared to the first blood sample, but remained within reference levels. I still was in good general health.

Conclusion: Unfortunately, my owner did not present me to the vets in the first hours and days after progesterone ingestion, so that they could not determine my progesterone concentrations right after ingestion. They think it might have been quite high. My body was obviously able to metabolize and excrete progesterone within a few days. My owner said she had not observed any clinical side effects.

The vets said that one report about a cat that showed signs of sedation after a potential progesterone intoxication had been published (1). In addition, some authors who studied the effect of high progesterone doses on humans concluded that modified sleep patterns (2) and a sedative and anxiolytic effect are possible (3).

I cannot say that all other dogs are as cool as I am, but at least I experienced no visible side effects. Let's see what I will try next .

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005 | Deslorelin implant to induce temporary infertility in tomcats

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Introduction and aim: The main reasons to induce temporary infertility in tomcats are undoubtedly unwanted urine marking, vocalization, other behavioural changes and poor appetite. Suprelorin® is a hormone implant used to induce temporary infertility. The implant contains deslorelin, a synthetic gonadotropin-releasing hormone agonist seven times more potent than natural GnRH. In cats all use of Suprelorin® is off-label, but the implant has been used in both males and females quite extensively. It seems to be very promising method for controlling domestic cat reproduction, but so far all the research has been done in laboratory environment and with quite small n-numbers. Deslorelin is mostly used by cat breeders wishing to remove a cat from breeding temporarily, for cats with increased anesthesia risk or in areas without access to surgical facilities.

Materials and methods: Study was done as an online survey to cat owners who have used the implant on their tomcats. Survey was distributed through different cat breeder groups and organizations.

Results: In total 486 answers were given from 28 countries and 31 different breeds. 97% of the answers came from Europe. Average age at implantation was 24.1 ± 14.6 months. 70.8% of the cats had offspring before the implant and 66.5% of the cats had offspring after the implant. Only 74.5% of all the cats were actually used in breeding after the implant and 89.2% of those cats got offspring after the implant. On average the effect lasted for 14.8 ± 6.0 months (no

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statistical difference between 4.7 mg and 9.4 mg implants, 14.5 ± 5.6 vs. 17.8 ± 8.5 respectively). Some of the cats were neutered after the implant due to excessive urine marking before fertility returned and some of the cats were still unable to produce kittens 4 years after the implant was inserted. The implant significantly reduced ($p < 0.0001$) urine marking, vocalization, aggressiveness, restlessness and weight loss. Smell of urine decreased in 87% and size of testicles decreased in 91% of the cats. Weight gain during the implant was reported in 77.5% of the cats. The implant had no observable effect in 2.7% of the cats and 19.5% showed mating activity towards females in heat even without any other signs of stud behaviour. 5.3% of the cats got offspring during the time when no stud behaviour was shown. In 2 cases deformed offspring were born shortly after the implant, but later one of them got normal offspring again. 30% of the cats had 2 implants and in 45.9% of the cats the effect lasted same as first one, in 17% of the cats the effect lasted longer than the first and in 27.4% the effect ended earlier. 93% of owners had positive overall experience of the implant.

Conclusions: In conclusion, the implant is easy to use and it can be used to induce temporary infertility in tomcats. However, the implant is not 100% effective and even if they don't show any stud behaviour, tomcats with the implant should be kept separated from females they are not allowed to mate.

006 | Feline testicular ultrasonogram before and after spermatogenic arrest

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Introduction and aim: Two-dimensional ultrasonography has been widely used in the male andrological exam. The availability of high-frequency and resolution equipment permits the accurate evaluation of the cellular composition of a tissue. Additionally, digital image analysis supports ultrasound findings to be an objective, quantifiable tool. Thus, echogenicity i.e. pixel intensity is described in terms of numerical values which range from 0 (absolute black) to 255 (absolute white) and heterogeneity in terms of pixel standard deviation of a tissue (1). These evaluations have been mainly carried out in farm animals' testes (2). Fine tuning of this technique in feline species may contribute to the improvement of cat reproduction. To describe the normal and abnormal feline testicular ultrasonogram findings, a pharmacological model of spermatogenic arrest was used. For this purpose a potent gonadotrophin releasing hormone (GnRH) antagonist (acyline), for which deleterious effects on feline spermatogenesis have previously been described (3), was selected as the disruptor.

Materials and methods: Five, 1 to 4 years old, cross-bred fertile cats were administered acyline 330 μg/kg SC once a week for 4 consecutive weeks. Testicular ultrasound evaluations of these cats were carried out one week before treatment (Pre) and one week after the last antagonist administration (Post) were undertaken by a single experienced evaluator using a real time B-mode ultrasound machine (Toshiba Nemio XG, Japan) with a 14 MHz lineal transducer. All machine settings were established at the first examination according to best image quality and remained unaltered for all remaining examinations (Gain: 100, focal depth: 2 cm). Acoustic gel was applied to the transducer and coupled directly to the clipped scrotum with minimum pressure to obtain the images. The testes were imaged in the longitudinal and transverse planes and dimensions were obtained from frozen images, using the ultrasound callipers. Total testicular volume was also calculated as described by Linn et al. (2009; 4). In the frozen digital images (jpg of 640 × 480 pixels) of the longitudinal sections, 3 regions of interest (ROI, 1 mm^2) were selected, between the central mediastinum and the testicular capsule (12 ROI/cat). Within each ROI the echogenicity and the heterogeneity of the testicular parenchyma were analyzed using Image J software (5, National Institutes of Health, Bethesda, Maryland, USA. Pre and Post ROI values as well as testicular volume were compared by a paired t test after normality was confirmed by Kolmogorov and Smirnov test. The level of significance was set at $p < 0.05$.

Results: Testicular volume (cc; 1.03 ± 0.09 vs. 0.86 ± 0.1; < 0.05) and parenchyma echogenicity (89.72 ± 12.90 vs. 84.70 ± 12.6; $p < 0.05$) but not its heterogeneity (13.97 ± 2.12 vs. 14.01 ± 2.33; $p > 0.1$) diminished after the GnRH antagonist treatment.

Conclusions: Quantitative analysis of ultrasonographic images of the cat testis could become a potentially useful tool in the diagnosis of spermatogenesis disruption. Further studies are being conducted.

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007 | Acute gangrenous mastitis in the bitch with clinical signs of sepsis

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Introduction and aim: Acute mastitis in a dam is the most common and severe post-partum inflammation of mammary glands. The disease is usually of bacterial origin occurring early in lactation and sporadically in the weaning process or pseudopregnancy (1). Clinical signs vary from mild local inflammation and local skin changes to

a debilitating acute condition with sepsis. This case report aims to describe gangrenous mastitis in a lactating dam as a cause of sepsis, alert veterinarians to the importance of early mastitis treatment and sepsis recognition and management, and to discuss a snakebite as a possible additional explanation for gross pathological changes in the inguinal mammary glands.

Clinical case: A 4-year-old primiparous Belgian Tervuren female was referred in lateral recumbency after acute onset of clinical signs. The dog experienced cardiac arrest and was resuscitated immediately. Afterwards, the pulse rate was 170 bpm, occasionally barely palpable. Electrocardiogram monitoring showed frequent supraventricular extrasystoles in addition to tachycardia. The main arterial blood pressure was 65–68 mm Hg but increased gradually the following day. Respiration was rapid and superficial. Mucous membranes were pale. Body temperature was 38.8–41.0°C. Diffuse gangrenous skin lesions were observed with most prominent changes in the inguinal mammary glands area. All the affected glands expressed yellowish to sanguineous changes in the colour of the milk. Microbiological examination of acquired midstream milk samples revealed a pure culture of haemolytic *Escherichia coli (E.coli)* and acute gangrenous mastitis with sepsis was diagnosed. Microscopically, we identified bacteria intra- and extracellularly with neutrophils and macrophages. The dam partially recovered after several days of intensive care and repetitive surgical removal of necrotic skin and subjacent tissue. Nevertheless, considering animal welfare, euthanasia was indicated 4 weeks after the disease onset due to irreversible proliferation and erosions in the oesophageal mucosal layer, with a subsequent stricture in the distal part of the oesophagus. In our own case, observation of the snake in the garden queries possible venomous snakebite interference.

Discussion: This case reports gangrenous mastitis interfered with sepsis. Gangrenous mastitis in the bitch is usually a consequence of inadequate acute mastitis treatment (1). Albeit rare, it can trigger sepsis. Microbial agents frequently isolated from septic milk are staphylococci, *E.coli* and streptococci (2, 3). When the immune system omits to provide an adequate response to a microbial trigger, a cascade of clinical signs better known as systemic inflammatory response syndrome (SIRS) occurs. Gram-negative enteric bacteria are the best-described and most common cause of sepsis (4). Although sepsis is a potentially life-threatening complication to different infectious diseases in a dog including mastitis, interference of these two conditions has been sparsely described in the literature. Described oesophageal changes could be attributed to a complication of anaesthesia induced for gland drainage attempt but also to the fact that sepsis may lead to clinically significant upper gastrointestinal bleeding (5).

Keywords: Mammary gland inflammation, sepsis, SIRS, canine, lactation, dam.

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008 | Anti-Müllerian hormone in young queens: Preliminary report

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Introduction and aim: Anti-Müllerian hormone (AMH) is a glycoprotein belonging to the transforming growth factors (TGF-β). In females, AMH is secreted by granulosa cells of small follicles in the ovary. Anti-Müllerian hormone serum concentrations have shown to represent the ovarian follicular reserve and the response to stimulation in assisted reproductive technologies in women, laboratory and production animals. Evaluation of AMH might also be useful for diagnosing of gonadal pathologies and response to contraceptive treatments. Up to the authors' knowledge, only 4 investigations of AMH have involved female domestic cats. Half of them have been carried out to demonstrate that AMH is useful to distinguish intact from gonadectomized cats (1,2), one to describe abnormal concentrations in a case of granulosa cell tumor (3) and other to predict the success of oocyte *in vitro* maturation (4). Nothing is known about the normal reference interval during reproductive "prime", the effect of the estrus cycle, body weight, age or photoperiod on AMH in the female cat. It was, therefore our objective to describe these aspects of AMH in young queens.

Materials and methods: Twenty-two, post-pubertal, 7 to 48 months old (mean 19.9 months), short-hair intact female cats were included in this study from June 2018 to March 2019 (9.8 to 14.30 light hours/day). The queens were healthy, privately owned and kept under outdoors- indoors home environments in the city of La Plata (34° South latitude and 57° West longitude), Argentina.

A single blood sample was collected from the jugular vein. Serum was stored frozen at −70°C until AMH and progesterone (P4; Elecsys Progesterone III, Roche Diagnostics, Mannheim, Germany) analysis. For AMH, an electrochemiluminescence immunoassay, Elecsys®, Cobas, Roche Diagnostics International Ltd., Switzerland) was used according to the manufacturer instructions. The sensibility and the

intraassay CV of the kit were 0.01 n/mL and < 5%, respectively. The stage of the estrus cycle (follicular phase, luteal phase or anestrus) was determined by observation of sexual behavior, vaginal cytology and P4 serum values.

Normality of AMH serum values was confirmed by Kolmogorov and Smirnov test. Anti-Müllerian hormone concentrations were statistically described and analyzed by analysis of variance including the main effects of estrus cycle, body weight, age and light hours per day. The level of significance was set at $p < 0.05$.

Results: Mean ± SD AMH serum concentrations were 5.43 ± 2.58 ng/mL with a range of 1.62 to 11.61 ng/mL. Similar mean values (9.27 ng/mL and 4.3 ng/mL for queens younger and older than 12 months, respectively) were described in a previous study using the same hormone assay (4). No effect of estrus cycle, body weight, age and light hours per day could be detected in this preliminary study in young queens ($p > 0.1$).

Conclusions: In spite of the quite homogenous group of cats, a large variability in AMH serum concentrations was found among these female cats. Further work in larger populations of queens is necessary to unveil the factors that affect AMH values during female reproductive "prime".

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010 | Comparison of the quantification of membrane-intact canine spermatozoa after a fluorescent computer-assisted spermatozoal quantification method, manual counting after nigrosin-eosin staining and manual counting after CFDA-PI staining

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Introduction and aim: The aim of this study was to compare the assessment of membrane integrity in canine spermatozoa using a newly developed and recently described method of fluorescent computer-assisted spermatozoal quantification (CASQ) after propidium iodide (PI) staining with manual counting after nigrosin-eosin (NE) staining, and after fluorescent staining with 6-carboxyfluorescein diacetate (CFDA) and PI.

Materials and methods: Firstly, CASQ was used to count membrane-disrupted spermatozoa (MDS) in both an untreated sample and a completely membrane-disrupted sample as described previously (1). The percentage of membrane-intact spermatozoa (MIS) in the sample was then calculated: (Total count − Untreated sample count) ÷ Total count × 100. Secondly, spermatozoa were stained with one-step NE stain (Society for Theriogenology), then at least 100 spermatozoa were manually examined under x1000 magnification and classified as MDS (stained with eosin) or MIS (non-stained). Thirdly, spermatozoa were stained with CFDA/PI stain using previously described methods (2), then at least 200 spermatozoa were manually examined under x1000 magnification and classified as MIS or MDS. Each spermatozoon was classified as membrane-intact only if it was stained completely green. The spermatozoon was classified as membrane-disrupted if it had been stained red with PI. Analysis was performed on both fresh (n = 6) and thawed cryopreserved (n = 102) canine semen. The CFDA/PI technique was assumed to be the most reliable technique and therefore data was subsequently analysed to measure the agreement between the CASQ and NE methods with the CFDA/PI technique using Bland-Altman methodology. Data were analysed together and subdivided into samples with ≥85% MIS and <85% MIS.

Results: The mean MIS% was similar (<3.6% difference) for the CASQ and CFDA/PI methods for all samples, and when the MIS% was ≥ 85% in the NE technique. The MIS% was higher for NE compared to CFDA/PI techniques in pooled samples and in samples with a MIS% <85% (Table 1). The agreement between methods was reasonable (within ± 16.5%) when the MIS% was ≥ 85% (Table 1). The agreement was unsatisfactory (>±25%) when the MIS% was < 85% (Table 1).

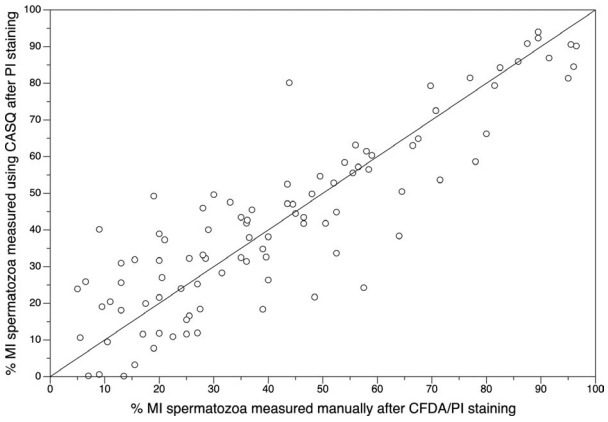

Figure 1. Percentage of membrane-intact spermatozoa using manual counting after CFDA/PI staining and using CASQ after PI staining, with a line of equality.

Conclusions: The generally higher MIS% seen with NE methodology compared to fluorescent staining at MIS% <85% has been seen previously (1, 3). A similar lack of agreement at low MIS% between a NucleoCounter® SP-100 (which uses PI staining) and a SYBR-14/PI method has been seen in stallions (3). Both the CASQ and CFDA/PI methods had similar mean %MIS, and variations between the two techniques appeared to be spread evenly

(Figure 1). Therefore, it may be possible that the agreement between the two methods can be improved with further refinement of the CASQ method. However, it should be remembered that the CFDA/PI and the CASQ methods may be performing two different assessments on the cell membrane so the results may always differ. All three methodologies give similar results at high MIS% (>85%) but not at MIS < 85%. Therefore, when reporting the percentage of MIS, it is very important to specify which methodology was used.

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TABLE 1. The mean difference and agreement of membrane-intact spermatozoa (MIS) measured with CASQ and nigrosin-eosin staining compared to manual counting after CFDA/PI staining.

	CASQ		Nigrosin-eosin	
	Mean % of MIS (Mean ± SE)	Limits of agreement (95%)	Mean % of MIS (Mean ± SE)	Limits of agreement (95%)
All samples	2.2 ± 1.3 (n = 100)	−24.2 to 28.5	−9.6 ± 1.9 (n = 108)	−48.4 to 29.1
Samples ≥ 85% MI	3.3 ± 2.2 (n = 9)	−9.7 to 16.4	−3.5 ± 2.0 (n = 9)	−15.7 to 8.7
Samples < 85% MI	2.1 ± 1.4 (n = 91)	−25.3 to 29.4	−10.2 ± 2.0 (n = 99)	−50.3 to 29.9

011 | Comparison of the quality of cryopreserved canine semen after thawing into an isotonic standard thaw medium and into the original hypertonic freezing medium

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Introduction and aim: The aim of this study was to determine whether reducing hypoosmotic shock at thawing would improve the post-thaw quality of cryopreserved, canine semen.

Materials and methods: One batch of Andersen's buffer with egg yolk (EYT, final concentration of glycerol 6.4%) was prepared for all of the experiments and stored at −20°C, and the osmolarity was measured in duplicate. The semen was cryopreserved into 0.5 mL straws after a dilution of 1:4 v/v in EYT. One batch of isotonic fructose control thaw media (THAW) was prepared according to the Uppsala protocol, stored at −20°C and used for all samples. EYT and THAW were thawed at 37 degrees C and maintained at that temperature in a water bath. To replicate the osmolality of frozen semen, the EYT was diluted 4:1 v/v with THAW and this diluted solution was used to thaw the spermatozoa. Two straws of semen were rapidly thawed at 70 degrees C for 8 seconds. One straw was emptied into 2 mL of the warm diluted EYT and the other into the THAW. The diluted semen was maintained at 37 degrees C in a water bath. Measurements of subjective motility (MOT%), VSL and VCL (of only the fast-moving spermatozoa) using CASA and the percentage of membrane intact spermatozoa (MIS%, counting 200 cells after CFDA/PI staining), were taken at 5, 60 and 120 minutes. Morphology was also measured at 5 minutes after nigrosin-eosin staining. Statistical analysis was performed using a 3-way ANOVA on the effects of the contributing dog, thaw medium and time on MOT%, VSL and VCL and MIS%, and a 2-way ANOVA on the effects of the contributor and thaw medium on morphology using the programme Minitab 17. Differences in the data were further analysed using a Fisher's least significant difference test with a 95% confidence level.

Results: The osmolality of the EYT was 1538 mOsmol/kg and the osmolality of the THAW medium was 311 mOsmol/kg. Therefore, the final EYT parameters would have been an osmolality of 1293 mOsmol/kg and a glycerol concentration of 5.12%. Semen samples were collected from five different contributing dogs and each sample was cryopreserved into batches of at least 10 straws. There was an effect of contributor ($p < 0.000$) on MOT% MIS%, morphology, VSL and VCL. There were no significant differences detected due to the type of medium on MOT% ($p = 0.148$), MIS% ($p = 0.094$) and morphology ($p = 0.110$). However, the VSL and VCL were higher ($p < 0.000$) in THAW than in EYT (Table 1). There was an effect of time ($p < 0.002$) on MOT%, MIS%, VSL and VCL (Table 1).

Conclusions: There was a significant osmotic gradient between the cryopreserved semen and THAW media and this would have exerted osmotic stress on the spermatozoa at the time of thawing. Although a reduction of hypoosmotic shock improved the quality of cryopreserved human spermatozoa (1), this effect was not observed in this study. The lack of benefit seen could be due to the relative resistance of canine spermatozoa to osmotic shock or to the presence of glycerol in the EYT medium which has toxic effects on spermatozoa (2). The highly permeable glycerol was included in the EYT thaw medium at the same concentration as

in the cryopreserved semen to stop its rapid movement across the cellular membrane which would change the osmolality of the thaw media and spermatozoa. Possible reasons for the lower VSL and VCL seen in EYT compared to THAW media include the lower cellular spermatozoal volume seen in hyperosmotic solutions resulting in less fluid displacement of the flagellum, and increased viscosity of the EYT compared to the THAW media. Further investigation would be needed to determine any benefit in reducing hypoosmotic shock in cryopreserved, canine spermatozoa at the time of thawing.

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TABLE 1. Effect of treatment and time on the parameters measured in this study. Data presented are mean values. Values with different superscripts are different (confidence level 95%, Fisher's least significant difference test).

Measurement	Time group (minutes)		
MOT%			
Treatment	5	60	120
EYT	75.8^{A}	33.6^{B}	21.2^{C}
THAW	71.0^{A}	40.6^{D}	26.0^{C}
MIS%			
Treatment	5	60	120
EYT	65.4^{A}	40.4^{B}	35.6^{C}
THAW	62.2^{A}	44.3^{B}	41.1^{B}
VSL			
Treatment	5	60	120
EYT	54.2^{A}	44.4^{B}	41.0^{B}
THAW	64.8^{C}	50.2^{A}	50.4^{A}
VCL			
Treatment	5	60	120
EYT	108.7^{A}	110.7^{A}	106.3^{A}
THAW	149.3^{B}	166.8^{C}	162.8^{C}

012 | CD20, but not CD45RA, a possible serum biomarker for malignant mammary tumours in bitches

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Introduction: Benign and malignant tumours of the mammary gland occur frequently in intact older bitches and are the most common type of cancer accounting for approximately 50% of tumours in bitches (1,2). Non-invasive, reliable and fast diagnostic techniques are required and blood-based tests are perfectly suited for this purpose. The aim of this study was to identify the diagnostic potential of CD20 and CD45RA as biomarkers in serum samples obtained from mammary tumour-bearing bitches.

Material and methods: In the present study, 30 bitches were included and split into 3 different groups: healthy (n = 3), benign mammary tumour (n = 13) and malignant mammary tumour (n = 14). CD20 and CD45RA expression in serum samples from each bitch was evaluated by Western blotting techniques. Obtained data were statistically analysed by means of two-sided Welch T-test.

Results: Our results showed a significant ($p < 0.05$) increase in CD20 expression in serum samples from healthy, benign and malignant groups (0.44 ± 0.03, 0.83 ± 0.45 and 1.59 ± 1.03, expressed in arbitrary units, in healthy, benign and malignant tumours, respectively). No statistical difference in CD45RA expression among healthy, benign and malignant samples was observed.

Discussion: CD20 and CD45RA were selected as they might help to elucidate the role of T- and B-cells during tumorigenesis as also serve as potential prognostic markers. Results suggest that CD20 could be used as possible biomarker of malignancy in canine mammary tumours. Benign and malignant samples have more heterogeneous expression of CD20 compared to healthy group. It can be explained through the different tumour types contained in each group. On the other hand, the sample size of healthy group was smaller than benign and malignant groups. To achieve improved statistical significance the sample size for each group should be increased. In addition, such heterogeneity within the same group indicates the differences on molecular level even though the tumours diagnosed and treated as the same disease confirming the urgency to improve cancer diagnosis using novel biomarkers. The immune system can positively and negatively influence tumorigenesis. Human breast carcinomas are frequently infiltrated by inflammatory cells (including CD20 positive B-cells) secreting considerable amounts of cytokines which regulate the activity of both immune and neoplastic cells. As both human and canine cancers have many similarities in regards to their immune system, cytokine, chemokine and CD-marker signatures may prove to be very helpful to establish stratification methods for canine mammary tumours.

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013 | Superfecundation in feral queens: Fact or fake? A preliminary study

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Introduction: Superfecundation is defined as the fertilization of two or more ova by spermatozoa from different males in the same oestrus. This phenomenon has been generally accepted as a proven fact in queens although it has been scarcely studied (1, 2).

Material and methods: In the present study, five feral queens belonging to a neutering program of stray cats were included. Ovariohysterectomies were routinely performed by mid-line laparotomy. Hepatic samples from each foetus, as well as a blood sample in an EDTA tube from each dam, were collected and kept frozen at −80°C until DNA analyses were performed. DNA analyses were performed using the technique of polymorphic microsatellite markers. This technique analyses 9 autosomal markers and compares the genotypes obtained from the mother and the foetuses, allowing to determine the presence of more than one father. In addition, an extra marker to determine the sex of the foetuses was included. Data were statistically analysed by means of a χ^2 test.

Results: Three of the 5 (60%) queens included in the present study presented superfecundation. No statistical difference between superfecundation, and monoparent fecundation was observed. When the sex of the foetuses was evaluated, 3/5 queens carried only females foetuses. The remaining queens, one was carrying 2/6 males foetuses and the other one 1/5 male foetuses. Thus, of a total of 25 foetuses, only 3 were male.

Discussion: This preliminary study brings to light that superfecundation is a fact in feral queens. This result is, up to some point, expectable due to the specific characteristics of feline reproductive physiology. The percentage in the present study is lower than those observed in previous studies that ranged from 70% to 80% (1,2). This difference could be due to human control on reproduction even in feral animals. In this sense, our neutering program has been running for several years and includes both feral females and males. That implies a descending number of possible available males, decreasing thus the probability of superfecundation. One weak aspect of the study is the low number of females included, which definitely needs to be increased to obtain a more accurate vision of superfecundation in feline feral population. It is noteworthy that most litters were only formed by female foetuses, and those of mixed sexes showed higher percentages of female foetuses as well.

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014 | Triplex Doppler ultrasonography in pregnant and non-pregnant bitches of different sizes to describe the uterine arteries after breeding

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Triplex Doppler ultrasonography to study the uterine arteries is widely used in human medicine to assess the fetal condition[1]. Haemodynamics of uterine vascularization is modified throughout pregnancy to meet the increasing demand of the growing fetuses. Blanco et al. have observed a progressive decrease in the resistivity and pulsatility indexes of the uterine arteries[2]. The aim of our study was to confirm this tendency in a larger number of bitches, to evaluate differences between bitches of different sizes and to study eventual pathological pregnancies.

Materials and methods: 44 bitches were included. All bitches were monitored during the estrous period to determine ovulation. Every 10 days from ovulation to 50 days post-ovulation, resistivity (RI) and pulsatility (PI) indexes of the right uterine artery (LOGIC F8, Scil, General Electric Healthcare) were performed together with usual assessment of fetal development and viability and follow up of the luteal function. At least 3 measurements of RI and PI per examination were obtained, always on the right artery (since no significant difference was observed between the two arteries in the published work[3]), and the mean value was calculated. Bitches were classified as pregnant (33/44) or not pregnant (11/44). Of these bitches, 4 categories were defined according to their weight: small (<10 kg), medium (11–25 kg), large (26–40 kg) and giant breeds (>41 kg). 6/33 pregnancies were retrospectively classified as abnormal (loss of more than 10% of the embryos [4]).

Results: Repartition of bitches were as followed: small: 8/44 total bitches but 5/27 normal pregnancies ([3–4] puppies); medium: 13/44 (11/27, [2–9]); large: 13/44 (6/27, [4–8]) and giant: 10/44 (5/27, [5–15]). In all bitches, throughout pregnancy, we observed a decrease of RI and PI, with these values being significantly ($p < 0.0001$) lower for pregnant bitches from 30 days post-ovulation. The RI and PI did not significantly vary with the size of the bitches. Also, we couldn't determine a significant impact of abnormal pregnancies on RI and PI ($p = 0.48$) but this may be due to the low number of abnormal pregnancies (6/33).

Discussion: To the best of our knowledge, this is the first study of this scale comparing bitches of different sizes. Nevertheless, Batista et al. suggested the existence of a difference between their values of RI and PI of uterine arteries in small breeds to the literature data

in other breeds[5]. Moreover, we observed a significant difference between pregnant and non pregnant bitches from 30 days post-ovulation. It could be interesting to confirm this tendency and to determine a more precise threshold with an even larger sample of bitches.

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016 | Low birth weight as a risk factor for kitten mortality: determination of breed-specific thresholds

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Introduction and aim: Identification of factors involved in kitten mortality is essential to improve their chances of survival. In canine species, low birth weight has been identified as an important risk factor for neonatal mortality (1). The objectives of this study were to evaluate the impact of birth weight on kitten mortality between birth and two months of age and to determine by breed, the critical thresholds of birth weight defining kittens at higher risk of death during this period.

Materials and methods: Weight, litter size, sex of kittens and date of parturition have been recorded by breeders and retrospectively collected on a voluntary basis. Only kittens from French catteries, with known birth weight and known status at two months (dead or alive) were included. First, generalized mixed models were fitted to determine factors affecting mortality during two different periods: 0–2 days and 2 days–2 months. The fixed-effects introduced in the models were: birth weight, litter size (total number of kittens born alive), litter heterogeneity (within-litter variation of birth weight, expressed as the coefficient of variation CV, ratio of the standard deviation to the mean), season of parturition and early growth rate (calculated, using the formula [(weight at 2 days − weight at birth) ÷ weight at birth × 100]; only for the period 2 days–2 months). Cattery and queen were introduced as random effects to deal with the non-independence of kittens sharing the same cattery and the same mother. Receiver operating characteristic (ROC) curves were used to identify optimal cut-off values for birth weight regarding mortality during the first two months of life specifically for each breed included. Areas under the ROC curves were calculated to estimate the ability of birth weight to discriminate between kittens of different status, i.e. dead or alive at two months of life.

Results: A total of 4152 live-born kittens from 13 breeds, 1106 litters and 136 French catteries were included. Sex ratio was 1.2 (1795 males to 1560 females). A total of 6.8% (95% confidence interval, 95% CI: 6–7.7) of live-born kittens died during the first two months after birth with significant variations between breeds (from 0% in Ragdoll to 15% in Russian Blue). From all parameters evaluated between 0–2 days, only birth weight was associated with mortality ($p < 0.001$). Mortality was significantly higher in kittens with birth weight lower than the first quartile: 14.2% (95%CI: 12.3–16.6) vs. 4.4% (3.6–5.2). Mortality between 2 days and 2 months of life was influenced by birth weight and early growth rate (both $p < 0.001$). During this period, mortality was significantly higher in kittens with birth weight lower than the first quartile: 8% (95%CI: 6.3–10) vs. 3% (95%CI: 2.4–3.7). Kittens with low birth weight and poor early growth rate (both parameters lower than the first quartile) were at higher risk of death between 2 days and 2 months after birth compared to kittens from other categories: mortality rate at 13.3% (95%CI: 9–18.7) and 3.8% (95%CI: 3.1–4.6). Birth weight critical thresholds have been established in 7 breeds (for which AUC ≥ 0.7): Abyssinian/Somali, 95 g; British group, 103 g; Chartreux, 107 g; Egyptian Mau, 85 g; Maine Coon, 120 g; Oriental group, 77 g; Russian Blue, 95 g. Interestingly, two breeds can have a similar birth weight distribution but significantly different critical thresholds (Oriental group vs. Abyssinian/Somali for example).

Conclusions: Birth weight critical thresholds, established in 7 breeds, would allow the identification of kittens with higher risk of mortality in order to provide them with appropriate nursing and medical care.

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017 | Impact of neonatal and adult factors on body condition of Labrador dogs

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Introduction and aim: Overweight, affecting 20–40% of the general canine population, is a growing global health concern since it promotes the development of numerous other diseases and decreases quality of life together with life span (1). Numerous risk factors for obesity have been described in the literature including genetics, sexual status, the amount of physical activity and the diet type (2). In human, several studies highlight the importance of the early-life environment in the development of adulthood overweight (3). The aim of this study was to analyse the association between neonatal

factors and overweight at adulthood in a population of pure breed Labradors.

Materials and methods: The data collection was conducted on dogs born within the same French breeding kennel (CESECAH, Lezoux, France). For each dog, information about neonatal period (birth weight, growth rate between birth and Day 2 and between Day 2 and Day 21) was recorded throughout a questionnaire. General information about dogs (sex, age, sterilization status, "Food motivation" score (4) ...), their life style (age of owner, walking duration per day...) was also recorded. Body condition score (BCS) was evaluated using the 9-point scale (5). After univariate analyses, only parameters with p-value lower than 0.20 or parameters with biological relevance were kept for multivariate analysis model, i.e. sterilisation (Yes/No), age of owner, "Food motivation" score, birth weight and growth rates (between birth and Day 2 and between Day 2 and Day 21). A generalized linear model was then fitted to determine factors affecting overweight (BCS > 6).

Results: A total of 85 Labradors (20 males and 65 females) raised under similar environmental conditions until two months of age were included in the present study. Dogs were from 6 months to 13 years of age (median: 3.8 years). The overall prevalence of overweight (BCS > 6) was 44% (95% confidence interval, 95% CI: 32–56). The main risk factor was the neutering (p = 0.009; relative risk RR: 3.5, CI: 1.8–7.1). For neutered dogs (males and females, n = 28), growth rate between 2 and 21 days was significantly associated with overweight (p < 0.001). Birth weight and "Food motivation" score tended to be significant (p = 0.079 and 0.074 respectively). Neutered dogs with a 2–21 days growth rate over 248% or a birth weight under 415 grams were at higher risk of overweight at adulthood than others (RR: 2.1, CI: 1.1–4.3 and RR: 1.7, CI: 0.8–3.8, respectively). A "Food motivation" score in the lowest values (low food-motivation) increased the risk of overweight.

Conclusions: These results suggest an influence of neonatal factors on the risk of overweight in addition to adult factors. Low birth weight puppies with high 2–21 days growth rate could be more susceptible to become overweight. More studies are needed to explore this relationship, to identify early-life predictive factors for canine overweight and obesity and to quantify the relative impact of early risk factors and environmental factors. These findings should help to reduce the current high prevalence of overweight and thus to improve health and welfare of companion dogs throughout an early management of puppies (from birth).

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018 | Kitten growth from birth to two months of age: breed-specific curves

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Introduction and aim: Weight-for-age charts are commonly used to evaluate pediatric development of infants (1). Deviation from a "normal" trajectory is associated with an increased risk of morbidity and mortality (2). Effective growth monitoring, a simple and easy-to-use tool for health management, requires accurate plotting on appropriate charts. Such curves are to date not available for kittens during the period they are raised by their breeder, i.e. from birth to the age of two months. The purpose of the present study was to draw reference growth curves in the feline species. Due to body-weight variability between feline breeds, data were plotted by breed.

Materials and methods: Purebred kittens were weighed by breeders from birth to two months of age (with various scales) and data were transmitted retrospectively on a voluntary basis and entered into an Excel file. Only kittens born in French catteries and declared alive at two months of age were included into the analysis. First, growth was described through seven parameters: weight at birth (D0), at D2, at D21 and at D60 and growth rates calculated between D0 and D2, between D2 and D21 and between D21 and D60. These seven parameters were compared between breed using the Kruskal-Wallis test and the Wilcoxon signed rank-test with Bonferroni correction. Then, breed-specific reference growth curves were drawn in two steps. First, box-and-whisker plots allowed to describe the weight at 14 different dates (D0, D1, D2, D4, D7, D10, D14, D21, D28, D35, D42, D49, D56, D60). Then, the weight values were fitted by a second-degree polynomial function, giving smoothed growth curves. On each graph, 13 parameters were represented: the median, the two quartiles, the eight remaining deciles and centiles 5 and 95.

Results: In total, 3639 kittens from 1010 litters were included. Twelve different breeds were represented: Abyssinian/Somali, Bengal, Birman, British group, Chartreux, Egyptian Mau, Maine Coon, Norwegian Forest, Oriental group, Persian group, Ragdoll and Siberian. The number of kittens included ranged from 101 to 640 per breed (median: 162). The number of litters per breed ranged from 27 to 199 (median: 60). The cattery of origin was known for 94% of kittens and 130 catteries were represented. The studied population included 1419 females and 1640 males (sex ratio: 1.2; 580 kittens with unknown sex). A significant breed effect was evidenced on all growth parameters (p-value < 0.001): birth weight ranged from 87 g (mean in Abyssinian/Somali) to 119 g (Maine Coon); weight at D60 ranged from 853 g (Oriental group) to 1174 g (Maine Coon); growth rate between D0 and D2 ranged from 16% (Birman) to 30% (Abyssinian/Somali); growth rate between D2 and D21

ranged from 174% (Chartreux) to 239% (Egyptian Mau). In contrast, growth patterns were similar between breeds with a quasi-linear curve. Differences in slopes combined with statistical differences in growth rates between breeds suggested that kitten growth description requires breed-specific reference growth curves described in this work.

Conclusions: These curves provide practical tools to breeders of twelve breeds for kitten follow-up. More data are needed to increase the precision of these twelve curves, to define reference growth curves for the numerous remaining breeds and to compare feline growth across different countries.

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019 | A single GnRH-antagonist treatment affects testicular expression of Steroidogenic Acute Regulatory protein (StAR) and steroidogenic enzymes in dogs

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Introduction and aim: Gonadotropin-releasing hormone (GnRH)-antagonists can suppress the production of gonadotropins from the pituitary gland. This results in a decreased production of testosterone and an impaired spermatogenesis in the testis. Therefore, GnRH-antagonists may have future potential for reproduction control in dogs (1). Steroidogenic acute regulatory protein (StAR) and steroidogenic enzymes are important factors for the synthesis of testosterone in the testis (2). However, the effect of GnRH-antagonist treatment on these factors has not been studied in dogs yet. This study investigates how a single treatment with the GnRH-antagonist acyline affects the expression of StAR and the steroidogenic enzymes cytochrome P450 side-chain cleavage (P450scc) and cytochrome P450 17α-hydroxylase/17,20-lyase (P450c17).

Materials and methods: Testicular tissues from nine clinically healthy and sexually mature male dogs (4–6 years, 8–11 kg) were included. Four dogs were treated SC with a single injection of acyline (330 µg/kg) and castrated surgically 14 days after the treatment (AG; n = 4). The remaining dogs served as a control group (CG; n = 5) and were castrated right after a clinical examination and semen collection/evaluation. Bouin's solution fixed and paraffin embedded testicular tissue was used to study the expression of StAR, P450scc and P450c17 by immunohistochemistry. Light microscopy was used to evaluate spermatogenesis and to determine the localization of immunopositive cells. By use of the digital image processing program ImageJ (https://imagej.net/Fiji), the percentage immunopositive area (PIA) and the intensity of the immunohistochemical staining (mean grey scale, MGS-value) in the interstitial compartment were calculated. Additionally, the prevalence of immunopositive peritubular cells was calculated in each sample by evaluation of 200 peritubular cells.

Results: Treatment resulted in a spermatogenic arrest. In regards to steroidogenesis, StAR, P450scc and P450c17 protein expression in Leydig cells was significantly affected 14 days after the treatment with acyline ($p < 0.05$). PIA was significantly lower for treated dogs compared to controls (StAR: $p < 0.05$; P450scc and P450c17: $p < 0.01$) and so was the MGS-value (StAR: $p < 0.001$; P450scc: $p < 0.01$; P450c17: $p < 0.05$). The mean prevalence of immunopositive peritubular cells was 1.0, 4.9 and 9.5% for StAR, P450scc and P450c17, respectively, in CG and 0.1, 0.9 and 1.6% in AG. Treatment resulted in a significant downregulation in the prevalence of immunopositive peritubular cells in the stainings for P450scc and P450c17 ($p < 0.01$).

Conclusion: The results of our study indicate that the entire steroidogenic cascade in the Leydig cells is downregulated after treatment with acyline resulting in a disruption of spermatogenesis.

The expression of StAR, P450scc and P450c17 in some of the peritubular cells indicates that these cells might be Leydig cell progenitors. To the knowledge of the authors, this has not previously been shown in dogs.

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021 | Breeders' chilling, a valuable option to cryopreserve dog semen collected in field conditions.

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Introduction and aim: Cryopreservation of dog semen is the best option for breeders to store or ship the genetic material of valuable individuals, but this service is usually only available in some specialized centers. In field conditions, as in local breeding kennels, small clinical practices or during expositions, the unavailability of specific equipment and liquid nitrogen makes freezing procedures unfeasible and breeders ask to collect the semen *in loco* and ship it cooled

to a cryopreservation facility. Although, before cryopreservation, prolonged semen chilling in laboratory setting gave different results (1–4), its effect on sperm characteristics when performed in field conditions has never been investigated. The aim of this study was to evaluate whether the cryopreservation of dog semen after 24 or 48 hr of storage in a chilling box results in a satisfying sperm quality. The ultimate goal is to suggest a valuable option to breeders for cryopreserving semen without moving animals.

Materials and methods: Ejaculated spermatozoa from 9 healthy stud dogs (mixed breeds, 2–13 years old) were collected by digital manipulation: one aliquot was evaluated as fresh control (FRESH) and one immediately cryopreserved (CRYO T0) by Uppsala method (5), whereas other two aliquots were diluted (1:1) with chilling extender (TRIS buffer, antibiotics and 20% egg yolk) and kept in a Minitube® styrofoam transport box for 24 hr (CRYO T24) or 48 hr (CRYO T48), until cryopreservation. Briefly, maintaining spermatozoa (CRYO T24 and CRYO T48) at the chilling temperature, the samples were centrifuged, the surnatant was discarded, and spermatozoa were diluted (1×10^5 sp/mL) with freezing extender (TRIS buffer, 10% glycerol, 1% Equex STM Paste, antibiotics and 20% egg yolk). At thawing, samples were analyzed for motility, morphology (Sperm Deformity Index, SDI: 6), membrane and acrosome integrity, and ability to interact with oocytes by Zona pellucida Binding Assay (ZBA). To also check whether dog age influenced the results, data were analyzed by ANCOVA (covariate = age) followed by Tukey's test for multiple comparisons (significance level set at $p < 0.05$).

Groups	Motility (%)	SDI (mean ± SD)	Acrosome integrity (%)	Membrane integrity (%)	Sperm bound to ZP (n.)
FRESH	90.0 ± 5.00 [a]	0.47 ± 0.25 [a]	89.1 ± 10.8 [a]	90.9 ± 7.4 [a]	17.7 ± 36.4
CRYO T0	50.0 ± 14.14 [b]	1.5 ± 0.17 [b]	49.4 ± 16.7 [b]	67.6 ± 16.9 [b]	14.3 ± 26.5
CRYO T24	45.6 ± 20.68 [b,c]	1.45 ± 0.21 [b]	39.0 ± 24.7 [b]	64.3 ± 22.1 [b]	14.8 ± 34.2
CRYO T48	30.0 ± 17.14 [c]	1.41 ± 0.17 [b]	53.0 ± 16.7 [b]	64.5 ± 14.9 [b]	17.2 ± 31.0

Results: In frozen spermatozoa, morphology, membrane and acrosome integrity were not affected by different treatments (CRYO T0-T24-T48). Although a decrease in motility in CRYO T48 was observed, the ability to adhere to oocytes zona pellucida was maintained at the same extent in both fresh and cryopreserved spermatozoa. Dog age did not influence sperm qualities of fresh or frozen samples.

Conclusions: Freezing of freshly collected semen is the optimal way to ensure a good sperm survival. However, present results showed that a cooled transport of 24 hr before freezing could be proposed to dog breeders to meet their demand for semen cryopreservation.

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022 | Cat vitrified oocytes culture in 3D liquid marble microbioreactors

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Introduction and aim: Liquid marbles, also known as pearl drops, are three-dimensional (3D) microbioreactors in which living cells can survive, proliferate and react to stimuli. The pillar on which this technology is established is the use of non-adhesive, hydrophobic molecules to create a casing that maintains the cells suspended in a small volume of inner liquid and has the right porosity to allow free exchange of gases (1). Microorganisms, tumor spheroids, red blood cells and embryonic stem cells have been cultured in liquid marbles, and their survival and growth were successfully obtained (1–3). This microbioreactor, that better resembles the in vivo conditions compared to two-dimensional (2D) cultures, might also be beneficial for female gametes, and especially for the low-competence cryopreserved oocytes. However, only one study with fresh sheep gametes (4) provided some information on the usefulness of this environment for in vitro maturation (IVM). In this study, the efficiency of liquid marbles as 3D microbioreactors for the IVM of vitrified domestic cat oocytes was assessed and compared with that of traditional microdrops of medium (2D).

Material and methods: Fresh ovaries (n = 30) from domestic queens were obtained after surgery and cumulus oocytes complexes (COCs) were collected. Sixty-five COCs were vitrified by Cryotop method (5) and, after warming, morphologically intact (6) vitrified oocytes (VOs, n = 59) were cultured in 3D liquid marbles (n = 30) or in 2D microdrops of medium (n = 29). To create the 3D microbioreactor, a chemically inert hydrophobic powder (polytetrafluoroethylene, PTFE; Sigma-Aldrich, St. Louis, MO, USA) was used following a published protocol (4). Oocytes were matured for 24 h in a controlled atmosphere (38.5°C and 5% CO_2 in air) in TCM199 supplemented with 10% FBS, 10 ng/mL EGF, 0.6 mM cysteine (Sigma-Aldrich) and 0.5 IU/mL FSH + 0.5 IU/mL LH (Pluset, Calier, Spain). Chromatin configurations were determined by bisbenzimide (Hoechst 33342; Sigma-Aldrich) staining, and data were analyzed by Chi-square test, with the level of significance set at $p < 0.05$.

Results: Vitrified oocytes resumed meiosis at similar proportions in 3D and 2D culture conditions (3D: 50% vs. 2D: 55.2%; p = 0.69), and

the same trend was observed for full maturation (3D: 13.3% vs. 2D: 13.8%; p = 0.96).

Conclusions: Liquid marble technology, even if promising for different types of somatic cells, in these experimental conditions had the same effect as traditional 2D culture on cat immature vitrified oocytes IVM. To fully restore and boost the developmental competence of feline cryopreserved oocytes other systems remain to be investigated.

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024 | Semen evaluation in pomeranian dogs

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Introduction and aim: Semen evaluation in dogs is one of the most important parameter to analyze the reproductive status in dogs. Semen parameters can reflect the fertility status in animals. This study was performed in order to evaluate semen characteristics from 36 male dogs, in three different times (three years distance from each other) and in two different kennels.

Materials and methods: Semen from 36 male adult and fertile Pomeranian dogs with average aged of 2.92 ± 1.52 years was collected, evaluated and the data was recorded. The dogs were divided into 3 groups as described: Group I - n = 15 dogs that had the semen collect and evaluated 5 years ago (2014), Group II - n = 11 dogs that had the semen collected and evaluated 3 years ago (2017) and Group II – n = 10 dogs, that had the semen collected and evaluated this year (2019). Group II and III were housed in the same kennel and Group I in another one. All dogs received the same commercial food and water ad libitum. Semen was evaluated by the same person for the following parameters: volume, motility, forward velocity, cell concentration and morphology. Volume measurement was done in the collection tube. Motility and forward velocity progression were estimated with a 20ul semen droplet on a pre warmed glass slide. To obtain concentration values, semen was diluted 1:20 and spermatozoa were counted in a Neubauer chamber. Finally morphological abnormalities were visualized in a colored smear (Panótico ® Laborclin, Parana, Brazil).

Results: Results are given as average ± standard deviation. There was no statistical difference (p < 0.05) in the seminal parameters from the dogs between the groups and in space of time. Results of semen evaluation were as follows: volume was 1.58 ± 0.99 mL (ranging from 1 to 4.5 mL); motility 79.71 ± 9.46% (ranging from 30 to 90%); forward progression velocity 3.65 ± 0.57 (grade from 0 to 5) (ranging from 3 to 5); concentration 20.89 ± 105.43 spermatozoa × 10^6/mL (ranging from 72 to 345 spermatozoa × 10^6/mL); total spermatozoa concentration 606.39 ± 366. 89 × 10^6/mL (ranging from 20 to 1096 spermatozoa × 10^6/mL) and normal cell morphology 92.24 ± 6.63% (ranging from 66 to 98%).

Conclusions: All the dog population was fertile according to our data. There are few publications about specific dog breeds reproductive parameters. Therefore, the contribution of this work showed no variation of seminal parameters from different population and space of time. That results provide a guideline for semen measurements of the population of Pomeranian dogs in this study.

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026 | Doppler ultrasound and fetal heart rate changes in pregnant bitches of different weight groups

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Introduction and aim: Although uterine and umbilical Doppler ultrasound and fetal heart rate (FHR) have proved to be useful parameters to monitor canine gestation (1,2) the effect of maternal body weight group on these parameters has not been described yet. The aim of this study was to compare uterine and umbilical arteries blood flow and FHR during the second half of pregnancy in small, medium and large canine breeds.

Materials and methods: Thirty-eight, 1–6 years of age, 3–45 kg of body weight (BW) purebred healthy pregnant bitches were recruited. Bitches were retrospectively assigned to one of the following groups according to their BW during estrus: small breeds (SB; <10 kg; n = 16), medium breeds (MB; 10–25 kg; n = 9) and large breeds (LB; >25 to 45 kg; n = 13). Pregnancy ultrasonographic diagnoses were performed on Day 25 and, then, both uterine and umbilical Doppler and M-mode ultrasound were carried out every 10 days from Day 30 to 60 of gestation. Umbilical Doppler and FHR were measured in the most caudal fetus of the right uterine horn. Peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured and resistance index [RI = (PSV−EDV)/PSV] was automatically calculated. Fetal heart rate was registered by M-Mode echocardiography. Values of PSV, EDV and RI of uterine and umbilical arteries and FHR were compared among groups by repeated measures ANOVA followed by Tukey test. To further characterize the studied parameters, the effects of maternal BW and litter size at birth were

analyzed by multivariate regression at each assessment time point (SPSS 17.0, SPSS Inc. Chicago, IL, USA). $p < 0.05$ was considered significant.

Results: No fetal losses were detected throughout the study period. All the bitches normally delivered 2–12 healthy puppies at term. During the second half of pregnancy, uterine and umbilical arteries PSV and EDV increased ($p < 0.01$), without differences among groups ($p > 0.05$). Conversely, uterine and umbilical arteries RI progressively and differently decreased in the three groups ($p < 0.01$) being lower in LB than in SB bitches from pregnancy Day 40 onwards ($p < 0.01$). Litter size but not maternal BW ($p > 0.1$) influenced uterine RI on Days 40 ($r = 0.39$; $p < 0.01$) and 50 ($r = 0.41$; $p < 0.01$). Conversely, on Day 60, maternal BW ($r = 0.6$; $p < 0.01$) had an effect while litter size did not ($p > 0.1$). FHR increased from 226 ± 3 bpm on Day 30 to 233 ± 1 bpm on Day 50. Then, it diminished to the end of pregnancy, reaching 216 ± 7 bpm on Day 60 ($p < 0.01$). FHR did not differ among groups at any time point ($p > 0.1$).

Conclusions: Uterine and umbilical blood flow differently increased throughout mid and late pregnancy in large and small breed bitches. These differences were influenced by litter size on Days 40 and 50, and by maternal BW on Day 60. Conversely, in this same period, fetal heart rate did not vary with different maternal BW groups. Physiological variations among bitches of different BW groups should be considered when gestational ultrasound examination is interpreted.

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028 | Supplementation of semen freezing medium with antifreeze protein type III and Equex STM paste improves dog sperm viability

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Introduction and aim: A cryoprotective agent (CPA) is needed to protect cell from cryodamage during freezing and thawing (1). Antifreeze Protein (AFP) type III, one of the CPA, can improve post-thawed sperm quality of buffalo and rabbit (1,3) but has not been yet elucidated in dog sperm. Addition of Equex STM paste to semen freezing medium provides beneficial effects on cryopreserved dog sperm (4). Hence, the purpose of this study was to find out the effects of AFP, Equex STM paste or a combination of them as CPA on cryopreserved dog spermatozoa.

Materials and methods: Twelve healthy beagles were included in the study. Ejaculates were collected and pooled from 3 dogs each time. Sperm motility was assessed subjectively with a warm stage at 38°C and semen samples with a minimum of 70% total motility were included in the study. The experiments were repeated 6 times. Two steps-dilution freezing methods (4) with 3 different tris-egg yolk based extenders (E1, E2 and E3) were used; E1 (AFP), E2 (AFP and Equex STM paste) and E3 (Equex STM paste). AFP (0.1 µl/mL) and/or Equex STM paste (1% v/v) were supplemented in the second step dilution. The final concentration of glycerol in E1, E2 and E3 before freezing was 5%. Sperm evaluation was done one week

TABLE 1. Mean ± SD of total motility (TM), PMI, PMFI, acrosome integrity and sperm velocity (VCL, VSL and VAP) in different extenders (E1, E2 and E3)

Time	TM	PMI	PMFI	Acrosome	VCL	VSL	VAP
E1							
0 h	$24.3 \pm 12.2^{a,x}$	$28.2 \pm 10.8^{a,x}$	$34.2 \pm 12.3^{a,x}$	$62.5 \pm 8.8^{a,x}$	$110.8 \pm 21.5^{a,x}$	$33.2 \pm 13.3^{a,x}$	$55.6 \pm 14.8^{a,x}$
1 h	$10.3 \pm 8.4^{b,x}$	$16.5 \pm 8.6^{b,x}$	$26.1 \pm 12.7^{ab,x}$	$58.2 \pm 16.3^{ab,x}$	$63.5 \pm 49.9^{b,x}$	$13.4 \pm 10.6^{b,x}$	$28.4 \pm 22.3^{b,x}$
2 h	$1.9 \pm 3.0^{b,x}$	$8.5 \pm 3.8^{b,x}$	$17.7 \pm 2.7^{b,x}$	$45.1 \pm 17.6^{ab,x}$	$25.3 \pm 39.2^{bc,x}$	$5.1 \pm 8.0^{bc,x}$	$11.2 \pm 17.4^{bc,x}$
3 h	$0.0 \pm 0.0^{b,x}$	$7.7 \pm 3.3^{b,x}$	$17.6 \pm 11.6^{b,x}$	$40.7 \pm 19.3^{b,x}$	$0.0 \pm 0.0^{c,x}$	$0.0 \pm 0.0^{c,x}$	$0.0 \pm 0.0^{c,x}$
E2							
0 h	$54.6 \pm 9.9^{a,y}$	$61.7 \pm 10.7^{a,y}$	$57.1 \pm 2.1^{a,y}$	$77.6 \pm 8.1^{a,x}$	$156.5 \pm 12.9^{a,y}$	$57.7 \pm 9.1^{a,y}$	$87.8 \pm 10.2^{a,y}$
1 h	$35.1 \pm 10.2^{b,y}$	$44.6 \pm 10.1^{b,y}$	$43.7 \pm 2.5^{b,y}$	$62.4 \pm 17.7^{ab,x}$	$123.2 \pm 14.7^{ab,y}$	$37.8 \pm 6.2^{b,y}$	$64.9 \pm 7.8^{b,y}$
2 h	$23.3 \pm 15.3^{bc,y}$	$37.4 \pm 10.1^{bc,y}$	$42.8 \pm 9.8^{b,y}$	$54.4 \pm 21.9^{b,x}$	$98.9 \pm 49.8^{b,y}$	$29.1 \pm 14.5^{bc,y}$	$50.7 \pm 25.4^{b,y}$
3 h	$19.8 \pm 10.9^{c,y}$	$31.0 \pm 7.2^{c,y}$	$34.3 \pm 7.7^{b,y}$	$48.7 \pm 14.6^{b,x}$	$91.4 \pm 47.3^{b,y}$	$23.6 \pm 13.5^{c,y}$	$44.6 \pm 23.6^{b,y}$
E3							
0 h	$53.3 \pm 7.1^{a,y}$	$50.5 \pm 8.0^{a,z}$	$57.2 \pm 12.9^{a,y}$	$74.7 \pm 4.2^{a,x}$	$154.9 \pm 8.2^{a,y}$	$58.3 \pm 2.2^{a,y}$	$87.5 \pm 4.9^{a,y}$
1 h	$28.0 \pm 8.5^{b,y}$	$35.7 \pm 10.8^{b,y}$	$43.6 \pm 12.1^{b,y}$	$60.7 \pm 13.1^{ab,x}$	$119.4 \pm 18.3^{ab,y}$	$37.4 \pm 8.9^{b,y}$	$62.1 \pm 11.4^{b,y}$
2 h	$25.0 \pm 15.2^{b,y}$	$34.2 \pm 11.5^{b,y}$	$30.4 \pm 4.1^{c,z}$	$52.83 \pm 16.8^{b,x}$	$102.9 \pm 52.1^{b,y}$	$32.3 \pm 16.6^{bc,y}$	$54.2 \pm 27.3^{b,y}$
3 h	$18.1 \pm 10.0^{b,y}$	$31.9 \pm 7.3^{b,y}$	$33.7 \pm 12.4^{cb,y}$	$45.8 \pm 14.5^{b,x}$	$87.7 \pm 43.3^{b,y}$	$22.6 \pm 11.3^{c,y}$	$42.8.2 \pm 21.3^{b,y}$

Different superscripts (a, b, c, d) between times in same extender and superscript (x, y, z) between semen extenders in the same time point indicate significant differences ($P<0.05$).

after freezing. After freezing and thawing, sperm motility and velocity were evaluated by Sperm Class Analyzer® (SCA Microptic SL, Barcelona, Spain). Sperm viability (plasma membrane integrity; PMI) (SYBR and EthD-1) and acrosome integrity (FITC-PNA and PI) were assessed by fluorescent microscopic examination. Plasma membrane functional integrity (PMFI) was evaluated by hypo osmotic swelling test (HOST).

Results: When data were pooled across observation times, regardless of time post-thaw, semen extenders containing Equex STM paste (E2, E3) showed better ($p < 0.0001$) sperm quality compared to E1 in all parameters except for acrosome integrity (Table 1). E2 significantly improved sperm viability compared to E3 when data were pooled across the times ($p = 0.02$) and immediately after thawing ($p = 0.02$). Different superscripts (a, b, c, d) between times in same extender and superscript (x, y, z) between semen extenders in the same time point indicate significant differences ($p < 0.05$).

Conclusions: Addition of AFP alone seems to be not sufficient to protect spermatozoa form the cryodamage. However, in combination with Equex STM Paste, APF can significantly improve viability of cryopreserved dog spermatozoa.

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029 | Is urine glucose concentration correlated with glycaemia in neonates? A preliminary study

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Introduction and aim: Neonates differ substantially from adults with regard to their ability to maintain normal blood glucose levels being in turn more prone to suffer hypoglycaemia. Therefore, meticulous monitoring of glucose is essential in neonates, but its small size and low circulating volume is often a limiting factor for frequent extractions of blood samples to measure glucose. At birth, the neonatal puppy and kitten kidney is immature in both structure and function. Functionally, the neonate has a lower glomerular filtration rate and renal plasma flow, lower filtration fraction and the urinary glucose reabsorption does not normalize until 3 weeks of age (1,2). Since glucosuria is common in neonates until 2 to 8 weeks of age (3), the present study aimed to determine the possible relationship between plasma and urine glucose concentration in neonate dogs in order to stablish if urine glucose concentration can be used as a new method for monitoring glycaemia.

Material and methods: Six healthy *Cane Corso* puppies from the same litter delivered by C-section were included in the present study. Blood and urine samples were obtained 4 hours and 7 days after delivery. Glucose concentration from both blood and urine samples were determined by means of a glucometer (Accu-Check®, Aviva, St Cugat del Vallès, Spain).

Results: Results are summarized on Table 1. On the first day of life, glycaemia ranged from 28 to 74 mg/dL, while glucosuria ranged from 54 to 295 mg/dL. On day 7, glycaemia showed normoglycaemia values ranging from 112 to 136 mg/dL and glucosuria from 26 to 175 mg/dL.

Conclusions: According to authors' knowledge, this is the first study that determines the concentration of urine glucose in neonates. Glucose concentration was higher in urine than in blood samples few hours after delivery in 4 out of 6 puppies, while on day 7, glycaemia values were higher than urine with the exception of one puppy. This specific puppy showed the lowest rhythm of weight increase. Thus, according to the present results, urine glucose seems not be correlated with glycaemia. It is noteworthy that all puppies showed blood glucose concentration below the normal rang few hours after delivery, maybe due to an insufficient ingestion of milk. On the other hand, one week after delivery, none of the puppies showed hypoglycaemia. Thus, further research is needed to establish the physiological values of urine glucose in both healthy and hypoglycaemic neonates before accepting or discarding this parameter as a possible diagnostic tool for hypoglycaemia.

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	Day 0		Day 7	
Puppy	Blood glucose (mg/dL)	Urine glucose (mg/dL)	Blood glucose (mg/dL)	Urine glucose (mg/dL)
1	46	240	126	175
2	28	-	112	26
3	74	67	125	27
4	34	54	151	35
5	65	56	136	51
6	40	295	130	36

032 | Serum Anti-Müllerian hormone during peri-ovulatory period in deslorelin-induced estrous bitches

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Introduction and aim: Although, the anti-*Müllerian* hormone (AMH) is associated with sexual development of fetuses, AMH is also produced during adulthood in male testes and female ovaries (1). Currently, knowledge on the clinical potential of AMH in the female dog is limited. In the ovaries, AMH is produced from granulosa cell of pre-antral and antral follicles. Accordingly, AMH concentrations might be related to follicle dynamics in the canine ovaries. To date, the GnRH agonist deslorelin has been used for estrous induction in small animal practice. Even though the results of estrous induction using deslorelin implantation are satisfactory, pregnancy rates vary among studies. To understand the follicular development of estrous induction using the GnRH agonist deslorelin, this study was performed to determine the association between AMH concentrations and follicular growth during the peri-ovulatory period in deslorelin-induced estrous bitches.

Materials and methods: Healthy, nulliparous, 2.5–4 years old Beagle bitches and weighing between 10.5–13.0 kg, were enrolled in this study (n = 4). The bitches were considered anestrus when their serum progesterone levels were below 1.0 ng/mL and the superficial cells from vaginal cytology was less than 10% (2). A 4.7 mg deslorelin implants (Suprelorin®, Virbac, Carros, France) was inserted subcutaneously in the post umbilical area in all anestrous bitches on the same day. Time of implantation was considered as day 0, and time from day 0 to estrous induction was calculated. The implant was removed at ovulation when progesterone concentrations reached 5.0 ng/mL. Blood samples were collected on days 0, 6, 8, 10, 12, 14, 16, 18, 20 and 22 after implantation. Sera were obtained by blood centrifugation for 10 min at 700 × g. According to the manufacturer's instructions, AMH concentration was determined by Canine AMH ELISA (Ansh Labs, Texas, USA). Progesterone concentration was determined by FEIA (TOSOH Corp., Tokyo, Japan). Data analyses were carried out by using general linear mixed models. The concentrations of AMH and progesterone were regarded as dependent variables. The statistical models included day of implantation as a fixed effect and bitch identity as a random effect. Least square means were obtained from each class of the factor and were compared by using least significant difference (LSD) test. The correlation between AMH and progesterone was determined by using Pearson's correlation. $p < 0.05$ was regarded to be statistically significant.

Results: On average, the serum AMH concentrations varied among bitches from 0.12 to 3.08 ng/mL during implantation period. No difference in the AMH concentration between day 0 and days 6, 8, 10, 12, 14, 16, 18, 20 and 22 after implantation were observed ($p > 0.05$). However, there was a significant difference in progesterone concentration between day 0 and days 14, 16, 18, 20 and 22 after implantation ($p < 0.01$). Serum AMH and progesterone concentrations in each bitch are presented in Fig. 1.

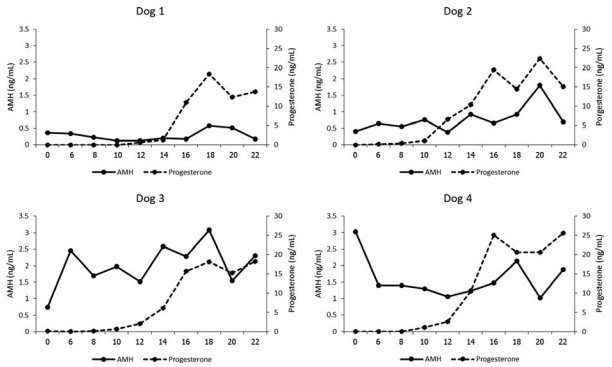

FIG. 1. Individual serum AMH and progesterone concentrations for four dogs from day 0 to day 22 following treatment with a 4.7 mg deslorelin implant in the periumbilical area.

Conclusions: In our study, deslorelin-induced estrus was successfully used in all bitches. Ovulation was observed during day 12 to day 16 of implantation in all bitches, in accordance with the previous study (3). The rise in AMH concentration appeared to be observed after LH surge (when progesterone is 2 ng/mL). This finding was in contrast with a previous study (4), demonstrating that AMH levels first increased two folds above the baseline observed before LH surge in natural estrous bitches. Changes in serum AMH concentrations of natural and deslorelin-induced estrus seemed interesting and further investigation with a larger number of animals is warranted. Variation in AMH levels in this study (n = 4) and a previous study (n = 5) (4) suggested that clinical application of AMH to monitor follicular development in estrous bitches remained to be elucidated.

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033 | Antibiotic use for bitches with apparent infertility: a retrospective study

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Introduction: During the last decades, human and veterinary professions share concerns about the use of antibiotics and the development of microbial resistance (1). Guidelines and recommendations have been published for treatment of various disease but are still lacking in the context of bitch apparent infertility. True infertility is

characterized by an inability to achieve conception. Bitches who did not produce puppies after breeding, are often qualified as "infertile". Bacteriological test and identification of Mycoplasmas can be performed. When highlighting an unbalance of the vaginal flora an antibiotic therapy is implemented. The use of antibiotics in this context and in absence of clinical signs is a current subject of controversy (2, 3).

The aim of this study was to provide an overview of the current situation in the area of Lyon, regarding antibiotic use for bitches with apparent infertility.

Materials and methods: All the oestrus follow-ups beginning with vaginal bacteriological tests were recorded since 2014. Breed, previous history of failure to conceive, test performed (bacteriological and/or Mycoplasmas examinations) and their results were recorded. Bacteriological test was considered abnormal when one (or two) germ(s) predominate; an antibiogram was therefore performed, and a three-weeks antibiotic therapy was established accordingly. Specific isolation of Mycoplasmas was associated with quantification. The presence of Mycoplasmas above a dilution 10^{-4} was considered as abnormal. In this case, a treatment based on josamycin (25 mg/kg, TID during 3 weeks) was implemented. When available, antimicrobial resistance and outcomes were recorded.

Results: In total, 152 consultations for bacteriological tests were recorded (150 bacteriological tests performed and 136 Mycoplasmas examinations). Between 2014 and 2018, the number of consultations for bacteriological tests and/or Mycoplasmas identification at the beginning of the oestrus was quite constant (33, 30, 25, 19 and 28 from 2014 to 2018). In early 2019, this number increased a lot: 17 consultations for bacteriological identification were noticed in only 2 months. Amongst these cases, the number of abnormal bacterial test varied greatly from year to year, representing 33 to 84% of bacteriological test performed. The number of tests with abnormal quantities of Mycoplasmas varied from 60 to 91% of mycoplasmas test performed depending on the years. Microbial resistance to at least one antibiotic was founded in 67 to 83% of bacterial test underscoring unbalance of the vaginal flora. Multi-resistant bacteria (resistance against 4 antibiotics families at least) were scarce. It represents 11 cases amongst the 150 bacterial tests performed between 2014 and February 2019. From 2014 to 2018, pregnancy diagnosis was available in only half of the cases (71/135 consultations). The pregnancy rate was 69% (i.e. 49 positive pregnancy diagnosis/71 pregnancy diagnosis). The mean litter size was 3.8.

Conclusions: Many situations result to an apparent infertility in bitch. Identification of infectious causes must be performed after excluding more common causes. Despite laboratory tests highlighting an unbalance of the vaginal flora, it is still difficult to affirm that "this caused the infertility". Use of antibiotics in absence of clinical signs is questionable. Increase of microbial resistance and multi-resistance is not obvious in this study. This can be reliable to the absence of strategy change for antibiotics treatments during these last 5 years. Data concerning outcomes (fertility and prolificity) should be interpreted carefully due to the absence of a control group.

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034 | Comparison of endoscopic-assisted transcervical and Norwegian insemination: a retrospective clinical study

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Introduction: Transcervical insemination is frequently requested by breeders and performed by specialists, especially in subfertile bitches. As well as using frozen semen, this method may provide higher whelping rates and litter sizes than vaginal insemination with fresh semen (1). Two transcervical techniques are described and recognized as viable technique: the use of Norwegian catheter and the endoscopic-assisted method (usually named TCI) (2). Direct comparison, with a large number of cases, between reproductive performance following these two techniques are still lacking. The aim of this retrospective study was to compare the two transcervical techniques, using fresh or frozen semen.

Materials and methods: All the bitches presented for oestrus follow-ups and artificial insemination from 2006 to 2018 were recorded. Most bitches were presented with history of failure to conceive or hypo-fertility, which may cause a bias. Inclusion criteria were: bitches inseminated transcervically with fresh or frozen semen and with ultrasonographic diagnosis of pregnancy. Only cases with complete history (number of puppies born) were selected to analyse prolificity. Bitches inseminated with both techniques were excluded. Statistical analysis were performed using Excel (2016) and RStudio (2018). The level of significance was set at $p < 0.05$.

Results: A total of 443 cases was recorded.

Regardless the technique used, the success rate with fresh semen was 79% and 62% with frozen semen. This difference was statistically significant ($\chi^2 = 11.058$, df = 1, p-value = 0.0008828). The mean litter size was 6.3 with fresh semen and 5.1 with frozen semen. This difference was significant.

Regardless the semen preparation, Norwegian catheter was used in 172 cases while TCI was performed in 271 cases. The success rate (percentage of pregnant bitches) was 73% with Norwegian catheter and 76% with TCI, with no significant difference. The number of puppies born was known for 318 cases (130 cases using Norwegian

catheter and 188 cases using TCI). The mean litter size was 6.0 using Norwegian catheter and 6.2 using TCI, with no significant difference. Considering the semen preparation, fresh semen was used in 337 cases and frozen semen was used in 106 cases.

* For insemination using fresh semen, Norwegian catheter was used in 137 cases, the success rate was 78%. TCI was used in 200 cases, the success rate was 80%. There was no significant difference. A full follow-up was available for 232 cases with 161 pregnant bitches. The mean litter size was 6.2 when Norwegian catheter was used and 6.7 when TCI was performed, with no significant difference.

* For insemination using frozen semen, Norwegian catheter was used in 35 cases, the success rate was 54%. TCI was used in 71 cases, the success rate was 66%. This difference was not statistically significant. A full follow-up was available for 86 cases with 46 pregnant bitches. The mean litter size was 4.9 when Norwegian catheter was used vs. 5.0 when TCI was performed. This difference of mean litter size was not significant.

Conclusions: These results suggest that TCI and use of Norwegian catheter are similar in terms of reproductive performance, with fresh and frozen semen. Both transcervical techniques proved to be suitable and effective in assisted reproduction, on the condition that inseminations are carried out at the right time. Further studies are needed to evaluate the impact of the technique employed when chilled semen is used.

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035 | Intra-testicular injection of GnRH-modified Chitosan Mediated Tumor Necrosis Factor Alpha Gene as a Nonsurgical Sterilization: A Rat Model

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Introduction and aim: Intra-testicular injection of chemical sterilants, for example zinc gluconate, calcium chloride, plays an alternative method in addressing the issue of animal overpopulation control. The big obstacle of this method is a severe adverse inflammatory reaction resulting in tissue necrosis. The challenges are to minimize the side effect using the natural compound for reducing the severity of tissue damage and testicular toxicity, while it remains effectiveness of permanent infertility. GnRHR are expressed in Spermatogenic cells including Leydig and peritubular myoid cells (1). Recently, we reported that gonadotropin-releasing hormone-modified chitosan (GnRH-CS) is a safe and efficient gene delivery vector for targeted DNA delivery to GnRHR-expressing cells (2). In addition, our previous study showed that GnRH-modified chitosan delivers a transgene of interest to spermatogonia cells (3). The present study aim at investigating *in vivo* changes in the testicular tissue after intra-testicular injection of GnRH-modified chitosan mediated tumor necrosis factor alpha gene (GnRH-CS/TNF-α) in the rat model.

Materials and methods: GnRH-CS was used as a gene vector mediated TNF-α to induce testicular cell death via intra-testicular injection. The research was performed according to procedures approved by the Chulalongkorn University Laboratory Animal Center (CULAC) (Protocol number 1773017). Sixteen mature male rats were divided

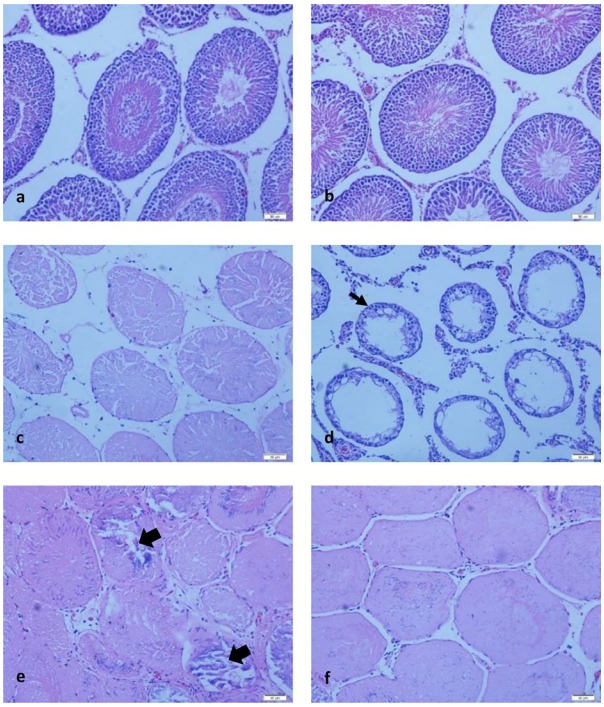

Figure 1. Microscopic appearance of the testes on Day 35 (×200 magnification, H&E). Testicular histological sections of a control rat (a) and a TNF-α treated rat (b) demonstrated normal seminiferous tubules and interstitial tissue. Seminiferous tubules obtained from a GnRH-CS treated rat (c and d) appeared normal interstitial tissue, intratubular coagulative necrosis (c), tubules lined by Sertoli cells remain (thin arrows) and tubular vacuolization (d). Intra-testicular injection of GnRH-CS/TNF-α (e and f) induced extensive intratubular hyalinization, coagulative necrosis, lymphocytic infiltration, collapsed seminiferous tubules, mineralization (broad arrows) and spermatid retention (f).

into 4 groups (n = 4 per group): control (without injection), TNF-α (naked DNA), GnRH-CS (naked nanoparticle) and GnRH-CS/TNF-α (nanoparticle/DNA). The animals were anesthetized before single bilateral intra-testicular injection with 700 μL of solution per testis. Body weight, testicular volume, testicular weight and serum testosterone were evaluated before (Day 0) and after intra-testicular injection (Day 7, 14, 28 and 35). The animals were euthanized on day 35 and the testes (n = 8 per group) were removed, weighted and examined histologically. Moreover, liver, kidney and urinary bladder were subjected to histological procedure because they also contain GnRH receptors.

Results: Neither a reduction in the body weight ($p > 0.05$) nor evidence of testicular inflammatory reactions (pain, redness or swelling) was observed throughout the study period. Decreases in testicular volume on day 7 were found in all treated groups (TNF-α, GnRH-CS and GnRH-CS/TNF-α), compared to the pre-treatment volume ($p < 0.001$). The testicular volume continued to reduce on day 14 ($p < 0.05$) and 28 ($p < 0.05$) in GnRH-CS/TNF-α and GnRH-CS groups, respectively. On day 35, testicular weight and volume of dissected testis were significantly lower in GnRH-CS/TNF-α and GnRH-CS groups ($p < 0.001$). Serum testosterone levels did not differ throughout the observation period ($p > 0.05$). Histopathologically, GnRH-CS/TNF-α and GnRH-CS induced testicular degeneration while TNF-α and control groups showed normal findings (Fig. 1). GnRH-CS/TNF-α treated animals showed higher severity degree of testicular degeneration compared to GnRH-CS treated animals (Fig. 1). Histological findings of the liver, kidney and urinary bladder were normal in all animal groups.

Conclusions: Intra-testicular injection of GnRH-CS/TNF-α provided an alternative method to induce testicular cell death without any severe side effects. The potential use of GnRH-CS/TNF-α for nonsurgical castration is promising, particularly the population control of dogs and cats. However, long-term efficacy of intra-testicular injection of GnRH-CS/TNF-α for male infertility remains an interesting issue for further research.

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036 | Clinical use of Sperm Chromatin Structure Assay in feline subfertility diagnosis – case reports

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Introduction: Basic semen analysis (sperm motility, viability, morphology, etc.) does not always provide enough information on the fertilizing potential of a male. Sometimes the male faces fertility problems despite good semen quality assessed by conventional methods (1). The reason is that some "hidden" defects, as sperm DNA fragmentation, may also lead to reproductive failure, suggesting that it should be evaluated as part of male infertility assessment. As numerous studies showed significant difference in sperm DNA integrity between fertile and subfertile bulls and men (2), tests as Sperm Chromatin Structure Assay (SCSA) are almost routinely used in infertility diagnostic procedures in human medicine. There are no information of the use of this test in clinical practice for cats.

The aim of this study was to present cases when SCSA was used to diagnose fertility problems in pure breed domestic cats.

Material and methods: Three male cats (Ragdoll and Maine Coon both 1.5 year old, and Siberian Forest Cat, 6 years old) were admitted independently to the Ambulatory of Department of Reproduction, Wroclaw University of Environmental and Life Sciences with fertility problems. In Ragdoll and Maine Coon the issues were small litter sizes and kittens born with congenital abnormalities. Siberian Cat, previously fertile, has been unsuccessful in several consecutive matings with different females. Cats were examined clinically and semen was collected via urethral catheterization after medetomidine administration (3). Sperm samples were assessed for motility, viability and morphology (after eosin-nigrosin staining), as well as for membrane (SYBR-14), acrosome (Lectin PNA) and chromatin (SCSA test) integrity evaluated with flow cytometry. Additionally, preputial swabs were taken for microbiological examination.

Results: All cats were clinically healthy. In young cats clinical examination and basic semen assessment revealed no abnormalities. However, in Maine Coon DNA Fragmentation Index (DFI%) was 18.8%, in Ragdoll DFI% was 5.14% and High DNA Stainability (HDS%) was shown by 33.3% of spermatozoa. Siberian Cat was overweight and showed high percentage of abnormal spermatozoa, including 10% of sperm cell with abnormal heads. DFI% in his sperm sample was 25.0%. Other sperm parameters were within normal range.

Conclusion: As the average DFI% in cat population measured in our other study was 3.2% and HDS% was 2.3% (4), and as in humans the threshold for DFI% is 19.9% (5), we can suppose that fertility problems in these tomcats were caused by abnormal DNA structure. These case reports prove the usefulness of SCSA test in the diagnosis of feline subfertility and highlight the need for advanced semen assessment in the cases when basic semen evaluation reveals no pathologies.

This study was supported by statutory research and development activity funds assigned to Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences

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037 | Neonatal mortality in Labrador Retriever puppies

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Introduction and aim: Neonatal viability continues to pose a great challenge for both veterinarians and breeders. Despite continuous efforts, mortality rates in dogs remain stubbornly high. The great variation in morphology, growth rates and weight amongst different dog breeds generates the need to develop criteria for neonatal health evaluation. This study aimed to measure and identify significant predictive factors for neonatal mortality in a Labrador Retriever kennel.

Material and methods: Our study included 148 Labrador Retriever puppies born from 16 different dams from the same breeder. Each parturition was monitored by the authors. At birth, puppies were classified according to the Apgar score (1). The time of birth of each puppy was registered. Their bodyweight and mortality were recorded from birth until 8 days of age. Early neonatal mortality was defined as the number of born-alive puppies that died before 8 days of age. Total perinatal mortality was defined as the total number of puppies (including stillbirths) that died before 8 days of age. Data analysis and statistics were performed using SPSS Statistics 23.0 ® for Windows. Binary logistic regression models were developed for the prediction of stillbirth, mortality until 48 h, mortality between Days 2 and 4 and mortality between Days 4 and 6. Receiver operating characteristic curves were analyzed to determine cut-off values for neonatal mortality.

Results: Total perinatal mortality was 27.70% (n = 41), of which 53.66% were still-births (n = 22). Early neonatal mortality was 12.84% (n = 19). The most significant predictive factor for stillbirth was the increase of inter-pup interval (p = 0.003; cut-off = 1 h 27 min), with an average inter-pup interval of 2h37 min ± 34 min. An Apgar score at or below 8 was identified as a significant predictive factor for mortality occurring until 48 h after birth (p = 0.01). A difference of bodyweight registered between birth and Day 2 below −1.11% was associated with a higher risk of death between Days 2 and 4 (p = 0.026). Similarly, a weight gain below 4.18% between Day 2 and 4 was a significant predictive factor for mortality between Days 4 and 6 (p = 0.026).

Conclusions: Mortality rates were higher than in other studies performed in the same breed (2), (3). In our study, data was collected through observation while other studies mentioned were questionnaire or registration-based. This might have partially contributed to the high mortality rates shown. Considering the average inter-pup interval was higher than the cut-off value for higher risk of stillbirth, it can be inferred that a significant amount of puppies could have survived had there been prompt veterinary assistance. The inter-pup interval cut-off values should become useful guidelines for the breeder for an earlier intervention in delivery management. Additionally, the cut-off values for weight gain and mortality between days 2 and 6, should be used as criteria to be considered for milk replacement supplementation and/or veterinary consultation.

The results shown highlight the importance of developing not only breed but also breeder-specific criteria for the identification of risk factors for neonatal mortality as they can become useful tools for overall improvement of kennel management and productivity.

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039 | Structure of *Escherichia coli* population in bitches diagnosed with pyometra

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Introduction and aim: Pyometra is one of the most common reproductive diseases in intact bitches. *Escherichia coli* is isolated from the uterus of up to 90% of the bitches diagnosed with pyometra. Pyometra *E. coli* isolates derive from the host's fecal and perineal flora and are mainly assigned to the phylogenetic group B2 and are characterized by a high number of uropathogenic *E. coli* (UPEC) VF genes and pathogenicity-associated islands markers (1). Peritonitis and urinary tract infection (UTI) are two possible complications of pyometra (2, 3). Therefore, the purpose of this work was to characterize the population structure of *E. coli* present in the uterine, urinary, extrauterine and fecal microbiota in pyometra bitches.

Materials and methods: A total of sixteen bitches with confirmed diagnose of pyometra due to *E. coli* were included in this study. For each animal four samples were collected: a rectal swab (before surgery), an extra-uterine swab (around the two uterine horns) and urine by cystocentesis, both during surgery and an intra-uterine swab, after surgery. Up to 10 colonies per sample (as available) of suspected *E. coli* were randomly picked from MacConkey plate agar medium, and confirmed by PCR screening for the presence of *E. coli* 16S rRNA. Phylogenetic group and clonal relationships among *E. coli* isolates were assessed by PCR and REP-PCR, respectively as described by (4) and (5). Results

The presence of peritonitis and UTI were found in five bitches (two of them had both co-morbidities). A total of 383 isolates were obtained, of which 160 were from uterine samples, 35 from urinary

samples, 42 from extra-uterine samples and 146 from fecal origin. Independently of the origin of the isolates, the most prevalent phylogenetic group was group B2 (uterine isolates: 93.8%; extrauterine isolates: 100%; urine isolates: 94.3%; fecal isolates: 79.5%). The 383 isolates were descriminated in 20 clones, with a mean of 1.25 clones/bitch. 81.3% of the bitches had only one B2 clone, common to all sample sources in which isolation was positive. Only one bitch had one B1 clone, common to uterine, urinary and fecal samples. Phylogenetic groups D and A were only identified in fecal isolates (in two bitches).

Conclusions: These results confirm that B2 *E. coli* isolates are more prone to cause disease in canine uterus. The fact that the majority of fecal *E. coli* isolates belonged to phylogenetic group B2 highlights the importance of companion animals as a reservoir of potentially extraintestinal pathogenic *E. coli*. In cases of *E. coli* pyometra with a concurrent subclinical urinary tract infection and/or peritonitis, it is likely that all infections are caused by the same bacterial strain.

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040 | Uterine stump adenocarcinoma with lung metastasis in a 14-year-old bitch

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Canine endometrial adenocarcinoma is rare, occurring mostly in intact middle-aged to geriatric bitches (1). A clinical case of a uterine stump adenocarcinoma is reported.

Clinical Case: A 14-year-old mongrel bitch (10.8 kg) was presented at the Teaching Hospital of the Faculty of Veterinary Medicine, with a persistent vulvar muco-sanguinolent discharge for the last two weeks, mild constipation and pollakiuria. She had been ovariohisterectomised five years before.

Findings on referral: At vaginoscopy, sanguineous fluid was noted, but no lesion was identified. At vaginal cytology, large agglomerates of pleomorphic cells and multinucleated vacuolated cells were observed. Rectal digital exam revealed a firm pelvic mass located dorso-caudal to the bladder. Complete haematology and biochemistry blood analysis revealed a slight increase in alkaline phosphatase values (238 U/L at 37°C; range 0 – 200). Serum progesterone concentration was basal. Abdominal ultrasound revealed and enlarged uterine stump (46 × 19 mm), with solid and fluid filled areas. A CT scan revealed an oval soft tissue density mass (44 × 25 mm) with heterogeneous contrast uptake with hypodense pockets, suggestive of collection of fluid or necrosis. The mass was partially localized in the caudal abdomen and pelvic cavity causing a dorsal deviation of the descending colon. A mild iliac lymphadenomegaly was observed with a homogeneous contrast uptake. A nodular lesion (13.7 × 9.2 mm) was detected in the lateral aspect of the left lung close to the costal wall, at the level of the 7th and 8th ribs. Both lesions were subject to an ultrasound-guided fine needle aspiration with similar cytological results. Cytology registered in both cases epithelial cells plaques with acinar-like arrangement; epithelial cells were basophilic with large nucleus; multinucleated vacuolated cells were also observed. The diagnosis was of uterine epithelial neoplasia metastasising in the lung. Despite the lung metastasis, surgery was agreed with the owner to alleviate the symptoms.

Exploratory coeliotomy: Abdominal access was performed after a midline xiphoid-pelvic incision. A well-marginated mass, slightly adherent to the bladder and terminally located at the level of the hysterectomy scar was identified. No remnant ovarian tissue was detected. Marginal excision of the mass was performed together with the enlarged iliac lymph node. Amoxicillin with clavulanic acid (15 mg/kg BID for 8 days) and robenacoxib (2 mg/kg SID for 3 days) were prescribed after surgery.

Histopathology: An endometrial epithelial neoplasia was identified with extensive areas of suppurative necrosis and the cells arranged in tubules and papillae. Tumour cells were pleomorphic with evident anisokaryosis; cytoplasm was scant and the nucleus ovoid and clear with a prominent nucleolus. Multinucleated cells were present. Mitoses were less than one per high power field. The neoplasia invaded the myometrium and there were no signs of vascular permeation and no neoplastic cells were observed in the lymph node.

Follow up: After surgery recovery, firocoxib (5 mg/kg SID), toceranib (3.25 mg/kg q48 h) and omeprazole (1 mg/kg SID) were prescribed. Two months after the surgery, the bitch started losing weight and became anorectic, prostrate, dyspnoeic and febrile. On ultrasound, the lung mass had increased in size (21.3 × 25.5 mm) showing evidence of central necrosis. Two days after hospitalization, the owners declined any further intervention and the bitch was euthanized.

Discussion: Although uterine neoplasia is rarely observed, it is usually found in intact female bitches (1, 2). Nevertheless, uterine adenocarcinoma may still be a differential diagnosis in a spayed female (3). In addition, this case highlights the importance for searching of metastases and draws attention to the significance of vaginal cytology in any case of vulvar discharge as an important diagnostic tool.

Funding: UID/CVT/00276/2019 from Foundation for Science and Technolo.

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042 | Melatonin implants to control estrus in Belgian breeding queens

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Introduction and aim: Seasonal anestrus in the queen is associated with high levels of melatonin. The use of subcutaneous melatonin implants developed for ovine estrus stimulation (Melovine® 18 mg; CEVA santé animale) has been tested in queens with variable results depending on environmental conditions. The aim of this clinical report is to evaluate the use of melatonin implants in field conditions in Belgian catteries.

Materials and methods: 13 melatonin implants (Melovine® 18 mg) were injected subcutaneously in the neck of 12 pubescent and 1 prepubescent queens. All queens were in interestrus or in prepubescent anestrus. Absence of estrus was confirmed by vaginal smear stained with a modified Harris-Shorr (Kit Diag-Oestro®, RAL Diagnostics, France). Queens were considered not in estrus if less than 70% of the cells were keratinized. The owner was asked to report signs of estrus during the 10 first days following the injection and the duration of the subsequent interestrus. Observed matings after return to cyclicity and potential pregnancies were also recorded. Implants were considered efficacious if interestrus lasted more than 6 weeks.

Results: Pubescent queens: implants were efficacious in 11/12 queens with a mean duration of action (from implantation to estrus) of 106 + /- 40 days. 4/11 presented mild signs of estrus with meowing for 2 to 3 days within 10 days post implantation but none was mated during these periods. The queen that failed to respond had a vaginal smear of 70% of keratinized cells at the time of implantation with a history of a 3 week-long estrus ending the day before. It was however decided to try implantation on this unclear case. The next estrus started the very next day to last for another 3 weeks. The breeder then decided to mate her and she got pregnant. Following this pregnancy, this same queen was implanted 3 times during the breeding season on a confirmed absence of estrus and the mean duration of the provoked interestrus was 55 + /- 8 days. One fourth implantation was done 6 weeks post-partum, in October, while she was lactating and a prolonged anestrus until mid-February (130.9 days) was observed. There was no interference with milk production and the kittens were weaned 3 weeks after implantation. A different queen was also implanted in October, when natural secretion of melatonin increases, and anestrus was also prolonged until mid-February. These two cases with increased endogenous secretion of melatonin may have affected the overall efficacy of implants to postpone estrus we report here. However, 3 other queens resumed cyclicity after implantation in November and December. After cyclicity resumption, 6 queens were successfully mated while another one, where no successful cover was observed, failed to get pregnant. That queen was subsequently neutered. The prepubescent queen was 6 months old when implanted, estrus appeared 52 days after.

Conclusions: Melovine® was safely used on one lactating queen but its innocuousness should be confirmed by larger studies. Our results also suggest that melatonin should be considered to prolongate post-partum anestrus. Mean duration of action is comparable to that previously reported in French and Italian catteries. Some queens showed signs of estrus in November and December while they should have been in seasonal anestrus. Late in the year implantation of melatonin may provide a promising tool to obtain seasonal anestrus in queens that cycle all year round. In our field conditions, the one prepubescent implanted queen showed first estrus signs at the age of 7.5 months, which is normal for puberty. This is in agreement with results obtained under controlled conditions, where no significant delay in first estrus occurrence was observed. Our results support the observation that fertility does not appear to be compromised by melatonin implants, making it a viable option for cyclicity control in catteries.

043 | Development of the digestive microbiota in puppies

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Introduction and aim: Bacteria of the digestive tract (digestive microbiota) are part of an intricate ecosystem playing a role in the development of the immune system, defense against pathogens and digestion. In cases of altered microbiota development, a newborn is at higher risk of death due to necrotizing enterocolitis (1). Not only short-term but also long-term diseases, such as chronic enteropathies and chronic lung disease, develop more frequently in adulthood in individuals with perinatal intestinal dysbiosis. Although development of the digestive microbiota has been recognized as critical for good health in humans, limited information is available describing the development of the neonatal digestive microbiome in dogs.

Materials and methods: Feces from puppies (n = 30; 10 different breeds; between 1 and 5 puppies for each breed) were collected at 2, 21, 42 and 56 days after birth via rectal swab. Feces from the puppies' mothers (n = 16) were collected in the same manner once within 24 hours after parturition. All samples were stored at −20°C until assay. All litters were housed within one breeding kennel with the same environmental conditions and the same diet was fed to all included bitches and their puppies (puppies from postnatal day 21). All puppies were born by natural delivery. DNA was extracted from fecal samples using ZR fecal DNA Mini Prep Kit (Zymo Research, Irvine CA, USA). 454-pyrosequencing was used to profile 16S rRNA genes

(QIIME pipeline) and quantitative PCR (qPCR) was performed to quantify bacterial groups underrepresented in sequencing data. Alpha and beta diversity, and Linear Discriminant Analysis Effect Size (LEfSe) were used to compare bacterial profiles within different timepoints.

Results: A total of 136 samples were analyzed in this study. In puppies, species richness (evaluated via alpha diversity) continued to increase significantly from 2 days of age until 42 days of age. Furthermore, microbial communities clustered separately from each other at 2, 21 and 42 days of age (evaluated via beta diversity). The microbial communities belonging to dams clustered separately from that of puppies at any given time point. Major phylogenetic changes were noted at all taxonomic levels with the most profound changes being a shift from primarily Firmicutes in puppies at 2 days of age to a co-dominance of Bacteroidetes, Fusobacteria and Firmicutes by 21 days of age.

Conclusions: This is the first time the digestive microbiota profile has been evaluated in growing puppies (2). Puppies go through great changes of their digestive microbiota, most probably corresponding to physiological changes, such as birth, then exclusive maternal milk ingestion during at least the first three weeks of life, then mixed food (maternal milk and kibbles) between 3 and 6 weeks of age and finally almost exclusive solid food (kibbles) at 8 weeks of age. Although in our study all puppies ingested solid food at 56 days of life, the microbial profile was still quite different from that of the mother. The age at which a stable microbial profile is established in the dog remains to be determined. Further studies are needed to elucidate the relationship between puppy microbiota development, physiological growth, neonatal survival and morbidity.

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044 | Exogenous uptake of estrogen as a differential diagnosis to Ovarian-remnant-syndrome

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Clinical case: This case report describes the clinical case of a 6.5 years old spayed crossbreed bitch weighing 8.4 kg which was exposed to exogenous estrogen over several months. The castration was performed in Romania within the first 6 month after birth. The bitch was presented on the 10th of January 2019 in the clinic because of a suspected ovarian-remnant-syndrome (ORS). She was of good general condition with no abnormalities in the clinical physical examination. She showed pruritus of the vulva and inguinal area, a prominent edema of the vulva, alopecia on both body sides and clear vaginal discharge. A vaginal cytology was performed which showed a high influence of estrogen because of the presence of anuclear cells and superficial cells exclusively. A vaginoscopy could not be performed because of the heavy defence reaction of the dog. A sonographic examination of the abdomen showed a prominent stump of the cervix. Caudal of both kidneys there was no signs of ovarian tissue. A blood sample was taken to examine the concentrations of anti-***M****üllerian*-hormone (AMH), luteinizing hormone (LH), progesterone (P4), estradiol-17ß (E17ß), blood profile and parameters of the kidneys (creatinine and urea) and the liver (ALT). The blood profile showed no abnormalities. The parameter of the liver was complying with the standard. The parameters of the kidneys were slightly increased with 169 µmol/mL creatinine (reference up to 159 µmol/mL) and 9.72 mmol/mL urea (reference up to 9 mmol/mL). The level of estradiol-17ß was 20.7 pg/mL. The progesterone concentration was to 0.36 ng/mL. Concentrations of AMH and LH were < 0.01 ng/mL and 0.1 ng/mL respectively. Throughout the conversation with the owner it was elucidated that she was using an estradiol-spray for nearly 1.5 years because of menopausal problems. Additionally, the dog was treated with delvosteron with no success which could be an explanation of the low LH concentration together with a negative feedback of the high estradiol concentration in the peripheral blood. This information together with the clinical and laboratory findings led to the suspected diagnosis of heat symptoms due to the influence of exogen estrogens. It was recommended to the owner to end the local estrogen therapy. To verify the diagnosis the dog was presented again on 6th of February 2019. At this time only a slight edema of the vulva was still present, there was no vaginal discharge and the vaginal cytology showed intermediate cells. Only the alopecia was still prominent. On the third consultation on the 18th of March the edema had completely disappeared, there was no vaginal discharge and the cytology showed only parabasal and basal cells. The alopecia was still obvious but a regrowth of the fur around the teats was noticeable. So it was obvious that there was no other source of estrogens besides the spray used by the owner.

Discussion: Based on this case report it could be shown that exogenous uptake of postmenopausal human estrogen drugs via the owner can be a differential diagnosis to ORS and should kept in mind when dealing with a dog with symptoms mentioned above.

046 | Who are dog breeders?

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Introduction and aim: Breeding practices in farm animals are well known and constantly improved for example thanks to dedicated national institutes. In companion animals, such as dogs, the institutional support given to dog breeders is rather poor and breeders' education background is most often not associated with animal breeding, making this sector underdeveloped. In order to supply breeders with recommendations adapted to kennel conditions, obtaining data on their everyday practices is needed. The aim of our study was to describe everyday practices of dog breeders in French kennels thanks to a series of questionnaires.

Materials and methods: Three questionnaires were sent to all followers of NeoCare group via Facebook and via mailing list (n = 3198 and n = 1515, respectively; mostly dog and cat breeders) in 2018. Each questionnaire consisted of a series of 10–15 questions in French on three different topics: breeder characterization, breeder education, puppies' selling.

Results: The number of participants in the different questionnaires was 151 (breeder characterization), 109 (breeder education), 125 (puppies' selling). Breeders were mostly from France (148/151; 98%), with 32.9% (48/146) of breeders having less than 5 years and 13.0% (19/146) having more than 20 years of experience. Only 9.3% (10/108) of breeders had no certificate allowing to breed dogs in France according to the French law. The majority of participants (62.7%; 94/150) bred only one dog breed, whereas only 2.0% (3/150) of them bred more than 5 breeds at the same time. Apart from breeding, some breeders provided also other services onsite such as boarding kennel (12%; 18/150), kibbles sale (8%; 12/150) or dog training (7.3%; 11/150). The average number of dogs housed per kennel (mean ± SD) was 13.4 ± 14.2, with 36% breeding bitches, 17% sires, 25% prebreeding stock and 22% retired. On average, 1.1 ± 0.4 persons worked at the kennel at full time. Staff working at the kennel consisted of breeder him/herself in 99.3% of kennels; family members in 19.9%; trainees in 5.3% and employees in only 2%. Mean number of puppies/kennel sold in 2018 was 20.0 ± 30.4. One fourth (25.2%; 31/123) of puppies left the kennel at 8 weeks (the minimal age at sale according to the French law), 30.9% (38/123) at 9 weeks, 25.2% (31/123) at 10 weeks and only 18.7% (23/123) above 10 weeks of age. Few breeders (1.6%; 2/123) sold puppies neutered while others (13.0%; 16/123) included a request of neutering as a general term of sales contract. Only 23.4% (92/124) of breeders sold puppies systematically insured.

Conclusions: This study allowed to better characterize French kennels and dog breeders in terms of their general profile (treated in the above study), but also other fields of canine breeding, which remain to be presented elsewhere. Such analysis of kennels could be the first step aiming to structure the canine breeding sector, followed by development of science-based recommendations.

047 | Physical, histological, endocrinological and steroidogenical evaluation of male cats postnatally exposed to sexual steroids

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Introduction and aim: To test the hypothesis that the administration of sexual steroids during the critical postnatal time window induces an impairment of domestic cat reproductive function (1,2), this study describes the clinical, endocrine, steroidogenical and histological effects of a single, high dose of a time-released androgen and a progestin on domestic male cat reproduction. Secondarily, the clinical safety of these pharmaceutical protocols was also evaluated.

Materials and methods: Twenty littermate male kittens were randomly assigned to one of the following treatment groups within the first 24 hours of birth: testosterone enanthate 12.5 mg total dose subcutaneously (TE; n = 8), medroxyprogesterone acetate 10 mg total dose subcutaneously (MA; n = 6), or Placebo: 0.05 mL corn oil subcutaneously injection (PL; n = 6).

The cats were behaviorally and physically examined until puberty when they were castrated and placed for adoption. Fecal samples were also collected on weeks 1 to 4, 7 and 10 for testosterone (T) measurement. The testes were processed for histomorphometrical examination and quantification of steroidogenic enzymes as well as androgen receptors (AR) by real-time polymerase chain reaction (PCR).

Results: In the first 4 weeks, T values were high, basal and intermediate in TE, MA and PL ($p < 0.05$), respectively. These differences progressively diminished and the three groups presented basal T concentrations at the 7^{th} and 10^{th} week ($p > 0.05$).

Kittens achieved puberty without differences among groups (190.4 + 24.2 vs. 162.4 + 6.3 vs. 221.7 + 23.7 days for TE, MA and PL, respectively; $p > 0.05$). One MA cat presented unilateral abdominal cryptorchidism and another a unilateral delayed descended testis. None of the animals presented other clinical side effects ($p > 0.1$).

Microscopic assessment revealed that all the males presented normal epididymal sperm motility and morphology. Histological evaluation of the MA ($p < 0.01$), but not of TE testes revealed decreased diameter ($p < 0.01$) and epithelial height ($p < 0.01$) of the seminiferous tubules due to the diminished tubular number of most of their cellular components ($p < 0.01$). Spermatids/Sertoli ratio and the Leydig cell nuclear area were also reduced in MA. Conversely, tubular/intertubular ratio was increased in TE animals ($p < 0.01$).

Quantitative real-time PCR analysis of mRNA expression revealed no significant differences among groups for steroidogenic acute regulatory (STAR)-protein and 17α- hydroxylase/ 17.20-lyase (P450c17). Conversely, TE caused substantial decrease in aromatase cytochrome P450 (P450arom) mRNA expression ($p < 0.05$). Neither the androgen nor the progestin affected mRNA level expression of AR ($p > 0.1$).

Conclusions: It was concluded that the postnatal progestagen initially suppressed the gonadal axis and caused an impairment of spermatogenesis and a 33% incidence of abnormal testicular descent. Conversely, the androgen treatment generated downregulation of the final steroidogenic cascade without provoking testicular alterations.

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048 | Anti- Müllerian hormone concentration in female gray wolf (*Canis lupus*) – preliminary results

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Introduction and aim: Anti-Müllerian hormone (AMH) is considered an attractive biomarker to assess fertility potential, gonadal function and follicular reserve due to the constant expression by ovarian granulosa cells (1). For domestic carnivores, AMH can be used as a potential predictive marker of fertility (2) and to predict the success of in vitro maturation (3). Data regarding AMH in exotic carnivores is limited, especially for canid species. In cheetahs, AMH concentrations are lower in older females and in females treated with contraceptives (4); no data for wolves are available. The objective of this study was to measure AMH concentrations in female gray wolves.

Materials and methods: Blood was collected from five female gray wolves during breeding (winter) and nonbreeding (summer) seasons for two consecutive years. A total of four samples were collected from each female. All females were under contraceptive treatment (deslorelin acetate) in 2016, however in 2017 all of them gave birth to healthy cubs, indicating that in 2017 breeding season they were no longer under deslorelin effect. AMH concentrations in the serum were measured using a canine AMH ELISA (Ansh Labs LLC, Texas, USA).

Results: AMH mean ± SD was 1.48 ± 1.59 ng/mL and 2.63 ± 0.93 ng/mL, during winter of 2016 and 2017, and 0.55 ± 0.70 ng/mL and 0.49 ± 0.26 ng/mL during summer of 2016 and 2017, respectively. Although AMH concentration was higher during breeding season, no differences were observed among both summer season and 2016 winter. Furthermore, 2017 winter had higher AMH levels when compared to 2016 and 2017 summer ($p < 0.05$). Since all females were under contraception in 2016, we believe that those low AMH levels during that winter are due to deslorelin effect. Similar results were described in cheetahs under contraceptive treatment (Place et al., 2017). In 2017, an AMH rise was detected in all females who had low AMH in 2016, indicating that AMH may be used to assess successful

contraceptive reversibility. Furthermore, our data reveal that AMH production in wolves is modulated by seasonality, as was also observed in Siberian hamsters (5) and beluga whales (6).

Conclusions: So far, these results indicate that, in female gray wolves, AMH production is lower during the nonbreeding season and in females are under deslorelin acetate contraceptive treatment. More samples will be collected in other to confirm these results.

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050 | Comparison of CEA and CA 15-3 levels in serum of the bitches with mammary tumors and in healthy ones

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Introduction and aim: Canine mammary gland tumors (CMT) are the second, most common neoplasia in dogs [1]. In addition, nearly 50% of CMT are malignant and therefore it represents a serious clinical problem. Due to similar immunohistochemical features of CMT and human breast cancer (HBC), evaluation of the same biomarkers may be considered. Biomarkers are substances (usually proteins), which levels can be measured in the blood as well as in other tissues (e.g. in tumor), and obtained results can provide us information about the presence of the disease, response to treatment and further prognosis for the patient. The most frequently studied HBC biomarkers are carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA 15–30 [2]. In veterinary medicine, there have only been a few studies in which detection of these biomarkers and initial determination of its concentration in both healthy as well as in bitches with CMT was done [2–4]. However, the comparison between concentrations of these biomarkers between healthy and bitches with CMT has not been done so far. The aim of the study was to determine the blood levels of CEA and CA 15-3 in bitches with CMT as well as in the blood of healthy bitches and to compare these results.

Materials and methods: 85 blood samples, of which 77 were taken from bitches with CMT (59 with malignant CMT, 11 with benign CMT, and 7 with non-neoplastic changes) and 8 samples from healthy bitches were evaluated using Commercial Canine CA 15-3 and CEA ELISA KITs (MyBiosource). The average age of bitches with CMT was 9.8 ± 2.4. The statistical analysis was done using Statistica 13.3 program (TIBCO Statistica). Quantitative variables were presented as mean and standard deviation (age) or median and interquartile range (CA 15-3 and CEA levels). CA 15-3 and CEA levels were compared between groups using Mann-Whitney U test (2 groups) or Kruskal-Wallis Test H (3 groups). The Pearson coefficient (r) was used to verify the lineal correlation between CA 15-3 and CEA levels. The significance level (α) was assumed to be 0.05.

Results: ELISA assays showed statistically significant difference in CEA and CA 15-3 levels in healthy dogs in comparison to dogs with CMT (its levels were significantly lower in healthy dogs, $p < 0.001$). There was no statistically significant difference in CEA as well as CA15-3 levels between malignant and benign tumors (CEA: $p = 0.908$; CA 15-3: $p = 0.608$). There was also no statistically significant difference in CEA and CA 15-3 levels in bitches with different grade of malignant CMT. There was no linear correlation between CA 15-3 and CEA in dogs with CMT ($r = 0.06$, p value = 0.606).

Conclusions: Statistically significant difference in CEA and CA 15-3 levels in healthy dogs in comparison to dogs with CMT may indicate than both CEA and CA 15-3 may be good biomarkers of an early diagnosis of CMT as well as of recurrence of the disease. Further studies in this direction are currently being carried out.

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051 | Comparison between computed tomography and ultrasonography of prostate gland volume in intact healthy male dogs

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Introduction and aim: Prostate gland is the most important accessory sex gland in male reproductive and urinary system that

completely encloses the proximal portion of the urethra. Diseases of the prostate are common in middle-aged to older intact dogs. Nevertheless, some studies revealed prostatic changes such as benign prostatic hyperplasia in young male dogs. It is known that prostate gland is an important reproductive organ and regular monitoring of the organ is recommended. The "golden standard" of diagnosing changes in prostate is rectal palpation and ultrasonography (USG). Meanwhile computed tomography (CT) is now presented as a new diagnostic method of prostatic disorders. The aim of this study was to compare CT and USG scans of the prostate gland and to determine which method is more precise for evaluation of the prostatic volume.

Materials and methods: 15 different breed, age and weight male dogs were examined in private practices. All males were confirmed as clinically healthy. At first the prostate gland was checked by digital palpation through rectum. Then all dogs underwent ultrasonography and CT scan of the gland. Volume of the prostate was measured in both studies. Ultrasound examination was performed in lateral and dorsal recumbency with Mindray DC-T6 Vet (Mindray Bio-Medical Electronics Co., Ltd.) ultrasound machine. Prostate was scanned with microconvex probe in transverse position. Using different parameters of the machine the best quality image was obtained and saved. Measurements of prostate gland where calculated automatically by "trace-area" volume counting option and presented in quadratic centimetres

CT scans were performed using helical 2 slice CT scanner (Somatom Spirit 2, Siemens, Germany). Scanning protocol was chosen: voltage – 130 kVp, current – 100 mAs, slice thickness - 3–5 mm (reconstructions – 1.5–2.5 mm). Scans were performed using bone and soft tissue algorithms.

CT scans were performed under general anaesthesia. All dogs were premedicated using medetomidine hydrochloride (Cepetor 1 mg/mL, CP-Pharma Handelsgesellschaft GmbH, Germany) at the dose of 10 μg/kg of body weight. Anaesthesia induced using propofol (Propoven 10 mg/mL, Fresenius Kabi AB, Sweden) at the dose of 2–4 mg/kg. Afterwards the patient was intubated and connected to an inhalation anaesthesia machine with isoflurane. Vital signs monitoring was present during all scanning time.

All patients were positioned and scanned in dorsal recumbency using radiolucent positioning aids to maintain stable positioning. Non-contrast and contrast scans were performed. Iohexol (Omnipaque 350 mg/mL, GE Healthcare AS, Norway) at dose of 600 mg/kg was used for contrast scan. Intravenous catheter was introduced into the peripheral vein (*v. cephalica)* and the bolus of contrast media was administered manually.

Non-contrast scans included regions started at the level of T13 until full visualization of both testicles. Slice thickness - 3–5 mm, reconstructions – 1.5–2.5 mm.

Post-contrast scans were performed at the same level, with the same slice thickness after i/v contrast media administration – arterious phase (~15 s after administration), venous phase (~60 s after administration).

Late phase (~120 s after administration) was performed at the region of interest (starting at the level of L3 until the full visualization of both testicles), with smaller slice thickness.

All data were collected and analysed using the OsiriX software (Pixmeo SARL, Bernex, Switzerland).

Statistical analysis where performed with SPSS statistical analysis software. ANOVA, and correlation methods where included in statistical analysis. The level of significance was set at $p < 0.05$.

The images of ultrasonography and CT scan of the same male dog are present in figure 1.

Results: Our results showed no significant difference between the means of measurements of prostatic volume using CT and USG. The correlation between these methods was statistically high ($p > 0.05$). The influence of age on mean prostatic volume is shown is figure 2.

There was no significant difference between breed size and ultrasonographic measurements of the prostate gland volume ($p > 0.05$). However, the significant difference was found between different breed sizes and computed tomography measurements of the prostate gland volume ($p < 0.05$). The changes of prostatic volume between different breed sizes is shown in figure 3.

Conclusions: Our results showed that different methods of prostatic volume measurements did not differ significantly and correlation was high. Ultrasonography is the "gold-standard" method to

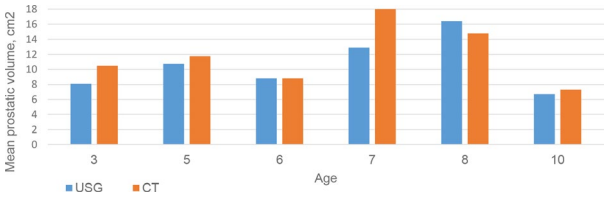

Figure 2. Changes of prostate volume by age.

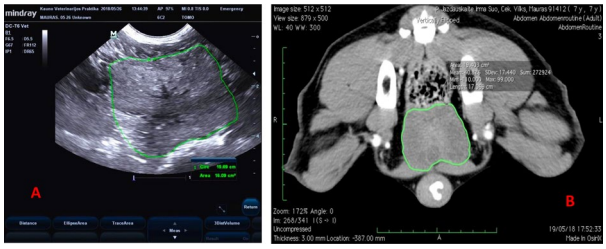

Figure 1. A - ultrasonographic scan of a Czechoslovakian wolfdog using microconvex probe. B - pre-contrast faze of CT scan of the same male. Prostatic cysts and calcifications are present in the picture.

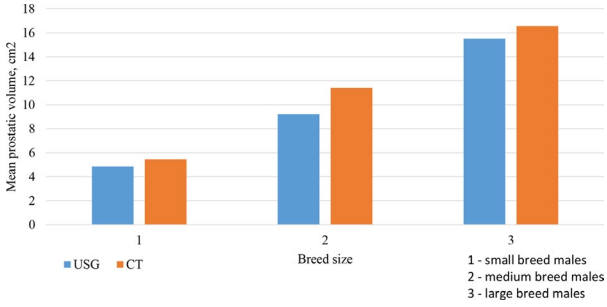

Figure 3. The changes of prostatic volume between different breed sizes.

diagnose prostatic diseases and changes, however there is a great potential for variation due to operator-dependent factors. In this part, CT eliminates some of this variation, which may allow for more consistent interpretation of examinations. Notwithstanding the fact that computed tomography in dogs requires general anaesthesia, all males in our study tolerated anaesthesia well and respiratory or cardiac disorders were not observed. Although all male dogs where clinically healthy, some of the results suggested presence of benign prostatic hyperplasia depending on the volume of the prostate gland. Regarding this, further research based on CT and USG examinations is needed to evaluate male dogs with prostatic disorders.

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053 | Systematic analysis of the literature regarding dystocia in the bitch

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Introduction and aim: High quality literature in the field of canine reproduction is rare (1). So there are no studies on the quality of the literature about dystocia in dogs. This is notable, because it is a heavily forensic charged topic.
The aim of this study was to analyze the literature regarding dystocia in the bitch with focus on caesarian section.
Material and Methods: Relevant articles and studies were collected with the help of the online databases PubMed, Medline and Google Scholar. It was focused on articles published between 1960 and 2014 in German and English. Work published earlier was not considered because of lack of comparability. The relevant papers were found by using the search terms "sectio caesarea", "cesarean section", "cesarean", "ceasarean" in combination with the words "bitch", "dog" and "small animal" and their plural forms together with the search item "anesthesia". Additionally, papers and book references from the library of the veterinarian department of the Justus-Liebig-University Giessen were added. The evidence of scientific work added was estimated to get a general view about the quality of the research with the schemes of Holmes (2004) and Greenhalgh (2003). This retrospective study analyzed the data base of 280 female dogs.
Results: 109 articles were included in the analysis: 69 opinions of expert, editorials and consensus reports, 21 case series, 5 clinical cases, 4 randomized, controlled studies and 3 otherwise controlled studies. Only one blinded, randomized controlled study, one cohort study and one case-control-study were included. Further 3 systematical reviews were added. The literature was further divided in different topics: 10 papers dealt with the topic physiological birth, 25 cited articles were about dystocia, 15 papers covered the topic of gaining a proper diagnose and 12 manuscripts pointed out breed predisposition. 43 articles covered the topic of proper preparation of surgery followed by 44 articles dealt with the surgery procedure. 21 of the articles discussed the different indications of a cesarean section. Further the general anesthesia was discussed in 57 articles. 2 papers dealt with the positioning of the dog during the surgery. The different kinds of cesarean section are the topic of 16 paper. Additionally the treatment of the bitch and the puppies during the recovery phase and postoperative medication were elucidated in 44 articles. In the selected literature is also one paper about stitching techniques, one about the responsibility of the veterinarian for clearing up and documentation and two case series about the risks of puppies of a cesarean section.
Conclusion: Improvement of the quality of research in the field of canine dystocia is required. One case of the low scientific quality at the moment may be that for ethical reasons, experiments are hardly possible.

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054 | Suckling behavior of puppies during the first 24 hours of life

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Introduction and aim: Within the very first hours after birth, colostrum ingestion, providing the newborns with both energy, is important for puppies' survival at short and long term [1] and immunoglobulins [2]. Establishment of an efficient suckling behavior during the first day of life is thus crucial but surprisingly it has never been described in canine newborns. The objective of this study was thus to describe the suckling behavior over the first 12 and 24 hours of life.
Materials and methods: Within one kennel, 34 Labrador puppies from 5 litters were included. Four dams were primiparous, one of parity 4, aged 18 months to 5 years. Births occurred by vaginal delivery 60–63 days after ovulation. At birth, puppies were identified by a plastic collar, weighted and sexed. They were then kept with their mother, in a heated whelping box 1.70 × 2.65 m. From birth to 24 hours of age, and for each puppy, the following informations were registered by permanent direct live visual observation: time at birth, birth rank,

time, duration of each suckling bout together with the position of the suckled mammary gland (from M1 thoracic gland until M5 posterior inguinal). Puppies were weighted at birth. Results are expressed as mean ± SEM. Normality of continued variables was tested by Shapiro-Wilk test, the influence of discontinued variables by Kruskal-Wallis and Student/Fisher test; correlations and associations were evaluated respectively by Spearman test and Khi 2 (XLSTAT software).

Results: Whelping lasted 54 min to 3.5 hours, with a mean interval of 28 minutes between two consecutive puppies. Mean birth weight was 389 ± 60 g. The mean delay between birth and first suckling was 1 h28 ± 1 h25 (min: 5 min; max: 6 h; median: 59 min). Each puppy experienced 10 ± 5 suckling bouts and 26 ± 9 respectively during their first 12 and 24 hours of life. The total duration of suckling was 80 ± 40 minutes over the first 12 hours of life (i.e. 11% of its life time), 213 ± 55 minutes over the first 24 hours (15% of its time life). Puppies suckled 5 ± 2 different teats over the first 12 hours and 9 ± 1 over the first 24 hours (p-value < 0.001). Puppies spent significantly more time suckling the left mammary chain (56 vs. 44% of suckling time; p-value = 0.021) and the most caudal mammary glands (30% suckling time on M5 vs. 17% M4, 19%M3, 16%M2, 18%M1; p-value < 0.001).

Birth weight did not affect delay from first suckling, but positively affected the number of bouts, the total suckling duration and the number of teats suckled over the first 12 hours.

Rank order did not affect delay from first suckling but negatively affected the number of bouts together with the total duration of suckling and the number of teats suckled over the first 12 hours.

Conclusions: This work described quantitative (duration, number of glands, number of bouts) and qualitative (time at first suckling, position of suckled mammary gland) aspects of suckling behavior, focusing on the colostral period in the canine species. It demonstrated the important variability of suckling behavior between puppies and between litters. Suckling is established very early after birth with no appropriation of any mammary gland by a given puppy. Birth weight and rank order are demonstrated as major variation factors of suckling behavior. The impact of the way of delivery on suckling behavior, together with differences among breeds would be interesting to explore, together with the impact of the different aspects of suckling behavior on puppies survival and growth.

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056 | Treatment options for ovarian cysts in the bitch – what do experts recommend?

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Introduction and aim: Decision making and giving good advice to patient owners is a central task of veterinary practitioners [1]. However, in some fields it is difficult to base our work on reliable research data, especially for conditions that we rarely see in practice and for which performing clinical research is difficult [2]. In that regard, we aim to design a database (working title REPROCASES) in which case reports on rare diseases in the field of small animal reproduction can be collected.

The first disease we want to exemplarily investigate are ovarian cysts in the bitch. This is a subject on which reliable information concerning the efficacy of medical treatment options, side effects and prognosis regarding fertility are hardly available [3]. So far, there are only few clinical studies that go beyond the individual case reports published [4]. In order to prepare a helpful case report form and provide some treatment guidelines we asked experts in the field of small animal reproduction which treatment regimens they would recommend for ovarian cysts in the dog.

Materials and methods: We used a survey with eleven questions that was performed via the online tool EFS Survey (https://www.questback.com/). The experts were contacted via the EVSSAR Newsletter and the mailing list cafereprod-l@list.cornell.edu in September 2018. Participation was anonymously and voluntarily.

Results: In total, 14 participants completed the survey until February 2019. The participants stated to be specialized without certification (n = 5), three participants had a national board certification, four had a Diplomate of the European College of Animal Reproduction, and two had a Diplomate of the American College of Theriogenologists. Work experience was between four and 33 years (mean 25.9 years). Being asked how many and which kind of cysts they had diagnosed throughout their working life, some participants stated to have seen only a few cysts (2–20). While one participant stated to have seen about 40 cysts, another one has seen 50 to 60 cysts. Two participant have seen as many as hundreds to thousands and one participant wrote “too numerous to count”. Regarding the question which therapy they recommend for the initial treatment, seven of the participants suggested hormonal therapy. Four colleagues recommended surgical therapy, two would have chosen a different approach “depending on actual progesterone concentrations” and one participant stated that no therapy is promising. Taking a closer look on hormonal therapies, four participants proposed a treatment with hCG and/or GnRH. However, recommended dosages and application regimes differed markedly and ranged from 500 IU hCG + 100 mg/kg GnRH every 48 hours, 500 IU hCG SC for 3 days (suggested by two respondents, respectively) to a protocol such as: Day 1: hCG + GnRH; Day 2: GnRH; Day 3: hCG + GnRH; Day 4: GnRH with dosages of 1000 IU hCG IV and 2.2 μg/kg GnRH IM. Another participant proposed one administration of 500 IU hCG and if no improvement is seen after 2 weeks to increase the dose. This participant also suggested administering cabergoline and prostaglandin 25 days later to avoid progesterone effects on the endometrium.

Conclusion: It can be concluded that there is no uniformly recommended conservative therapy for bitches with ovarian cysts. The participants suggest different dosages of hormonal medication or different time intervals for administration. Thus, more research or

case collections – even if difficult to perform - is warranted to learn more about treatment success, side effects and subsequent fertility.

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057 | Standardization of the assessment of exfoliative vaginal cytologies

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Aim and Introduction: It is the aim of this study to standardize the procedures and evaluation of the exfoliative vaginal cytology in the bitch. Investigated was the influence of magnification on the examination of exfoliative vaginal cytologies. The results of cell classification carried out at 200-fold and 400-fold magnification were compared. Also investigated was whether a significant difference of the cell distribution between three different lines of each smear, as well as between the thirds of one line could be detected. Through the use of a statistical program, the minimum number of epithelial cells which should be examined, for an accurate assessment in an exfoliative vaginal cytology was determined.

Material and methods: Slides of 16 cytologies were examined at 200-fold and 400-fold magnification. Additionally, plasma progesterone and oestradiol-17β concentrations were measured. Four slides from the cycle phases proestrus, estrus, metestrus and anestrus were assessed. The cytologies were viewed in a meandering pattern. For every slide cells were counted and classified for each line and within each third of one line. The cells were examined and classified as follows: cluster, anuclear, superficial, intermediate, parabasal, basal and foam cells. Further analysis of the data occurred in groups, which were established using the average occurrence of each cell class for each slide. Data analysis was carried out using a three factorial variance analysis with repeat measurements considering the parameters; magnification, line and thirds within a line.

Results: Main effects: No significant difference in the classification of cells could be found when comparing the results of the examination with a 200-fold and 400-fold magnification. Significant differences in cell distribution between the lines of a sample could be noted for cytologies with a high amount of anuclear cells ($p = 0.019$). Remarkably more anuclear cells were detected at both magnifications (200-fold and 400-fold magnification) in the third line compared to the first and second line. The observed difference of each line from the mean are minor and therefore have no practical relevance. The distribution of the different cell classes within each third of each line showed no statistical significance.

Double-interaction: Statistically significant differences in cell distribution between the lines depending on the applied magnification were not noted for any of the analyzed cell classes. Also, no difference of the cell distribution between the thirds of each line depending on the applied magnification were detected. The cell class "cluster", showed a significant difference in the distribution between the thirds of the first line of a slide ($p = 0.012$). Since the noted differences between the thirds of the first line are minor, they do not have practical relevance.

Sample size calculation: To accurately assess the occurrence of each cell class in a vaginal smear at least 200 cells should be counted. The expected share of a cell class should not be less than 5%. Assessment of 100 cells is only sufficient for smears with an expected occurrence of a cell class of more than 50%.

Conclusions: Assessment of exfoliative vaginal cytologies in the bitch can be carried out using either a 200-fold or 400-fold magnification. Differences in distribution of cell classes between the lines and thirds of one line have no practical relevance. It is recommended to count at least 200 cells of a smear to correctly assess a vaginal cytology.

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058 | Enrichment of "in vitro" dog capacitated sperm through "swim-up" technique

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Introduction: The aim of the study was to obtain an enriched fraction of capacitated dog sperm cells by separation of them from non-capacitated cells by means of the swim-up technique, which is based on the differences in motility between capacitated and non-capacitated sperm (1).

Material and methods: Freshly obtained sperm-rich fractions from 19 dog ejaculates were gently placed in the bottom of a tube containing 10 mL of a specific *in vitro* capacitation (IVC) medium (2). Cells were incubated in this medium for 4 h at 38.5°C avoiding any movement. Afterwards, the 10 mL of sperm suspension were divided in 5 fractions, from which F4 was the 4 mL placed at the top of the tube, the F3 the second 4 mL, F2 the next 1 mL, F1 the cloudy, next-to-bottom section and F0 the bottom sperm suspension. Percentage of viability, morphological abnormalities, sperm concentration, capacitation-like chlortetracycline (CTC) staining, as well as

computer-assisted analysis (CASA) of motility were carried out on all of the above described fractions as well as in the initial ejaculate.

Results: Percentage of morphological abnormalities showed no significant difference among the different fractions obtained by swim-up, while the percentage of viability was significantly lower in F2, F3 and F4. Sperm cells concentration showed increasing values from the top to the bottom of the swim-up tube. Focusing on CASA analyses, all fractions showed the presence of 4 different sperm subpopulations. Of these, sperm from subpopulation 3 showed the highest percentage of cells with hyperactive, capacitation-like motion characteristics. The highest percentage of sperm included in Subpopulation 3 was observed in F2 (53% of the total motile sperm in the fraction), with percentages only slightly lower in F3 (47%) and F4 (43%). Concomitantly, the percentage of capacitation-like CTC staining was significantly ($p < 0.05$) higher when compared with the other fractions in F2 (93.9%) and F3 (81.3%).

Conclusions: The application of swim-up together with IVC does not allow to physically split the separate motile sperm subpopulations present in a dog ejaculate. Meanwhile, the combined analysis of results showed that the greater percentages of sperm with capacitation-like characteristics were mainly distributed among fractions F2 and F3. In conclusion, the combination of IVC with a swim up technique allows us to obtain an enriched fraction of capacitated sperm from dog samples, facilitating thus the study of sperm capacitation in this species.

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059 | Gonadectomy of dogs and cats in france: Motivations and knowledge of the owners of male and female pets

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Introduction and aim: In France, around 70% cats (males and females), 17% male dogs and 40% female dogs are neutered [1]. The aims of this work were i) to describe the motivations of the owners to ask for the neutering of their pet, ii) to understand the ways through which the owners collected information about this surgery and iii) to evaluate their knowledge about the indications and long term complications.

Materials and methods: A questionnaire was distributed to dog and cat owners asking for a consultation to get their animal neutered at Toulouse National Veterinary School. On a voluntary basis, the owners filled the questionnaire during the waiting period before the consultation, before any discussion with vets, vet students or nurses. Over the study period, 101 questionnaires were collected for tomcats, 25 for male dogs, 73 for queens and 95 for bitches.

Results: Tomcats were aged 10 months (±9.4; mean ± SEM; from 3 to 61 months), much younger than male dogs (27.6 ± 27.5 months; from 6 to 100 months). Mean age was 12.1 months (± 9.8; 4 to 88 months) for queens and 19 months (± 18.3) for bitches (from 5 to 100 months), with respectively 47% and 60% of females pubertal. Respectively 15% and 7% of the owners did not whether the female was pubertal or not. Among pubertal females, 38% queens and 61% bitches had free access outside out of surveillance; 33% queens and 61% des bitches were in frequent contact with a non neutered male. Only 7.4% queens and 4% bitches were previously treated by progestagens; 13% queens and 8% bitches had already been pregnant (16 mismatings out of 19). For a low proportion of owners (15% bitches; 17% queens) believes that pregnancy is positive for the global welfare of their female pet.

About the way they searched information about neutering, 27.2% of owners declared to not have looked for before the consultation, 35.4% previously discussed with a vet, 16% collected data on the website of a vet clinic and 21.4% elsewhere on the internet.

The main motivations of owners were: in tomcats, avoiding urine marking (33%), roaming and accidents (23.2%) and reproduction (18.4%); in male dogs, avoid roaming and accidents (26.6%), suppress fertility (20.2%) and decrease aggressive behaviors against other animals (10.5%). The two motivations for neutering of a queen was to avoid the management of pregnancy, queening and litter (47.6%) or estrus (13.6%). The owners of bitches were motivated by the prevention of pregnancy (27%), of mammary tumors (13.4%), and for a global positive impact on health (9.4%).

Regarding potential negative side effects, between 50 and 80% of owners declared not to be aware of the effect of neutering on their relative risk, with the exception of overweight: 77% of male owners and 58% of female owners were informed from the increased risk after neutering. Among the owners having an opinion, 48% are convinced that neutering decreases the risk for prostatic tumor, 30% for other prostatic diseases; the risk for urolithiasis was believed to be decreased by 9% of tomcat owners and increased by 4%. Only 14% of bitch owners were aware about an increased risk for urinary incontinence, 26% for the global risk of neoplasia; only 50% knew about the protective risk on mammary tumors, uterine infections and pseudocyesis.

Conclusions: Pet owners are far from well informed about the consequences of gonadectomy on the animal health. Precise information needs to be provided, for which the vet remains the main source, in direct or through a website. Taking into account the recent epidemiological data on the health impact of neutering [1, 2], preneutering consultation needs to be informative for owners in order to get a real informed consent.

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060 | Fatty acid profile of canine colostrum, milk and industrial milks

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Introduction and aim: Milk fatty acids (FA) represent major sources of energy but also of essential nutrients for newborn puppies. They are involved into growth, neurological development, immunity and numerous metabolic pathways (including insulin sensitivity). The aim of this work was to compare FA profiles of 18 industrial milks (IndMs) designed for puppies with that of canine mammary secretions (CMS) of the first week of lactation.

Materials and methods: Colostrum and milk samples were collected within one multiracial kennel. In bitches freely suckled by their puppies, CMS (from all glands) were collected at Day 1 (D0 = whelping; n = 23), D3 (n = 18) and D7 (n = 26) and stored at −20°C. IndMs (n = 18) were diluted following the manufacturer's instruction. FA were quantified by gas chromatography [1]. For the comparison between CMS and IndMs, 11 FA were selected. For one given FA, concentrations in IndMs were considered as "*satisfactory*" if above the 25% lowest (Q1) concentrations measured in CMS from D1-3 (colostrum). A second comparison was performed with Q1 of concentrations measured in CMS from D7 (milk). Results are expressed as mean ± SEM. Statistical analysis was conducted with repeated measures ANOVA and Principal Component Analysis (PCA).

Results: Total fatty acids (FA) concentration did not significantly vary over the first three days after whelping (D1: 40.2 ± 8.7 g/L; Day 3: 38.8 ± 6.8 g/L) and then increased markedly (D7: 56.8 ± 9.2 g/L) ($p < 0.001$). The concentration of some FA families (saturated FA, unsaturated FA, n-3 and n-6 FA) and some individual FA (alpha-linolenic acid ALA, eicosapentaenoic acid EPA, docosahexaenoic acid DHA) followed the same pattern. In contrast, the increase in DPA concentration is limited, and arachidonic acid AA concentration as well as polyunsaturated/saturated ratio (59 ± 8.0%) and n-6/n-3 ratio (10.6 ± 1.6) remain stable over the first week of lactation.

PCA evidenced that all FA profiles from CMS (whatever the delay from whelping) are closely clustered, whereas an important diversity was noticed among IndMs FA profiles. Compared to D1-3 CMS, total FA concentration was satisfactory for 11 out of 18 IndMs. In total, over the 11 parameters evaluated, IndMs reached the requirements for only 2 parameters (median; Q1: 2; Q3 3). The FA concentrations were satisfactory in most IndMs for saturated FA (SFA; 14/18 IndMs), and less frequently satisfactory for ALA (6/18), n-3 FA (3/18), EPA and DHA (3/18 for both), polyunsaturated FA (PUFA) (1/18), n-6 (1/18), AA (1/18), DPA (0/18). Compared to D7 CMS, IndMs were satisfactory for 2 parameters (Q1 = 1, Q3 = 2). They are in excess of SFA (x1.9) and at the opposite, in deficit of PUFA (: 2.7) compared to CMS, with a more pronounced deficit in n-3 than in n-6 FA. Most IndMs contain almost null concentrations in AA (15/18), EPA (14/18), DHA (15/18), DPA (15/18).

Conclusion: As industrial milks are made from bovine milk, the striking differences between their fatty acids profiles and those from canine mammary secretions was expected. Deficit observed for some FA are similar to those evidenced in formulas for human infants, especially in AA [2]. Since IndMs display major differences between them, users may be advised to prefer those found satisfactory for total fatty acid concentration, saturated FA concentration (of easier metabolization), DHA and EPA (important for neurological development), with a n-6/n-3 ratio equivalent to the one of mammary secretions. This work contributes to provide objective data to choose milk replacer optimal for the newborn puppy but has to be confirmed with comparison with other CMS collected from bitches fed other regimens.

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061 | Evaluation of progesterone measurements with the Speed Reader®

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Introduction and aim: With the new development of in-house progesterone tests, there is a new economically attractive and fast way to measure progesterone in small animal practices. In 2017 the immunochromatographic method for the quantitative measurement of P4 in canine blood samples was introduced into the market (Speed Progesterone™ using the Speed Reader®, Virbac, France). Nerveless, the radioimmunoassay is still the golden standard to measure progesterone (1).The aim of this study was to compare the results of the measurements with the Speed Reader® and a well-established in-house radioimmunoassay (1).

Material and methods: Blood from 45 healthy bitches, which were presented for the determination of the optimal time of breeding, were taken. Serum was obtained and divided into two aliquots, Progesterone was measured with the RIA and Speed Reader®, respectively. The methods were statistically evaluated by pairwise linear regression analysis. Due to the measuring range of the Speed Reader®, only samples between 1 and 20 ng/mL were included in the study.

Results: The pairwise linear regression analyses revealed a highly significant ($p < 0.001$) positive correlation. The correlation coefficient (R) for the Speed Reader® vs. RIA was R = 0.94. The regression line for the Speed Reader® vs. RIA was close to the identity line (slope: 0.93, intercept: 0.53). In one of the samples a P4 concentration of

19.7 ng/mL was measured using the Speed Reader®. The progesterone concentration measured with RIA was 25.5 ng/mL. This might indicate that the P4 concentration exceeded the measuring range defined for the Speed Reader®.

Conclusion: Our results confirm that Speed Reader® allows the precise measurement of P4 in canine blood samples in a rage of 1 to 20 ng/mL. Due to the high positive correlation of the RIA and the Speed Reader®, measurements with the Speed Reader® seem to be good new alternative for in-house measurements of progesterone.

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062 | Echostructure analysis of canine benign prostate gland hyperplasia and chronical prostatitis reveals significant differences

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Introduction and aims: Benign Prostate Gland Hyperplasia (BPH) and chronical prostatitis are frequent problems of the aging dog. Diagnosis can be supported by advanced sonographical techniques, like Doppler sonography (1,2) and elastography (3). In the present study, we aimed to improve diagnostics by means of echostructure analysis.

Animals and Methods: We compared the echostructure of prostate glands of young dogs with the echostructure in case of BPH and chronical prostatitis; furthermore the echostructure in BPH before and two weeks after treatment with antiandrogens. The sonographical pictures of the healthy prostate glands were from patients introduced for examination before castration (controls, n = 14 years, age: 1.5 ± 0.4 years) All others were introduced because of bloody preputial secretion and/or urination/defecation problems (BPH, n = 21, age: 9.5 ± 3.4 years; prostatitis, n = 6, age: 6.5 ± 3.5 years). A case history was taken and after clinical examination, sonography of the prostate gland was performed. The diagnosis chronical prostatitis was supported by bacteriological examination of urine or semen and a blood picture. A total of 412 pictures were evaluated (control: n = 145, BPH: n = 123, prostatitis n = 144). Digitized images of the prostate gland were divided into four equal quadrants. On each quadrant, a quadratic region-of-interest (ROI) was chosen randomly on B-mode images and echotexture analyses were done using a customized program (ImageJ; Wayne Rasband, National Institutes of Health, Bethesda, Maryland, USA). For the computer-assisted analyses, echotexture of the tissues using the parameters mean grey level (MGL), mean gradient (MG), homogeneity (HOM), entropy (ENTR), contrast (CONT) and grey value (GV) was evaluated. Statistical analyses were done by using the Shapiro-Wilk-Test for distribution; since data showed no normal distribution, the Wilcoxon Signed Ranks Test was used for comparison between groups. All values are given as means ± standard deviations, a p-value < 0.05 was considered statistically significant.

Results: Statistical analyses revealed that *energy*, *correlation*, *mean grey value and entropy* differed significantly between the healthy controls on one side, and BPH and prostatitis on the other; while a diseased gland showed decreased energy and correlation, the entropy and mean grey value was increased (all $p < 0.01$). Furthermore the decrease in *homogeneity* contributed to differentiate between healthy and prostatitis dogs ($p < 0.01$); *homogeneity* values were in addition significantly lower in prostatitis than in BPH patients ($p < 0.05$). Between the first examination of BPH patients and the last control after treatment, a significant increase in *energy* ($p < 0.05$) and *homogeneity* ($p < 0.05$), and a decrease in *entropy* ($p < 0.01$) was observed.

Conclusions: Echostructure analysis was helpful to diagnose BPH and chronical prostatitis, and to differentiate between the diseases, when all parameters were evaluated and compared with values from young, healthy dogs. Furthermore the effect of an antiandrogen therapy can be observed.

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(2) Zelli et al, Reprod Domest Anim 2013; 48(5):768–73.
(3) Jeon et al, Vet Radiol Ultrasound 2015; 56(4):425–31.

065 | Computed tomography and ultrasound examination of subcapsular prostatic cyst in a five-year-old dog with severe benign prostatic hyperplasia

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Clinical case: A five-year-old Bullmastiff dog was initially presented for staging of an extra skeletal osteosarcoma on the right hip. The dog had a history of intermittent haematuria for months that had never been investigated. Abdominal computed tomographic examination (CT) incidentally revealed an enlarged (8 × 7.6 × 5 cm) and heterogeneous prostate with contrast enhancement and periprostatic fat stranding. Several hypoattenuating areas were observed in the parenchyma and its periphery, some of them outlined by a rim enhancement on post-contrast scan. Prostatitis and prostatic abscesses associated with secondary steatitis were suspected. Bilateral mild medial iliac lymphadenopathy was present. Ultrasound examination (US) showed an

enlarged (7.6 cm width, 4 cm height in cross section), bilobed prostate. Anechoic dilated ducts radiated from the middle of the gland and connected with an anechoic, septated and irregular-shaped area with distal enhancement between the parenchyma and the distended prostatic capsule of the right lobe. The surrounding fat was hyperechoic, suggesting secondary steatitis. No abnormality was found in the testicles. Cytology from fluid collected by prostatic lavage (PL) showed red blood cells and prostatic cells but no inflammatory cells or bacteria. Urinalysis was within standard limits. Ultrasound-guided fine needle aspiration (US-FNA) of the subcapsular cavity and prostatic parenchyma were performed and 0.2 mL of serous fluid were collected from the cavity. No signs of inflammation were observed on cytological examination. A severe benign prostatic hyperplasia (BPH) and a subcapsular cyst were diagnosed. No US-FNA of the iliac lymph nodes was performed. The dog underwent castration at the same time as surgical removal of the hip mass. Regardless of his BPH, he received cefalexin and carprofen during one week. The dog showed no urinary symptoms during the three weeks after surgery and no ultrasound control was performed.

Discussion: Small intraparenchymal cysts are common findings in BPH. Obstruction of ducts by cellular hyperplasia and hypertrophy leads to accumulation of non-inflammatory prostatic secretions within the parenchyma. We report that, while most cysts associated with BPH are located within the parenchyma, they can also collect peripherally between the prostatic parenchyma and the capsule. In this case, CT showed severe enlargement and heterogeneity of the prostate. CT is reported to be more accurate than US for evaluating prostatic size. Prostatic height may have been underestimated by US. Heterogeneous tissue structure and changes in attenuation on CT after contrast agent injection seem to be very sensitive and to detect earlier prostatic changes than US[1]. However, these features do not specifically differentiate between different prostatic conditions and should not be over-interpreted. Prostatic surrounding reactive fat at US or CT is usually not observed in BPH. Severe enlargement of the prostate could have led to surrounding steatitis. US is the gold standard when investigating the prostate. However, cytology is essential to distinguish between cysts and abscesses[2] and to confirm a prostatitis. Cytology of fluid obtained by PL shows a good correlation with histology in case of inflammation. Dilution should be avoid by centrifugation of sample before examination. Contamination by cells of the urinary tract may occur. US-FNA shows the strongest correlation with histological diagnosis, even if aspiration of fibrotic tissue can lead to poor cellularity[3]. In this case, both methods lead to a BHP diagnosis. Increased serum canine prostatic specific esterase (CPSE) concentrations have been reported in dogs affected by HBP, prostatitis or prostatic carcinoma. CPSE could be useful to detect early prostatic disorders but further evaluations are needed to differentiate between prostatic diseases. According to anamnesis and clinical findings, cytology should be performed to refine the imaging diagnosis and propose the most accurate treatment.

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067 | Hermaphroditism in six related beagle dogs

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Clinical cases: This case report describes a disorder of sexual development in six beagle dogs resulting in hermaphroditism. All female dogs came from the same breeding kennel and belonged to the same family. The phenotype of all dogs was female with a protruding hard structure from the vulva consistent with an enlarged clitoris and penile bone (*baculum*). Animals were between one and two years of age. A transabdominal ultrasonography revealed the presence of ambiguous gonadal structures and uterus. Gonadectomy was performed between 7 to 12 months of age and the gonads were sent for histopathological examination. One month after castration, the presence of enlarged clitoris was evaluated and no difference was recorded. Among the bitches, 4/6 were surgically managed with partial clitorectomy.

Histological analysis revealed the presence of bilateral ovotestis and uterus in 5 animals, the last dog presented bilateral testis. Among the 6 examined dogs with sex development disturbances, one presented oestrus at the age of 12 months.

Cytogenetic analysis in 5 bitches showed a normal female karyotype (78, XX). Amplification of *SRY* gene did not show any result in comparison with a control gene (*NROB*) which suggest that all animals are *SRY*-negative. Further genetic studies will be performed on these animals.

In summary, we confirmed the presence of bilateral ovotestis and 78, XX - *SRY*-negative sex reversal consistent with a diagnosis of true hermaphroditism in 5/6 dogs and 1/6 pseudohermaphroditism dog.

Discussion: Disorder of sexual development (DSD) is a rare affection in dogs and may result from disturbances in sex chromosomes, genes or a failure in development of gonads [1–4]. Testis induction is normally due to the expression of the Y-linked *SRY* gene, along with *SOX9*, an autosomal testis-determining gene. Some other genes like *Nr5a1*, *Wt1* or *Rspo1* have been shown to regulate the expression of *SRY* or *SOX9* during fetal differentiation of gonads. However, the presence of testis has been described in human patients and domestic animals with *SRY*-negative XX sex reversal.

Autosomal genes that cause testis induction in this disorder in dogs are presently unclear. Only one study of familial cases of XX sex reversal in American Cocker Spaniel established an association between sexual differentiation failure and an insertion in *CFA9* [1]. In human medicine, various genetic mutations have been described causing XX sex reversal such as a missense mutation in *NR5A1*, homozygous splice site donor of *RSPO1* or *FOXL2* mutation [5] .

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068 | A multiparametric ultrasonographic approach to assess testicular tumors in dogs

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Introduction and aim: Testicular tumors are very frequent in dogs (1), but their preoperative diagnosis remains challenging. Conventional ultrasonography (US) does not differentiate benign from malignant testicular lesions, while the diagnostic accuracy of Doppler ultrasonography is still under review because it has been tested only in few studies (2). Thus, to improve the assessment of testicular tumors in dogs, in the present study we evaluated a multiparametric approach consisting in the use of two-dimensional (2-D) ultrasound combined with Color Doppler (CD), Pulsed Wave (PW), and Power Doppler (PD) ultrasonographic techniques, B-flow imaging, strain sonoelastography, and Contrast-Enhanced Ultrasound (CEUS).

Materials and Methods: We retrospectively reviewed the multiparametric imaging patterns and parameters of 22 consecutive dogs with 34 testicular tumors. After 2-D ultrasound examination of each testicle, using different Doppler and B-flow techniques, we evaluated the qualitative and quantitative parameters of the blood flows in the spermatic cord and marginal regions as well as within and/or around the tumor lesion, when present. For each dog, by sonoelastography we compared semi-quantitatively the tissue distortion in a region of interest (ROI) drawn within the tumor lesion with that of a reference ROI in a normal parenchyma area. By CEUS, after injection of a bolus dose of contrast agent (Sono-Vue), we evaluated contrast enhancement patterns as well as quantitative parameters (Peak intensity, Time-to-peak, and Area under the curve) in the same two ROIs described above.

Results: Dogs' age and weight ranged from 5.5 to 16 years (median 12) and from 5 to 35 kg (median 26), respectively. Ten dogs had unilateral lesions, whilst the other 12 dogs had bilateral lesions. The focal testicular tumors (17.4 ± 14.5 mm in size) included 12 seminomas, 2 sertoliomas, 15 leydigoma, and 5 mixed tumors. By PW Doppler, blood flow parameters did not differ between the spermatic cord and marginal arteries except for the Resistance Index that was higher ($p < 0.05$) in normal than in pathological testicles (0.60 ± 0.04 vs. 0.43 ± 0.14, respectively). By PD, the mean pixel values tended to be higher in tumor lesions than in normal parenchyma (4893 ± 2798 vs. 3903 ± 2278, respectively). By B-flow, 80% of leydigoma showed a predominant vascularization surrounding the lesion, whilst 93% of all the other tumors evidenced only intralesional vessels. By semi-quantitative sonoelastography, all tumors presented a harder pattern, with higher stiffness values compared to normal parenchyma (3.1 ± 0.5 vs. 2.1 ± 0.9, respectively). By CEUS, testicular tumors showed an inhomogeneous, hyperenhanced pattern during the wash-in and a slow inhomogeneous washout; the peak intensity was higher ($p < 0.05$) in tumor than in normal testicular ROIs ((−51.9 ± 5.99 vs. −57.5 ± 4.98%, respectively).

Conclusions: Our results, although based on a limited array of tumor types, suggest that the multiparametric ultrasonographic approach for the evaluation of the blood flow can help to the detection of testicular tumors and to their differential diagnosis in dogs, at least for the cases of leydigoma.

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069 | What happens to the bitch uterus after ovariectomy? Histological observations and tomographic appearance

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Introduction: Ovariectomy (OVE) has always been taught as the elective procedure to sterilize bitches at the Veterinary University of Turin (Italy) and in many other Italian Universities. Ovariohysterectomy (OVH) has historically been recommended in the United States and the most frequently used surgical textbooks do not even describe OVE. However, long term observation studies do not report uterine pathologies in ovariectomized bitches (1,2). The aim of this study was to describe morphology, histology and computed tomographic appearance of the canine uterus following

OVE and to compare uterine diameters in ovariectomized and intact bitches.

Materials and Methods: Anatomical specimens were collected either at necropsy or at practical exercitations for veterinary students. Tissue samples from uterine horns and body were formalin fixed, paraffin embedded and sections were Haematoxilin-Eosin stained.

The records of intact or sterilized bitches that had undergone computed tomographic (CT) examination of the whole body or of the abdominal tract in the last 4 years were extracted from the database of the Veterinary Teaching Hospital of the University of Turin. The tomographic aspect of the uterus was examined in the ovariectomized bitches. The diameters of uterine body and horns, were compared between ovariectomized and intact bitches (Wilcoxon rank sum test after weight and age homogeneity assessment through two-sided Wilcoxon rank sum test).

Results: The number of cases that could be included in the study was rather limited. A complete uterus could be removed from seven bitches at necropsy. The organs did not show any gross anomaly, had an homogeneously firm consistence, and appeared atrophic. Histologically, few inactive uterine glands, increased stromal cellularity and myometrial basophilia were observed in all except one case, showing mild cystic endometrial hyperplasia (CEH) together with rather thick eosinophilic myometrium.

The CT records of 216 sterilized bitches and 45 intact ones were examined. After exclusion of pathologies interfering with reproductive tract visualization, 171 sterilized bitches and 22 intact ones were left. Thirteen out of 22 intact bitches showed uterine collections interpreted as hydrometra. 163 out 171 sterilized bitches had been ovariohysterectomized (95.3%), leaving 8 cases suitable for inclusion. Three of these showed a minimal liquid collection either in the uterine body or horns, for a maximum diameter of 0.5 cm in dogs weighing 20–38 Kg. The uterine diameters of the 5 cases without collection were compared with the diameters of the 9 cases of intact bitches without collection. The uterine horns diameter resulted significantly smaller in ovariectomized bitches (3.3 ± 1.40 mm; mean ± SD) than in intact ones (5.4 ± 1.2 mm). No significant difference was found in uterine body.

Discussion: Our data show that, notwithstanding the Academic approach, OVH appears to have become the standard procedure in Italy, at least in the past years. From our observations, the uterus left *in situ* shows atrophic tracts both macroscopically and histologically although some tissue dystrophy like CEH can persist years after gonadal removal and minimal liquid collection can be appreciated in the uterine lumen. These conditions, probably existing before OVE, are not likely to have clinical importance after gonadal steroid deprivation. Steroid receptors have been shown to be present at least six months after OVE (3), but observations on uterine tissues after longer periods of time are lacking.

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070 | Vaginal microbiota of healthy anestrus bitches: culture vs. genomic analysis results

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Introduction and aims: Cultural techniques currently used to investigate microbial community harbored by canine vagina lack effectiveness in detecting the complexity of resident microbiota, in contrast to the more powerful metagenomic approach obtained *via* NGS techniques.

Aim of the present work was to compare results obtained from culture dependent and culture independent approaches in describing the vaginal microbial community in healthy anestrus bitches.

Materials and Methods: A total of 11 healthy anestrus bitches of different breed, parity (0–4) and age (3.8 ± 2.2 years mean ± SD) were included in the study. Vaginal samples were collected using sterile swabs through a sterile speculum. Anestrus was confirmed through vaginal cytology and serum progesterone concentration analysis.

Bacterial isolation was performed according to standard lab culture techniques. Taxonomic identification was obtained by MALDI-TOF MS. Total DNA was extracted from vaginal swabs and quantified. After PCR amplification of V3 and V4 hypervariable regions of the rRNA16S coding gene and library preparation, DNA was sequenced by Illumina MiSeq platform. The Greengenes database was used to assign taxonomy to all identified Operational Taxonomic Units (OTUs). Alpha and beta diversity were calculated after normalization of samples via rarefaction.

Results: *Cultural dependent assay*: *Streptococcus* spp. was the most represented *Genus*, isolated from 7/11 bitches (63.6%) -with *S. canis* being the most frequently isolated species- followed by *S.pseudintermedius* (45.4%). *Haemophylus* spp., *E.coli* and *Enterococcus* spp. were isolated from 36.4% of samples whereas coagulase negative *Staphylococcus* spp., *Micrococcus* spp. and *Proteus* spp. were found in 18.2%of cases. *Arcanobacterium* spp., *Bacillus cereus*, *Pasteurella canis*, *Trueperella bernardie*, *Pediococcus acidilactici* were isolated 9.1% of samples. *Mycoplasma canis* was isolated from a single animfal.

Cultural independent assay: Metataxonomic analysis identified 164 *Genera* belonging to a total of 97 *Families*. *Staphylococcus* spp. and *Enterococcus* spp. were identified in 59.1% and 18.2% of samples, respectively, whereas *Proteus* spp., *Trueperella* spp., *Pediococcus* spp. and *Arcanobacterium* spp. were only identified in 9.1% of samples. *Bacillus* spp. and *Micrococcus* spp. resulted to be lowly present also according to culture-independent analysis, with a percentage of isolation of 4.5%. *Haemophylus* spp. and *E.coli* were not detected in any of the tested samples. Finally, contrarily to the cultural-based assay, NGS-based approach highlighted a diffused presence of both *Mycoplasma* spp. (72.7% of samples) and *Ureaplsma* spp (40.9% of samples).

Diversity analysis showed a large variability among bitches in terms of both species richness and evenness (alpha diversity) and total microbial community composition (beta diversity).

Discussion: Interpretation of culture results from bitches vagina is not univocal and the pathological role that one or more microorganisms can play in hypo/infertility issues is not clear (1).
The genomic approach highlighted a very high inter-individual variability of the microbial community, making the definition of a baseline for vaginal community of healthy bitches in anoestrus state not straightforward (2). Culture dependent and independent assays results display a high level of overlap although further studies are necessary to assess the clinical implications of the microbiome analysis.
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071 | Comparative study between endometrial brushing and lavage techniques to diagnose uterine inflammation in the queen

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Introduction: Endometritis has been demonstrated to induce infertility in several species such as cows, mares and bitches. Diagnostic tools include endometrial bacteriology, cytology and biopsy. The aim of the present study was to compare endometrial brushing and endometrial lavage as possible techniques in the diagnosis of uterine inflammation in the queen.
Material and methods: Twenty-eight queens were included in the study and split into two groups, the brushing (B; n = 15) and the lavage (L; n = 13) group. Females were referred for a routine ovariohysterectomy. After being removed, B-uteri were incised with a scalpel and a sterile swab was taken for microbiological purposes. Then, an endometrial cytology was obtained by brushing a sterile swab against the endometrium, followed by a full-thick biopsy. On the other hand, L-uteri were infused with PBS. Recovered PBS was split into two samples, one for microbiological purposes and the other one to perform an endometrial cytology. Finally, a full-thick biopsy was also taken. Endometrial swabs were stained by the modified Gray-Giemsa technique and 10 fields at x40 were evaluated for total number of neutrophils (PMN). Samples were scored as none, +1 (1–10), +2 (>10, but isolated) and + 3 (large clumps) (1). Uterine biopsies were used as a control of the real degree of endometritis and were evaluated according to Klein et al (2016) (2).
Results: Microbiological results were all negative. Regarding to cytologies, brushing samples yielded higher scores of neutrophils than lavage samples. Thus, in L-cytologies, 30% of them were classified as 0, 40% as + 1, and 30% a + 2. On the other hand, in B-cytologies only 16.7% were classified as 0, 33.3% as + 1, and 50% as + 2. In addition, B-cytologies also showed higher morphological abnormalities. Finally, biopsies showed no signs of acute or chronic inflammation.
Conclusion: According to the present results, lavage samples provide closer PMN-scores to biopsy than brushing samples, suggesting that they are more reliable than brushing samples, although biopsy and further histology is always more reliable. These results are somehow expectable since the friction of the swab against the endometrium can induce the break of endometrial capillaries allowing the release of neutrophils. On the other hand, cells obtained by brushing techniques showed a higher degree of morphological abnormalities than those obtained by uterine lavage. This increase can be also explained by the friction of the swab against the endometrium during the sample obtaining.
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074 | APGAR score in newborn kittens related to survival, birth weight and growth until 12 weeks of age

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Introduction and aim: The aim of this study was to examine if APGAR scoring can be used as a reliable method of determining neonatal health in newborn kittens – both by breeders at home and by staff at veterinary clinics after cesarean sections. Another aim was to examine if there was a connection between APGAR scores and survival and APGAR scores and birth weight in kittens, as well as long time survival and growth until 12 weeks of age. Materials and methods
Data from litters born at home was collected by questionnaires sent out to breeders. The questionnaires included questions regarding the queen and the parturition, as well as questions regarding the kittens including health, weights and APGAR score. The study included 17 litters resulting in 78 kittens. Data were analyzed with the mixed procedure and Fisher's exact test in SAS 9.2 and regression analyses in Minitab 17. Results
Mean gestation length was 65.1 ± 0.5 days and mean litter size was 4.6 ± 0.4 kittens. 7.7% were stillborn or died within the first week. Mean birth weight of kittens born alive was 89.8 ± 1.5 g. 5.1% were born with congenital defects. Median time interval between expulsion of each kitten was 30 (range 5–2870) minutes. Viability and birth weight had a significant association ($p < 0.0001$). Larger females gave birth to relatively smaller kittens (median birth weight of the litter/mother's body weight) ($p = 0.004$). There was no significant relationship between expulsion time for each kitten and APGAR score. Because most kittens scored high in APGAR and survived for 12 weeks there was little diversity in these data contributing to lack of significance in analysis of the influence of APGAR on viability. Conclusions

This study is, to the authors' best knowledge, as up to today the first evaluation of the use of APGAR scoring in newborn kittens. As experienced from this study, APGAR scoring is practical in newborn kittens. This study suggests that a widely implemented use of APGAR scoring in queening, both by breeders in home environment and in the veterinary field, could positively affect the breeding management and neonatal care. Veronesi et al (2009) showed a significant association between APGAR score and viability in newborn puppies. Unfortunately, a relationship between APGAR score and viability could not be investigated in kittens in this study, due to low mortality in the included data. The significant relationship between viability and birth weight is probably due to the fact that kittens born dead had significantly lower birth weight than kittens born alive.

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075 | Cardiovascular changes of bitches suffering from cystic endometrial hyperplasia – pyometra complex

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Introduction and aim: The cystic endometrial hyperplasia-pyometra complex (CEH-P) is one of the most prevalent uterine diseases in the female dog (1). Cardiac structure and function adjustments associated with uterine infection have not been reported so far. Therefore, the aim of the present study was to evaluate cardiac dimensions and function as well as systolic blood pressure in bitches suffering from CEH-P before and after ovariohysterectomy.

Materials and methods: Seven, 4 to 12 years old, diestrous intact bitches were included in this study. In all the animals, primary cardiac diseases were ruled out. The bitches were slightly febrile and dehydrated and had a 3–7 days history of decreased appetite. CEH-P was clinically and ultrasonographically confirmed (5 open-cervix vs. 2 cosed-cervix). All the female dogs presented a mild to severe uterine enlargement. The bitches were rehydrated and amoxicillin – clavulanate was administered twice a day at 12.5 mg/kg orally. Twenty-four hours later, the animals underwent ovariohysterectomy (OVH; Day 0) and antibacterial therapy continued for 10 days. The animals were clinically and echocardiographically evaluated on Days -1, 3, 7, 14, 21 and 28 (2). Interventricular septum thickness in diastole (IVSd) and systole (IVSs), left ventricle internal diameter in diastole (LVDd) and systole (LVDs), and left ventricular free wall thickness in diastole (LVFWd) and systole (LVFWs) were measured. End diastolic (LVVd) and systolic (LVVs) left ventricle volume were also obtained. Shortening fraction (SF), ejection fraction (EF), stroke volume (SV) and cardiac output (CO) were calculated. Hearth rate was obtained by electrocardiographic monitoring. Mitral E wave (E) and A wave (A) peak velocity, deceleration time (Dt) of E wave and E/A ratio (E/A) were measured by pulsed wave Doppler. Systolic blood pressure (SBP) was determined with a Doppler flow detector (3). Values of IVSd, IVSs, LVDd, LVDs, LVFWd, LVFWs, LVVd, LVVs, SF, EF, SV, CO, E, A, Dt, E/A, HR and SBP were analyzed by repeated measures ANOVA followed by Tukey test (SPSS 19.0; SPSS, Chicago, IL, USA). $p < 0.05$ was considered significant.

Results: By Day 3, all 7 animals were afebrile and had normal appetite. SF ($p < 0.01$) and EF ($p < 0.01$) increased markedly on Day 3, while E ($p < 0.01$) and A ($p < 0.05$) showed a progressive increase during the study period. In contrast, LVVs ($p < 0.05$), CO ($p < 0.01$), HR ($p < 0.05$) and SBP ($p < 0.01$) decreased progressively throughout the study. IVSd, LVDd, LVFWd, IVSs, LVDs, LVFWd, LVVd, SV, Dt and E/A remained unchanged ($p > 0.05$).

Conclusions: It is concluded that bitches suffering from CEH-P presented a reversible depression of systolic and diastolic function, and a reversible increase in SBP and HR. Left ventricle dimensions were not modified by CEH-P. The cardiovascular system should be considered during the postsurgical monitoring of pyometra cases.

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076 | Parturition monitoring with human cardiotocography in the bitch

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Introduction and aim: Monitoring uterine contractions and foetal heartbeat are important in follow-up of labour in human medicine. For bitches, the WhelpWise Veterinary Perinatal Specialties service in the USA provides tocodynamometric surveillance for home use with portable equipment (1). Tocodynamometry may be a useful tool to detect uterine inertia in bitches (2, 3). The aim of this study was to test the suitability of human cardiotocography (CTG) for monitoring parturition in bitches, specifically uterine contractions with external tocodynamometry and foetal heartbeat with an ultrasonic probe.

Materials and methods: We monitored uterine contractions in the following 47 pregnant bitches: prepartum (n = 13), during the first (n = 7) and second stages of normal parturition (n = 17, N), in complete primary uterine inertia (n = 3, CUI), in partial primary uterine inertia (n = 8, PUI), in obstructive dystocia (n = 3, OD), and in medically treated dystocia (n = 6, MD). Contractions with amplitude

change > 10% units and duration > 1 minute were calculated. Foetal heart rates were recorded. We also tested the monitors in non-pregnant bitches to detect the effect of body movements on pressure changes. The mean weight of the bitches was 21 kg (range 5 to 65 kg). The mean ± SD number of puppies was 6.1 ± 2.7. The bitches represented 21 breeds, including one mongrel. The cardiotocographies used were Sonicaid FM 820 and TeamCare (Algol Diagnostics, Vantaa, Finland). The position of the probe was fixed with a belt caudally to the costal arch and laterally to the midline.

Results: Ranges of the individual uterine contraction monitoring times in minutes (mean) were the following: prepartum 8–170 (39), first stage 7–165 (57), N 13–140 (86), CUI 20–45 (32), PUI 10–110 (42), OD 20–45 (29), MD 14–56 (34). Ranges of the contraction numbers during the monitoring time (mean) were the following: prepartum 0–3 (2.0), first stage 0–11 (3.9), N 1–31 (8.3), CUI 0, PUI 1–14 (5.0), OD 3–4 (3.3), MD 1–7 (34). Ranges of the pressure changes in % units (mean) were the following: prepartum 10–15 (13.8), first stage 10–30 (21.7), N 20–45 (32.6), CUI < 10, PUI 10–30 (17.5), OD 20–40 (30.0), MD 15–45 (29.1). Ranges of the intervals between the contractions in minutes (mean) were the following: prepartum 4–9 (6.5), first stage 4–21 (10.6), N 1–14 (6.2), PUI 4–13 (7.3), OD 2–8 (5.0), MD 3–4 (3.5). Ranges of the contraction durations in minutes (mean) were the following: prepartum 1–3 (2.1), first stage 1–4 (2.6), N 2–5 (3.1), CUI < 1, PUI 1–4 (2.3), OD 2–4 (3.0), MD 2–5 (3.0). Heartbeats were detected in caudal puppies in most of the cases but were often missed with cranial puppies.

Conclusions: Both monitors are suitable for detecting uterine contractions in bitches. Uterine inertia can be diagnosed. The crucial limitation for monitor use is nervousness of the dam. Attention should be paid to ensure correct calibration of the basal line for the contraction curve. Adjustment of the probe position and tightness of the belt after puppy expulsion should also be considered. For foetal heartbeat monitoring, the probe should be carefully adjusted as the probe easily detects the heartbeat of the dam.

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077 | Evaluation of a vaginal examination simulator prototype

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Introduction and aim: The inclusion of simulators in the training of veterinary students aims to improve teaching outcomes and students' practical skills [1]. Vaginoscopy is an essential examination technique, and understanding of the anatomy of the female reproductive tract is important [2].

Training of gynecological examination of living patients can be challenging due to movements of the animal and particular requirements of vaginoscopy in dogs. For vaginoscopy, a lubricated vaginoscope should be placed between the vulvar folds and then passed with the tip acutely angled up toward the spine of the dog [3]. The vaginoscope is then advanced through the vestibule to the vulvar commissure, where the vestibulovaginal junction and urethral opening can be identified. The aim of our project was, to construct and evaluate a simulator for the training of the vaginoscopy in dogs, including vaginal inspection and taking of swabs.

Materials and methods: Basis of the vaginoscopy simulator was a fibre glass Great Dane that was commercially available for decoration purposes. Two areas were cut out of the fibre glass body to enable a view into the abdomen and to be replaced by a silicon vulva, respectively. The latter we developed and produced in our own simulator workshop. In addition, we developed and produced a complete silicone genital tract which allows an insertion of specula or rigid endoscopes till the cervical tubercle.

For a systematic evaluation, questionnaires with five statements (see table) were provided.

Results: The simulator has been in use for six months now. Students in the third year attending the course clinical propaedeutics and in the 5th year attending the clinical rotation were trained using the simulator. Every student had the opportunity to insert a lubricated speculum (Storz, vaginoscope 20 mm diameter, 15 cm) into the simulator and also in real life patient dogs.

The survey revealed the following opinions (n = 20 fifth year students)::

Statement	Fully agree	Agree	Neutral	Disagree	Fully disagree
The approach to train examinations before performing them on patients is useful in terms of…					
…animal welfare	11	7	1	0	1
…training in a non-hectic atmosphere	17	2	1	0	0
…having good skills when performing the examination in an animal the first time	14	4	2	0	0
The simulator is well appropriate to recapitulate the anatomy of the canine genital tract	2	6	11	1	0
The simulator is well appropriate to train vaginoscopy in the dog	4	14	0	2	0

During our own tests with the simulator and also based on the feedback of the students we learned that the angle of the silicone vestibulum was not steep enough and has to be adjusted.

Conclusion: The prototype of the vaginoscopy simulator is a good tool for improving manual skills in gynecological examination in a non-hectic atmosphere. However, we need to adjust the anatomy of the vestibulum to better meet the anatomic situation in real female dogs.

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078 | Influence of dog-appeasing pheromone (ADAPTIL®) on maternal behavior of bitches up to three weeks postpartum.

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Introduction and aim: The end of pregnancy and the first weeks of postpartum may be considered a challenging time for the bitch. In general, the bitch is transferred to a maternity area around one week prior to whelping. In addition, the bitch will face physiological, behavioral, hormonal and metabolic changes from the peripartum to the neonatal period; making these two phases a crucial time to be addressed. This stressful time may have noticeable negative effects. Although some stress is part of whelping, overstressed bitches might be more prompt to problems during parturition and maybe less likely to develop an optimal maternal bond. Since maternal behavior seems to play an important role on puppy behavioral development (1, 2, 3), decreasing stress could help the bitches to display better maternal care. We hypothesized that the use of a dog-appeasing pheromone diffuser (ADAPTIL® Calm) around parturition and during the first three weeks postpartum could modulate the maternal behavior of bitches, compared to a placebo. ADAPTIL® (Ceva Santé Animale, Libourne, France) has been shown to have calming effects in both young and adult dogs under a wide variety of stressful situations (4).

Materials and methods: 48 bitches from 13 breeding facilities were included in a double blinded study. Forty-one completed the study, exposed to Adaptil (n = 20) or Placebo (n = 21) from the peripartum period up to three weeks postpartum. The treatment efficacy was evaluated using three major methods. 1) An electronic device (FitBark dog activity trackers, Kansas City, MO) monitored the bitch's daily activity and resting time. 2) Maternal behavior assessed by the observation of video recordings of 75.38 minutes in average at four time points (W0- within the first 48 hours of whelping, W1 – one, W2 – two and W3 – three weeks after parturition). Specific behaviors were considered such as time in close contact with the puppies (CON), oro-nasal interaction – (ONI) and nursing position and time (NUR-P and NUR-T). 3) Overall assessment of maternal behavior and puppies' well-being by breeders measured using a visual analytical scale (VAS) at the same time points.

Results: There were non-significant differences in the bitches' activity time between both groups (p = 0.341). For all observed maternal behaviors (CON, ONI and NUR-T), there was a steadily decrease in levels as the puppies were more developed as already described (1, 2 and 3) with no difference between groups. However, bitches exposed to ADAPTIL tended to nurse significantly more in lying position, while those exposed to the placebo nursed more in a seating position, especially at W1 (p = 0.06) and W3 (p = 0.005). For the breeders' opinion based on the VAS evaluation, attention scores towards puppies were significantly higher in ADAPTIL than in placebo group at each time point (p = 0.,01). Moreover, a difference according to parity was observed (p = 0.004), with greater attention score displayed by primiparous bitches exposed to ADAPTIL compared to placebo on W0 (p = 0.02), W1 and W3 (p < 0.001). Global mother-puppies relationship was also perceived as significantly better (p = 0.0002) by breeders of bitches exposed to ADAPTIL, with significant differences at W2 (p = 0.01) and W3 (p = 0.001).

Conclusion: Exposition to the dog-appeasing pheromone had positive effects in the bitch during the peripartum period, improving maternal behavior and attention toward the puppies over the first weeks of life. These benefits were perceived by breeders, while being blinded towards the treatment given to their bitches.

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079 | Different effects of egg yolk plasma and catalase in lecithin and TRIS based diluents on frozen-thawed canine spermatozoa

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Introduction and aims: To determine effects of 1) egg yolk plasma and 2) catalase added to two different freezing extenders on frozen-thawed semen characteristics in dogs.

Animals and Methods: A total of 17 healthy stud dogs of different breeds, aged 4.2 ± 3.1 years, were available. Fractionated semen

collection was done once per dog and after quality assessment (computer assisted sperm analysis: CASA; morphology: Hancock fixation; viability: CYBR14/PI fluorescent stain), the sperm rich fraction was divided into 3 aliquots (I-III) without centrifugation. The aliquots were diluted with different cooling extenders: (I) was diluted with a TRIS-fructose-egg yolk extender (TRIS-EY) containing 20% egg yolk (EY) and 3% glycerol (1), (II) with TRIS extender but 20% egg yolk plasma (TRIS-EYP, 2) instead of 20% EY. EYP was made by mixing 20 mL EY + 80 mL TRIS buffer and centrifugation at 10000 g for 45 min, 3 times; only the supernatant was used. (III) was diluted with a lecithin extender (Minitüb, Tiefenbach, Germany) containing 20% EYP and 3% glycerol. All aliquots were diluted 1 + 1 with the cooling medium and equilibrated 1 h at + 4°C in a water bath. Afterwards the freezing extenders were added (1 + 3): (I) was diluted with TRIS-EY enriched with 7% glycerol and 1% Equex STM paste (1); (II) was divided in 2 aliquots and one part diluted with the TRIS-EYP containing 7% glycerol and 1% Equex, and the other (IIa) with TRIS-EYP, 7% glycerol, 1% Equex, and 300 I.U./mL catalase (Sigma Aldrich, Vienna, A). (III) was also divided in 2 aliquots and one part diluted with the lecithin extender containing 20% EYP and 7% glycerol, and the other (IIIa) with the lecithin extender containing in addition 300 I.U./mL catalase. The samples were frozen with a computer-assisted freezing automat in 0.5 mL straws (1). After thawing at 38°C for 15 sec., samples were analysed by CASA and sperm chromatin structure assay (SCSA, 3). In addition, reactive oxygen species (ROS) were determined by using dichlorofluorescin-diacetate (DCFH-DA) and dihydroethidium (DSH) staining (4, Sigma Aldrich, Vienna, A), and sperm were analysed for apoptosis with the Annexin V-FITC Apoptosis Detection Kit II (BD Pharmingen, San Diego, Ca, USA). Statistical comparison between groups was done by using a mixed linear model (Sidak's multiple comparison test). All data are given as means ± SE, $p < 0.05$ was considered significant.

Results: After thawing, TRIS-EY (I) showed higher progressive motility (P:72.8 ± 0.9%) in comparison to all others (II:67.4 ± 1.1%, $p < 0.05$, IIa:63.5 ± 1.3%, III:65.9 ± 1.1%, IIIa:55.4 ± 3.7%, all $p < 0.01$). VCL did not differ among groups. Viability did not differ between I (77.2 ± 1.1%) and II (73 ± 0.9%), but addition of catalase (IIa:69.6 ± 1.3%) and both lecithin extenders (III:70.4 ± 0.8%, IIIa:62.8 ± 3.1%) decreased the values in comparison to I (all $p < 0.01$). Percentage of DNA fragmentation and morphological aberrations did not differ between groups, however, TRIS-EY (I) revealed the lowest % of acrosome damages ($p < 0.05$). When ROS were measured using DSH, samples with EYP (II) showed more ROS than with EY (I, $p < 0.01$), addition of catalase and lecithin did not change the values in comparison to TRIS-EY (I). Samples with TRIS-EY (I) showed lowest ROS content (I: 45.9 ± 2.6, II: 64 ± 2.9, IIa: 60.8 ± 6.0, III: 60.2 ± 4.3,IIIa: 60.9 ± 4.1) and highest percentage of intact cells (44.2 ± 3.5, $p < 0.05$ against II). Percentage of dead cells was highest in IIIa (lecithin+EYP+catalase: 43.5 ± 4.0%, $p < 0.05$ against I and $p < 0.01$ against II). The percentage of early apoptotic cells did not differ among groups, late apoptotic cells were highest in II ($p < 0.01$).

Conclusions: The TRIS-EY extender was superior regarding P and viability, ROS and live cells. However, when EYP was used, similar results were obtained except a higher amount of ROS and late apoptotic cells. Worst results were obtained with the lecithin extenders; addition of catalase had no effect on ROS. The fertilization capacity of these diluents remains to be investigated.

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080 | Comparison of different materials for self-pressurized vitrification of feline oocytes

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Introduction and aim: Cryoconservation of gametes is fundamental to preserve genetic diversity in threatened species. Unfortunately in felids oocyte vitrification is still an experimental technique, and survival remains far from adequate. Self-pressurized vitrification of cells in metal tubes showed an instant cryofixation, avoiding ice crystal formation and providing high cooling/warming rates (1). We aim to find out whether this technique could be beneficial for cat oocytes.

Materials and methods: After routine ovariohysterectomy, high quality cat cumulus-oocyte-complexes (COCs, n = 189) were sorted. Batches of 2–3 COCs were vitrified at immature stage using our 3-step protocol, consisting of Dulbecco's PBS supplemented with ethylene glycol, dimethyl sulfoxide, fetal calf serum, Ficoll PM-70 and trehalose. They were introduced in the tubes with an adapted stripper tip and before plunging them into liquid nitrogen both sides of the tubes were tightly closed. Three tube materials were tested: aluminium (outer/inner diameter 0.6/0.3 mm), silver (outer/inner diameter 0.6/0.3 mm) and titanium (outer/inner diameter 0.51/0.35 mm) cut to a length from 10 to 15 mm. After warming, *in vitro* maturation was performed for 24 hours. Maturation rate was evaluated by the presence of the first polar body under an inverted microscope, and mature oocytes were consequently fertilised by ICSI with fresh testicular cat sperm. Zygotes were checked every 24 hours for embryo development. Due to a larger availability of male gametes, they were used for toxicity tests by comparing the loss of motility through the time in two different treatments: sperm cells in contact with fresh cryoprotectants (CPAs) against gametes in contact with CPAs that have been preincubated in the tubes. Measurements were performed by computer-assisted sperm analysis with AndroVision software. Toxicity tests with fresh COCs evaluated the developmental competence after the same treatments, in the cases where toxicity was suspected.

Results: COCs vitrified in silver tubes showed the lowest maturation rate (17.5%, n = 63), followed by similar rates in aluminium (23%, n = 61) and titanium (23.1%, n = 65) tubes. Embryo cleavage was lower in the aluminium tubes group (14.3%), next the silver group (18.2%) and higher with titanium tubes (20%). Embryos cleaved until the 6-cell stage. Sperm toxicity tests presented a much higher decrease of motility after 4 minutes in the group of CPAs preincubated in aluminium tubes than in the fresh CPAs group (62.3% vs. 37.9%). Accordingly, oocyte toxicity tests also reflected a significant decrease (Fisher's exact test, p = 0.03) in developmental competence in the same treatment group (59.1% vs. 13%, n = 45). No relevant differences were found between treatments in sperm toxicity tests, neither for silver (18% vs. 26.4%) nor for titanium (18% vs. 25.4%).

Conclusions: Previous experiments performed with the same vitrification protocol combined with Cryotop (2) had higher maturation (24.5%) and embryo cleavage rates (30%), with the embryos developing until morula stage. Here, no improvement was appreciated in spite of the intrinsic benefits of the method. Unfortunately the use of aluminium in combination with the CPAs seems to wash out some toxic components of the tubes. This toxic effect was not observed with silver, nevertheless silver has been long related with antibacterial properties and silver nanoparticles are known to produce several dysfunctions in cellular processes as well as to cause embryotoxicity. Titanium is widely used as implant material and it appears to be biologically inert. Accordingly, we found also no toxic effect, and it showed the highest survival and cleavage rates. It pointed out to be the most suitable material, but future studies need to focus on increasing the success of the method.

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083 | The effect of dilution ratio, equilibration period and freezing rate on the quality of frozen-thawed canine semen in OptiXcell® using the contact freezing method

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Introduction and aim: Cryopreservation of semen consists of different steps. Among them are proper concentration levels, equilibration periods and freezing rates. The objective of this study was to compare three different concentration levels, three equilibration periods and three freezing rates in OptiXcell® using the contact freezing method.

Materials and methods: Ejaculates were collected manually from 66 dogs of 37 different breeds and different ages. The ejaculates were diluted to a concentration of 100 × 10^6 spermatozoa/mL. After dilution, the semen was equilibrated for 6 hours at 4°C, and then put into 0.5 mL straws, which were frozen by the contact freezing method. To compare dilution rates, the ejaculates were diluted to a concentration of 50 × 10^6; 100 × 10^6 and 200 × 10^6 spermatozoa/mL. To compare equilibration periods, the semen was equilibrated for 3; 6 and 24 hours. To compare different freezing rates, the semen was frozen using the contact freezing method with a 1; 2 or 3 mm foam mat. Semen motility and morphology were evaluated at two points: before and after freezing. A comparison of difference in motility and morphology between before and after freezing was made. The results of the dilution rate test were analysed by regression analyses. Both the equilibration period test and the freezing rate test were analysed by repeated measures analyses.

Results: The mean motility of the fresh semen was 83% and ranged from 70% to 95%. The mean morphology of the fresh semen was 84% and ranged from 70% to 98%. For the dilution ratio test, the loss of motility ranged from 8.2% to 73.3%. Through regression analysis, p = 0.572 was found. The loss of motility for the equilibration period ranged from 3% to 73.3%. The difference in mean loss of motility between three equilibration periods was only 5%. The probability values were calculated: between 3 h and 6 h: p = 0.900; between 3 h and 24 h: p = 0.754 and between 6 h and 24 h: p = 0.001. The mean loss of motility for the three freezing rates only differs 5% between the three used mats. The probability values were calculated: between 1 mm and 2 mm p = 1.000; between 1 mm and 3 mm: p = 0.001 and between 2 mm and 3 mm: p = 0.002. No significant differences were found in morphology in all tests.

Conclusions: The dilution ratio had no significant effect on the loss of motility in the semen. An equilibration period of 24 hours showed a significantly lower loss of motility than the equilibration period of 6 hours. Between 3 and 6 hours or 3 and 24 hours, no significant difference was found. The freezing rate test showed significantly higher motility by using the 3 mm foam mat. No significant difference was found between 1 and 2 mm foam mats. Dilution rate, equilibration period and freezing rate had no significant effect on the morphology.

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084 | The vertical vaginal septa diagnosed in five Newfoundland dogs – a case report

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: Between April and December 2018 the five cases of vaginal septum and subsequent fertility problems were diagnosed in Newfoundland dogs presented to the Department of Reproduction, Clinic for Small Animals in Wroclaw. The dogs were between 2.5 and 6 years old. In 4 out of the 5 recorded cases the unsuccessful mating or insemination using fresh or frozen semen were documented. All the investigated females had history of a recurrent vaginitis. In one case the septum was accidentally noticed during natural delivery. Each of the reported septa were vertical. In the one case the septum was so thin and fragile that was broken during routine vaginal examination, whereas in 3 dogs the septa were approximately 3–5 millimeters wide. Yet, the most complicated was the last one, which was very broad and strong. It was around 2 cm long and dividing the caudal vagina in two sections, while the medial and cranial parts of the vagina with paracervix and cervical opening were normally formed. In this case the urethra was also divided by a septum. Its endoscopic examination revealed that the left part was blind and the right one was patent allowing the urine flow. There are two methods recommended for the septum resection: using scissors (2) or using a diode laser (1). In each of the investigated cases (apart from the one with a spontaneous tear) the septum was ligated and dissected using scissors without any complications. Up to date 3 of the bitches were successfully artificially inseminated (AI) during the first estrus after septum removal, in one bitch the AI was unsuccessful and the last one has not had estrus, yet.

The thin membrane separating the vagina from the vestibule usually regresses at the time of puberty (3). Occasionally, remnants of the hymen obstruct some parts of the vaginal lumen. These abnormalities may cause inability to mate naturally due to pain and if mating is successful there maybe problem with natural delivery (5). Some of these septa can be palpated easily during routine vaginal examination, but sometimes vaginoscopy is necessary to diagnose the condition, to assess the size of the septum and to remove it (4). The septa may promote challenging to diagnose subclinical cystitis, vaginitis and metritis.

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085 | Characterisation of extracellular vesicles in the canine ejaculate: Preliminary results

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Introduction and aim: Extracellular vesicles (EVs) are endogenous nano- and micro-sized membrane-bound vesicles secreted in the extracellular medium by prokaryotic and eukaryotic cells (1). They serve as mediators of intercellular communication by transporting cellular content (cargo) from parental to neighbouring or distal target cells. Their cargo is composed of a variety of bioactive and regulatory molecules such as proteins, lipids and genetic material (e.g. mRNA, miRNAs, and DNA) (2). Extracellular vesicles have been identified in the ejaculate of various species, including dogs (3). Canine seminal plasma contains epididymosomes (EVs present in the epididymal fluid) secreted by epididymal cells and prostasomes (EVs present in the prostatic fluid) secreted by prostatic cells. Epididymosomes are involved in the maturation of the sperm cell along the epididymis while prostasomes enhance progressive motility, avoid premature capacitation as well as acrosome reaction and have an antimicrobial, antioxidative and immune-regulatory effect (2). Nevertheless, studies on EVs from canine seminal plasma are limited and knowledge about hypothetical differences between epididymosomes and prostasomes is still lacking. Hence, the aim of this study was to describe the characteristics of both epididymosomes and prostasomes from canine seminal plasma by means of Western Blotting (WB), Nanoparticle Tracking Analysis (NTA) and Transmission Electron Microscopy (TEM).

Materials and methods: The second and third semen fraction of a healthy normospermic dog were collected separately, subjected to centrifugation (720 × ***g*** for 5 min) to remove spermatozoa and further processed by Izon qEV size exclusion column (Izon qEV, iZON science, Cambridge MA) for EV isolation. Presence of epididymosomes and prostasomes was assessed by WB using an EV-specific marker protein (CD63). By means of NTA information on EV size, distribution and concentration was obtained. Finally, TEM was used to obtain morphology and EV size details from both samples.

Results: Epididymal and prostatic fluid showed a positive result for EV-specific marker CD63. Epididymosomes sized between 50–450 nm with a mean diameter of 134.0 ± 3.5 nm. Similarly, prostasomes sized between 30–500 nm with a mean diameter of 140.5 ± 10.6 nm. However, EVs were 100 fold more concentrated in epididymal fluid compared to prostatic fluid with a concentration of 1.14×10^{13} particles/mL for epididymosomes and 1.21×10^{11} particles/mL for prostasomes. These results were confirmed by TEM, with similar morphology and size for both samples, but a higher EV concentration in epididymal fluid.

Conclusions: This study confirms the presence of EVs in epididymal fluid and prostatic fluid of dogs and provides new information

about their ultrastructure. These preliminary results point out a similar morphology and size distribution between epididymosomes and prostasomes but show differences in concentration. The high concentration of EVs in the epididymal fluid supports the important role of epididymosomes in semen maturation. Data from a larger population and further characterisation are however required to describe these EVs into more detail.

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086 | Inquiry on the attitude and experiences of owners towards neutering their dog

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Introduction and aim: Neutering pets has become routine in the last decades and is now the most common surgical procedure carried out by companion animal veterinarians (1). The initial drive behind the widespread practice of spaying dogs was to control pet population and prevent diseases from the reproductive tract (gonadal and mammary tumours, pyometra, etc.). Nonetheless, some recent studies suggest a number of drawbacks of neutering on long-term health parameters in dogs (2). Therefore, the topic of neutering dogs and the appropriate timing for it, is sure to spark hot debate. Besides teaching the scientific data, we are convinced that it is utmost important that last year veterinary students gain more insights in expectations and appreciations of veterinary clients concerning neutering their dog.

Materials and methods: Participants were included in the waiting room of the small animal department of the faculty of veterinary medicine from September 2018 till February 2019. They were asked open-ended questions concerning the reasons for having the dog neutered and the obstacles or negatives for neutering. Furthermore, we informed if they experienced downsides and unexpected outcomes after neutering their dog.

Results: Information from 65 participants was collected (31 owners of a neutered male dog, 34 owners of a neutered female). At the moment of the survey, the mean age of the dogs was 7.2 year (9 months–15.5 years). The mean age at castration was 2.8 years (0.5–9 years) for the male dogs and 3.2 years (0.5–12 years) for the female dogs. For the females, neutering occurred predominantly (68.6%) after puberty. The time after castration was on average 4.1 year for the male dogs and 4.3 years for the females. The reason to neuter the dog was curative in 26.2% of the cases (because of pyometra, mammary neoplasia or severe pseudo-pregnancy for the females, cryptorchidism, prostate hyperplasia, balanoposthitis and testicular neoplasia for male dogs). 'Behaviour improvement' was mentioned in 27.7% of the cases as a reason, but was mainly an issue for male dogs (54.8% vs. 2.9% in female dogs). For 29.6% (5/17) of these males, the effect of neutering on the behaviour was first tested by chemical castration. 'Prevention of breeding' (21.5% in total; 3.2% in males vs. 35.3% in females) and 'preventive health care' (20.0% in total; 9.7% in males vs. 32.4% in females) were stated as reasons predominantly in females. In females, 29.4% mentioned 'prevention of heat symptoms' and 8.8% 'because the vet told us so' as reasons for neutering. During the decision-making process to spay their dog, half of the participants (56.9%) were not aware of any side effects related to neutering, the other participants mentioned 'the risk of the anaesthesia and surgery complications' (18.5%), 'weight gain' (15.4%), 'changes in behaviour' (7.7%), 'coat changes' (6.2%) and 'urinary incontinence' (1.5%) after neutering as an obstacle for them to perform the procedure. After surgery, 41.5% of the participants did not observe any downsides of neutering for their dog. 'Weight gain' and 'coat changes' were mentioned as a downside in 33.8% and 18.5% respectively. Other downsides were 'induction of anxious behaviour' (6.2), 'no changes in behaviour' (3.1%), 'urinary incontinence' (3.1%), 'costs' (1.5%), 'post-op care' (1.5%) and 'no pups anymore' (1.5%). For 78.5% of the participants no unexpected outcomes occurred, whereas 'behaviour changes' (6.2%), 'coat changes' (6.2%), 'urinary incontinence' (1.5%), 'weight gain' (1.5%) and the fact that the behavioural problem was not solved (1.5%) were reported as unexpected by other participants.

Conclusions: By talking to dog owners, the last year vet students realized that, although neutering is considered as a routine procedure, a good communication between vet and client is of utmost importance. Half of the participants mentioned that they were not aware of any complications related to the procedure, which might be caused by insufficient knowledge of potential side-effects (on short or long term). Side-effects were observed in almost 60% of the cases, and for 21.5% of the participants some of these outcomes were unexpected.

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088 | Reproductive potential of male wolves (*Canis lupus*) in Sweden, based on examination of testes and epididymis

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Introduction and aim: The population of grey wolves (*Canis lupus*) in Scandinavia consists of family groups and pair-bonded wolves located both in Sweden and Norway (1). The population was practically extinct in Scandinavia in 1966 (2), but has increased over the

last half-century so that more than 400 wolves were estimated to live in Scandinavia in 2018 (3). Mating season for wolves in Sweden occurs from late February to the beginning of March (4). Wolves in Scandinavia usually mate for the first time at the age of 22 months, which has been considered as the age when they reach sexual maturity (4, 5). To our knowledge, no study has been conducted where sexual maturity in grey wolves have been evaluated based on presence of spermatozoa in the epididymis. The aim of this study was to evaluate the reproductive potential of male wolves in Sweden by examination of testicles and epididymis collected by the Swedish National Veterinary Institute (SVA) between the years 2003 and 2018.

Materials and methods: Male gonads from 221 wolves were examined in this study. All organs had been kept frozen at SVA since necropsy and were thawed before examination took place. The testes and epididymis were separated from each other and both parts were measured and weighed. Biopsies from testes and epididymis were fixed in formalin, cut into thin sections and stained with Hematoxylin and eosin. The sections were examined using a light microscope to determine the presence of elongated spermatids in the seminiferous tubules of the testes and spermatozoa in the cauda epididymidis. Individuals were considered to be fertile when spermatozoa were present and infertile when no spermatozoa were to be found in the sections. Information regarding the individual wolves (body weight, date of necropsy, age) were collected from SVA's databases. The age of the wolves had been determined at Matson's laboratory (6) using cementum annulus counts. The wolves were divided into three age groups: younger than 1 year, 1 year and two years or older. An ANOVA (one way and general linear model) was used to compare testicular weight, testicular volume and body weight between different age groups. Spearman rho was used for correlation analysis and groups of data were compared using Tukey's test.

Results: 212 out of the 221 wolves that were examined were included in the study. Ten wolves had notes in their files stating that they were cryptorchid. 145 wolves in the study were assessed to be fertile and 51 as infertile, whereas 16 samples could not be properly evaluated. Six of the cryptorchid wolves were considered to be fertile. Testicular weight was found to have a positive correlation to body weight and testicular volume. Almost every wolf had reached sexual maturity at the age of one year, and some wolves younger than one year were found to be fertile as well. A significant difference in testicular weight and testicular volume between fertile and infertile wolves was shown.

Conclusions: In a wild wolf population it is likely that breeding does not occur before the age of 22 months for most males. However, this study has shown that male wolves in Sweden have spermatozoa present in the cauda epididymidis at the age of one year or earlier. The significant difference in testicular volume between fertile and infertile individuals could be used in future studies to estimate fertility in wolves in vivo. Based on the findings in this study, the reproductive potential of male wolves in Sweden is considered good.

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089 | Pseudopregnancy and mammary tumors development in the bitch

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Introduction and aim: Pseudopregnancy lactation (PL) is frequent in the canine species with an estimated incidence up to 75%. Its diagnosis is clinical and based on the stage of estrous cycle, behavior and presence of a milky secretion in the mammary gland of a non-pregnant or non-nursing bitch. Mammary tumors represent 25 to 50% of all tumors in female dogs for a global incidence of 0.2 to 1%, with a mean age of 8.4 years at the time of diagnosis. Approximatively half of them are malignant. Previous studies have speculated that there may be an association between recurrent PL and mammary tumors in bitches. The aim of our study was to identify a possible association between PL and mammary tumor development in bitches.

Materials and methods: A retrospective cohort study was performed on 70 bitches presented in our clinical department between the 1st of January 2004 and the 31st December 2010. Bitches included into the cohort were bitches that came for the first time in our consultation and had no suspicion of any mammary tumor at that time, or any history of a previous mammary tumor earlier in their life. Breed, parity, age or previous use of progestins were recorded. The exposure of interest was the presence of clinical sign of PL at the time of the consultation. Furthermore, in 2017, a phone call was made to all owners of bitches that fulfilled the inclusion criteria, in order to know if these bitches had developed further mammary tumors. A survival analysis was conducted using the Kaplan-Meyer method comparing the bitches with or without PL on the occurrence of further mammary tumors.

Results: On the 2157 bitches presented in our consultation during the studied period, 736 were excluded for anteriority criteria (already presented in our consultation before, with or without suspicion of mammary tumor) and 968 were excluded because of inadequate consultation expectancies (ovulation monitoring, ovarian pathology, pregnancy follow up…). For 149 bitches, the clinical database was not sufficient to be sure about the accuracy of the clinical examination. Furthermore, in 2017, 134 owners did not respond to the phone call. So the 70 remaining female dogs composed the cohort.

The most frequent reason of the initial consultation was clinical examination before routine sterilization (80%). The mean age at the first consultation was 2.5 years (IQR [1.4; 4.7]) and the mean time of follow up after first consultation was 7.9 years (IQR [6.6; 9.4]). Of the 70 bitches, 24 (34%) presented at least one episode of PL. During

follow-up, mammary tumors occurred in 6 bitches (9%) with a mean age of appearance 8.5 years (IQR [7.8; 10.3]). In the Kaplan-Meyer study, the bitches with lactation at the moment of consultation seemed to develop mammary tumors after a shorter time: three years after consultation. 15% of bitches that presented PL at the time of the initial consultation later developed mammary tumors, compared to none of bitches in the group of bitches that did not present PL.

Conclusions: The association between PL and the time to occurrence of mammary tumors was not significant. With our results, it is not possible to conclude that the presence of PL is associated with a further development of mammary tumors. Due to the small size of our sample, further studies on a larger scale should be performed to demonstrate an association between mammary tumors and the presence of PL.

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090 | How to interpret serum progesterone values in female dogs, obtained by different determination methods: ELFA versus CLIA

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Introduction and aim: For the detection of ovulation in the female dog, determination of progesterone (P4) concentration in the peripheral blood is the gold standard (1). Under practical conditions, progesterone measurement is additionally used to monitor pregnant dogs with suspected luteal insufficiency, to follow up treatment with natural progesterone, or to verify luteolysis prior to parturition (2). The enzyme-linked fluorescent assay (ELFA), performed on MiniVidas®, was developed as an automated analyzer which provides quantitative results within 45 min. A number of commercial laboratories offer quantitative determination of P4 (chemiluminescent immunoassay (CLIA) or radioimmunoassay (RIA)), whereby serum samples have to be transported to the lab. RIA is often replaced by non-radio isotopic immunoassays, to avoid the problem of radioactive waste (3). CLIA or ELFA therefore seem to be reliable, accurate and safe methods to detect quantitative P4 concentrations (4). The purpose of this study was to compare concentrations of P4 measured with ELFA and CLIA in a large population.

Materials and methods: For this study, 250 serum samples were collected from 72 bitches of different breeds during estrous, the luteal phase in pregnancy and the nonpregnant-cycle, respectively. First, ELFA was performed on the MiniVidas® analyzer in fresh serum samples with values between 0.25 and 80.0 ng/mL. Subsequently, all samples (stored frozen at −80°C until second measurement) were compared to a CLIA carried out at a commercial laboratory (Laboklin, Germany). The values were grouped and analyzed according to their range measured with ELFA (group a = 0–1 ng/mL, group b = 1.01–3 ng/mL, group c = 3.01–6 ng/mL, group d = 6.01–10 ng/mL, group e = 10.01–20 ng/mL, group f = 20.01–30 ng/mL, group g = 30.01–40 ng/mL, Group h > 40 ng/mL).

Results: Serum progesterone concentrations measured by ELFA were significantly higher than progesterone concentration measured with CLIA in groups d (n = 16, 8.20 ± 1.1 vs. 4.36 ± 1.40), e (n = 56, 14.93 ± 2.95 vs. 7.47 ± 4.36), f (n = 41, 24.54 ± 2.61 vs. 10.16 ± 1.93), g (n = 31, 34.43 ± 2.56 vs. 13.85 ± 2.49) and h (n = 48, 50.58 ± 10.98 vs. 20.06 ± 4.82). The presented data show that ELFA measured values are nearly twice as high as measured with CLIA for groups d-h on the one hand, but also higher by trend for groups a, b and c (p = 0.15). The serum progesterone concentration measured by ELFA correlated well with those of CLIA (Pearson`s r = 0.943, n = 250). Taking the animals (n = 20) with ovulation values (4–7 ng/mL) determined by ELFA, the comparison with the CLIA values of the same samples showed that in 85% (n = 17; range 1.9–3.6 ng/mL in CLIA) of the cases the optimal breeding time would have been chosen too early (1–4 days), and in 55% (11 cases) no additional test would have been proposed because of determined values over 5 ng/mL in ELFA, but below 5 ng/mL in CLIA. The appropriate factor to compare both methods depends on the progesterone concentration (factor *0.6* for range from 0–6 ng/mL; factor *0.5* for range from 6.01–10 ng/mL; factor *0.45* for range from 10.01–20 and factor *0.4* for values > 20 ng/mL).

Conclusions: The determination of serum progesterone concentrations with ELFA (MiniVidas®) provides rapid and reliable results, but the significant higher values (twice higher and more) compared to other methods, like CLIA (IMMULITE) should be considered in the clinical interpretation, esp. in interpreting ovulation time, required follow-up examinations and to fix correct and fertile mating data. The use of factors multiplied by ELFA values could be a possible solution to compare the methods and therefore the individual concentrations in a proper way.

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091 | Successful relocation of pregnant horn trapped in inguinal hernia – a case report

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The 6-year-old Ca de Bou bitch was presented to Clinic of Department of Reproduction of Wroclaw University Environmental

and Life Sciences due to the painful enlargement in the left groin that recently enlarged quickly. On clinical examination no other abnormalities were detected. The bitch was 5 weeks pregnant. The ultrasound examination revealed the enlargement in the left groin was a hernia with trapped uterine horn. Four fetuses – two in the uterus horn located in hernia and another two in the abdominal cavity – all with detectable heart ratio. Due to the fact, that the bitch was a pedigree and very valuable, the relocation of the horn back to the abdominal cavity was attempted. The prognosis was guarded because of the anesthetic risk for the fetuses, possibility of damaging uterus, fetuses or placentas and a good chance of relapse owing to the burden of the content and the pressure of abdominal content on the stitches. For premedication, meloxicam (0.2 mg/kg), midazolam (0.1 mg/kg) and propofol (2 mg/kg) were used. Anaesthesia was maintained on isoflurane. During the procedure fentanyl (2 ug/kg) was administered twice for analgesic effect. The skin incision was made in the left groin at the basis of hernia, avoiding mammary gland. Under the skin, the approximately 25-centimeters long uterine horn was visible as well as the bifurcation and the expanded mesentery. The inguinal canal was broaden, the horn and mesentery were relocated. The canal was closed using interrupted suture pattern (Monosyn 0). The ultrasound examination after the surgery shown four alive fetuses located in abdominal cavity. On the follow-up, 4 days later, four alive fetuses with well-defined heart ratio were still visible. No pathologies or sings of inflammation inside the uterus were present. The owners were advised to limit the activity of the dog, administer highly digestible diet and come for control visits every week. The planned Caesarean section was done on approximately 61st day after the mating. There were four alive puppies delivered. The uterus was removed during the surgery at the owner's request.

The reason of inguinal hernias is the existence of inguinal rings defect which happens to be a gate for abdominal content. Inguinal hernias occur more often in females. It was suggested they might be related to impact of estrogen, as they are usually diagnosed during estrus or pregnancy and rarely affect spayed bitches (1). Erdogan et al. have presented the case of a Boxer bitch which suffered inguinal hernia containing uterus horn. The ultrasound examination confirmed that the dog was 40 days pregnant. Ovariohysterectomy has been performed (2) in that case. There have been a number of reports on pyometra with inguinal herniation and Sontas et al. described a case of the hernia with hydrometra/mucometra in a poodle bitch (3, 4). The usual treatment procedure is ovariohysterectomy in such cases.

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093 | Aerobic bacterial flora isolation from the prepuce of stud dogs

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Introduction and aim: There are numerous bacteria that normally reside in the preputial cavity in a male dog. These bacteria are normal inhabitants and their presence in low to moderate numbers ensures a healthy mucosal environment. When these bacteria invade the reproductive tract, if the animal's immune system does not function properly, they can proliferate and cause disease (1). The normal preputial microflora consists mainly of aerobic bacteria that can also be isolated from canine semen, as well as from dogs with bacterial prostatitis, orchitis and epididyimitis. The preputial bacterial flora include: *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Escherichia coli*, *Pseudomonas* sp., *Bacillus* spp. and *Mycoplasma* species (2). Mycoplasma can be isolated from both healthy fertile dogs and dogs with genital diseases (3, 4). Aerobic bacteria might be transferred between a stud dog and a female dog at mating. The aim of this study was to investigate the preputial aerobic bacterial flora in healthy stud dogs and to examine antibiotics susceptibility.

Materials and methods: The preputial samples were taken from 121 healthy stud dogs. The obtained samples were cultured aerobically on selective, special and indicator nutrition media. Microorganisms were identified according to biochemical and antigenic characteristics. Coagulase-positive staphylococci isolates were identified using multiplex polymerase chain reaction (M-PCR). Commercial tests Liofilchem®, Italy, were used for isolation of mycoplasmas and identification of minimal inhibition concentration (MIC). Microorganisms' isolates were tested for antimicrobial susceptibility using Kirby and Bauer standardized method. Eight antimicrobial agents were tested in this study. Polymerase chain reaction was used to detect genes associated with resistance beta-lactams (*blaZ*). p-values of $p \leq 0.05$, $p \leq 0.01$ were considered to be significant.

Results: Aerobic bacteria were isolated in 82.64% of cases from 121 healthy stud dogs. The following bacteria were isolated from prepuce of dogs: *Staphylococcus pseudintermedius* – 41%, *Escherichia coli* – 15.70%, *Staphylococcus aureus* – 14.47%, *Mycoplasma* spp. – 11.57%, *Ureaplasma* spp. – 4.95%, *Pseudomonas* spp. – 4.95%, *Proteus* spp. – 4.1% and *Streptococcus* spp. – 3.3%. The highest resistance of the isolated *Staphylococcus pseudintermedius* was found to amoxicillin (61.22%) and tetracycline (65.3%). The most susceptible antimicrobials were amoxicillin/clavulanic acid (71.43%) and enrofloxacin (65.31%). The most susceptible antimicrobials of the *S. aureus* isolates were enrofloxacin (73.7%) and cefalexin (63.2%). The highest resistance was detected to amoxicillin (52.6%). *BlaZ* gene was detected in 6.12% strains of *S. pseudintermedius* and 2.1% of *S. aureus* isolated from prepuce

of stud dogs. We determined that amoxicillin/clavulanic acid was more effective than amoxicillin ($p < 0.001$) *in vitro*. Mycoplasma isolates were the most susceptible to enrofloxacin (≤8 μg/mL) and doxycycline (≤4 μg/mL). The highest resistance was observed to azithromycin (≥8 μg/mL).

Conclusions: The most frequently isolated microorganism in the prepuce of stud dogs was *Staphylococcus pseudintermedius* – 41%. The highest sensitivity of *Staphylococcus pseudintermedius* was observed to amoxicillin/clavulanic acid and enrofloxacin. Bacterial isolation in healthy dogs is important because these bacteria can become pathogenic when an enabling condition is created.

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097 | Fertility-sparing surgery in a pedigree queen

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The routine clinical approach in cases of uterine pathology is spaying of the animal. However, there are situations when saving of fertility becomes a crucial issue for the owner or the breeder. There are some reports on single horn removal (cornuectomy) performed in dogs and cats that did not affected the subsequent fertility (1, 2). In the reported case a pedigree queen was subjected to left uterine horn removal due to its pathology, which had no impact on her future health and fertility.

Clinical case: A 1.5 year old Maine coon queen was presented 11 days after normal parturition and delivering 5 healthy kittens. It was her second pregnancy, the first one uneventful with 9 (1 dead) kittens. On the 11th day after parturition the owner noticed a yellow-pink fluid coming out of the vulva. On clinical examination the cat was bright, the body temperature was normal (38.9°C), the abdomen slightly was tense cranially and there was small amount of yellow discharge from vulva. The US scan revealed 2 dead fetuses in the cranial abdomen. The preliminary diagnosis was made of retained fetuses and the exploratory laparotomy was performed. The uterine body and both horns were small (0.5–1 cm) indicating the advanced puerperal involution. At the cranial tip of the left horn, near to the ovary there was a large, kidney-shaped mass (15 × 8 cm). The queen was very valuable for breeding purposes, so it was decided to perform a unilateral hysterectomy (cornuectomy). The incision was made on the border between the horn and uterine body and the affected horn with the mass and the ipsilateral ovary were removed. The incision was sutured the same way as for caesarian section (Monosyn 3/0). The histopathological examination of the mass revealed it was a uterine hernia with two separate fetal sacks, two placentas and two fetuses (about 50 days old). The post-op recovery was uneventful and the litter was weaned without any problems. The queen showed first symptoms of heat 2 months after the operation, thereafter the heats were regular (every 21 days) with typical symptoms. She received Melovine implant to suppress estrus symptoms, but half a year after the operation she was mated, got pregnant and spontaneously delivered 2 kittens. The pregnancy, delivery and weaning were normal. Subsequently, she was implanted with Melovine to prolong time before the following pregnancy and was mated 6 months later (16 months after operation) and delivered 4 alive and 1 dead kitten. She was again implanted with Melovine, mated 10 months later (28 months after operation) and delivered 7 alive and 1 dead kitten. She showed no disorders during any of her pregnancies nor deliveries, she delivered spontaneously altogether 12 healthy kittens and 2 dead ones in 3 consecutive unicornual pregnancies.

Similar to the reports in dogs (2) and one cat (1) in the presented case, the partial removal of the reproductive tract might be a very rewarding procedure, maintaining the ability to reproduce and having no adverse effects on the female. Interestingly, in the reported case the reproductive efficiency seemed not to be significantly affected, as the queen delivered in total 14 kittens in 3 unicornual pregnancies, which is 4,6 kitten per pregnancy. Based on the presented clinical case the authors suggest that in individual, justified cases when the fertility of the female needs to be preserved the unilateral cornuectomy should be considered, as the appropriate and save treatment option.

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099 | Prostatic adenocarcinoma in neutered male dog: A case report

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The dog is one of the few species in which spontaneous prostate cancer occurs (1). The incidence of prostate cancer was found to be 2.6% of dogs with prostatic diseases in male dogs (2), which can occur in both intact and neutered dogs. Although prostatic neoplasia is uncommon, due to a lack of pathognomonic symptoms of each prostatic disease, similar clinical signs may occur.

Clinical case: An 11-year-old neutered male mixed-breed dog was referred to Watcharaphol Animal Hospital with suspected prostatitis.

After the history taking, physical examination and transrectal digital palpation, enlargement of the prostate was found. Ultrasonography and radiography were performed for examination of the prostatic structure and to determine the size, shape and location of the prostate. After that, the final diagnosis was made by prostatic massage and wash to obtain samples for cytology.

Results and Discussion: The dog had an asymmetrical, irregular and enlarged prostate and pain when transrectal digital palpation was performed. The notable clinical signs of this dog were indicated by problems with defecation and urination. The defecation problems consisted of tenesmus and constipation, which may occur due to compression of the rectum by the irregular contour and enlargement of the prostate. The urination problem was stranguria, which may reflect pain or local invasion into the lumen of prostatic urethra with partial obstruction by inflammation of prostatitis (1) or neoplasia. Another clinical sign was anorexia. Ultrasonographic and radiographic examination showed enlargement of the prostate and mineralization of the parenchyma. Usually, the normal size of the prostate from radiography should not be over 50% of the width of the pelvic inlet and if over 90% indicates likely prostatic cancer, abscess or cyst (3), but in this dog, it was over 100%. It was also shown that the rectum was compressed by the enlargement of the prostate and lung metastasis. From the examination data, the tentative diagnosis was prostate cancer. Then, prostatic massage and wash were performed to obtain samples for cytology. The sample from the prostatic wash is more likely to inform the final diagnosis than an ejaculate sample, especially in a neutered male dog from which prostatic fluid cannot be obtained (4). Finally, the cytology revealed small clusters of carcinoma cells and that the tumor cells were a large cuboidal shape, with light basophilic cytoplasm and large pleomorphic round nuclei with prominent nucleoli. Final diagnosis was prostate gland adenocarcinoma. Prostate gland adenocarcinoma and transitional cell carcinoma are the most common prostatic tumors in dogs and are usually found in 8- to 10-year-old dogs (4). The dog was treated with palliative treatment, and Piroxicam, a non-steroidal anti-inflammatory drug (NSAID), was used in this dog. The mechanisms of the NSAID towards tumors are not clearly defined; they are likely multi-factorial and may involve cyclooxygenase-2 (COX-2) inhibition and consequent apoptosis (1). Tramadol and Hyoscine were used for relief of pain, and fluid therapy for correcting dehydration. After being treated for approximately one month, the dog died. During the final stage, this dog showed lethargy, weight loss and dysuria, and during the last week of life, the urine was drained by inserting a catheter. Diagnosis can detect the advanced stages of the cancer, but survival time is very short, ranging from days to months after diagnosis, and many patients are euthanised at the time of diagnosis (5). Other treatments are prostatectomy and radiation therapy, but these have complications; therefore, no treatment has been shown to increase survival times (4), and the cancer is aggressive and highly metastatic.

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100 | The autologous ovarian tissue cross side transplantation enhanced by platelet rich plasma (PRP) performed in a rat model

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Introduction and aim: Ovarian tissue cryopreservation is a currently available option for young women undergoing anti-cancers therapies for fertility maintenance. Unfortunately tissue autotransplantation still needs to be ameliorated concerning revascularization time, onset and rate and follicular reserve survival. The aim of this pilot study was to investigate possible surgical technique for autologous ovarian tissue cross-side transplantation enhanced by addition of platelet rich plasma (PRP) on a rat model.

Material and methods: 15 female WAG rats in diestrus (confirmed by cytological smear) were anesthetized and underwent the standard ventral ovariectomy. The ovaries were released from ligament (lig. suspensorium ovarii) and the main blood vessels (A. and V. ovarica) were secured by absorbable sutures. The ovaries were collected, washed in sterile PBS and cleaned from the surrounded adipose tissue. One of each ovaries was placed in sterile NaCl and served as a control whilst another was moistened in PRP. The organs were replaced on the opposite side of the uterus then sutured to the vascular uterine mesentery and inserted into the uterine horn wall where a special pouch was made. The animals were divided into 3 groups (n = 5 each group) and euthanized at Day 3, 7 and 14 day after surgery, respectively. The organs were collected into 4% formaldehyde solution and analyzed by immunofluorescence for apoptosis level and vascularization using specific antibodies (TUNEL-based assay, PECAM- platelet endothelial cell adhesion molecule antibody). Follicle count was performed using standard hematoxylin & eosin stained histological preparations. Results

The ventral cross side ovarian autotransplantation performed in rats seems to influence the neovascularization and follicular survival. Increased new blood vessels density (up to 30%, 14 days after autotransplantation) and a conserved primordial follicular pool was observed in ovaries treated with PRP in comparison to the controls.

Conclusions: The study showed a slight enhancement of neovascularization and an improvement of follicular reserve preservation in ovaries treated with PRP after autologous cross-side transplantation.

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101 | Descriptive analysis of the abstracts printed in the successive proceeding books of the EVSSAR congresses from 1998 to 2015, which haven't been published in international peer-reviewed journals.

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Introduction and aim: Each year, several abstracts are printed in the proceeding books accompanying the EVSSAR congresses. Some are presented as short communications and others as posters. Not all of them give rise to further publications in international peer-reviewed journals. The aim of our study was to quantify and make a descriptive analysis of unpublished abstracts that have been presented during the successive congresses of the EVSSAR between 1998 and 2015.
Material and methods: We initially decided to exclude from our study the abstracts that concerned other species than dog and cat, or abstracts of fundamental research in the field of biotechnology of reproduction. For each other abstract, using keywords found in the title, but also the names of the different authors we used 4 scientific databases (PubMed, Scopus, Vetmed Resource and Google scholar) to find any potential scientific publication that could be related to it. All apparently unpublished abstracts were classified according to different criteria and a descriptive analysis of their content was made.
Results: 832 abstracts related with dog or cat reproduction were found in the successive proceeding books. From these, 375 seem to have remained unpublished (45.1%). 84/276 (30.4%) short communications and 289/556 (52.0%) posters seem to have remained unpublished after their presentation in the EVSSAR congresses. 324/375 (86.4%) of the unpublished abstracts concerned dog reproduction and 45/375 (12.0%) concerned cat reproduction – other studies concerned both species. The studies on female reproduction (gynaecology, obstetrics, pathology of the genital organs and the mammary gland, fertility, endocrinology) were over-represented within unpublished abstracts (242/375 – 64.5%). Other unpublished abstracts were related with male reproduction in 107/375 (28.5%) of the cases or neonatology in 26/375 (6.9%) of the cases. 36/375 (9.6%) of unpublished abstracts were related with fundamental research, 247/375 (65.9%) were clinical studies based on a precise research protocol and 92/375 (24.5%) were case reports. More than 50% of the abstracts that have remained unpublished were presented at the EVSSAR congresses after 2010. Neonatology is a recent topic that appeared in the proceedings after 2013. Considering that EVSSAR is a European society, we found that 260/375 (69.3%) of the unpublished abstracts were presented by authors from European teams. There were also unpublished abstracts from 14 other countries outside Europe. There was a tendency for the main topics of the abstracts to vary depending on the geographical origin of the authors (for example 29% of the unpublished studies from Asia concerned the cat vs. 12% for overall studies).
Conclusions: 45.1% of the abstracts found in the EVSSAR proceeding books between 1998 and 2015 seem to have remained unpublished. More posters remained unpublished compared with short communications, which may be related with a better scientific content of the latter. There were a minority of studies on cat reproduction compared with dog reproduction (12.0 vs. 86.4%), which may mean a lack of knowledge in the field of feline reproduction. Nevertheless, the number of unpublished abstracts has increased over the last years, together with the number of abstracts presented at the EVSSAR congresses. Further studies in order to analyze the reasons of the non-publication of so many abstracts have to be conducted.
References: Proceedings books of the EVSSAR congresses (Barcelona 1998, Lyon 1999, Oslo 2000, Milano 2001, Liège 2002, Dublin 2003, Barcelona 2004, Amsterdam 2005, Budapest 2006, Estoril 2007, Vienna 2008, Wroclaw 2009, Louvain-la-Neuve 2010, Milano 2011, Whistler 2012, Toulouse 2013, Wroclaw 2014, Hannover 2015.

102 | Spaying dogs before or after the onset of puberty: Different risk for acquired urinary incontinence?

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Introduction and aim: Acquired urinary incontinence (AUI) affects up to 20% of spayed dogs. While breed, body weight and size, tail docking, obesity and urethral length are proven risk factors for AUI, the timing of spaying relative to the onset of puberty is still controversially discussed. Spaying puppies before three months of age increased the risk for AUI, but dogs spayed shortly before the first estrus had only about half the risk for AUI compared with dogs spayed after puberty. In other studies time of spaying in relation to the onset of puberty was not revealed as a risk factor for AUI (1). The aim of our study was to investigate the risk factor "time of spaying relative to the onset of puberty" on AUI by controlling for possible confounders.

Materials and methods: A retrospective matched-pair cohort study was carried out on data from 1285 dogs spayed for ≥5 years at two veterinary hospitals. The dogs were matched for breed, body weight, age, age at spaying and time interval since spaying. In each pair, one dog was spayed before and one after the onset of puberty. AUI was assessed using an owner questionnaire. A conditional logistic regression for matched pairs for AUI was performed on 131 pairs of dogs. Additionally, observation of canine mammary tumors (CMT) by the owners of the dogs were recorded.

Results: The dogs were 5.4–16.9 (9.9 ± 2.6, mean ± SD) years old and had been spayed 4.9–15.6 (8.8 ± 2.5) years before. Bodyweight varied between 4.5 and 74 kg (26.0 ± 11.7) and body condition score (BCS) between 1.0 and 7.5 (4.7 ± 1.1; assessed on a 9-point scale). These parameters did not differ between the two spay groups, however, dogs spayed before the onset of puberty were younger at the time of spaying compared to those spayed after puberty (0.3–1.4 (0.6 ± 0.2) years vs. 0.3–8.2(1.8 ± 1.3) years). Time of spaying relative to the onset of puberty was identified in the conditional logistic regression model as the only risk factor for AUI ($p = 0.007$), while age, time interval since spaying, bodyweight and BCS posed no risk for AUI. AUI was reported in 30 (22.9%) dogs spayed before puberty, but only in 14 (10.7%) dogs spayed after the first oestrus. CMTs were observed in only 5 dogs, which were all spayed after the onset of puberty between the ages of 0.9–8.2 years.

Discussion: Spaying before the onset of puberty was the only risk factor for AUI in our study. However, as matching of pairs was performed for all possible confounders to clearly delineate the effect of the time of spaying relative to onset of puberty on AUI, it is not surprising that these parameters were not identified as risk factors. CMT were only found in dogs spayed after puberty. Timing of spaying has long been known as a risk factor for CMT development (2). Although the scientific evidence was questioned in a systematic review (3), the CMT-sparing effect of spaying before the age of 2.5 years was confirmed recently (4). Due to the low number of pairs included in the study, differentiation between the risk factors "years of ovary exposure" and "time of spaying in relation to the onset of puberty" was not possible.

Conclusion: Spaying before puberty increases the risk for AUI, however postponement means increased risk for CMT. Our results underline the importance of individual counselling not only about pros and cons of spaying, but also in regard to the time of spaying relative to the onset of puberty. Breed predispositions, dog keeping practices and personality of the dog and the owner should be also taken into account.

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103 | The concentration of IL-2 in peripheral and uterine blood of bitches with pyometra

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Introduction: Pyometra is a common reproductive disorder affecting female dogs. Intoxication leads to a strong immune reaction. As a consequence, interleukins are produced, which support the immune system. Interleukin 2 (IL-2) has a key function in the immunity by its direct effects on T cells.

Aim: The aim of the study was to compare the concentration of IL-2 in peripheral and uterine blood of bitches with pyometra.

Material and methods: Thirty-seven bitches with pyometra were enrolled in the study (9 with open cervix pyometra, 23 with closed cervix pyometra, and 6 healthy controls). During the ovariohysterectomy peripheral blood was collected from the cephalic antebrachial vein and uterine blood from the uterine vein. Serum was prepared by centrifugation (10 min, 4000 g), placed in micro centrifuge 1-mL tubes and stored at −71°C until use. IL-6 concentrations were determined using a ELISA Kit for IL-2 (Cloud-Clone Corp. SEA073Ca). IL-2 concentrations were compared between paired samples (veins) using the Wilcoxon's signed-rank test and between unpaired samples (groups) using the Kruskal-Wallis test with Dunn's post-hoc test. A significance level was set at 0.05. The analysis was performed by the TIBCO Statistica 13.3 program (TIBCO Software Inc.).

Results: Average concentration of IL-2 in dogs with pyometra with open cervix was 1.8 pg/mL in the uterine vein and up to 22.3 pg/mL in the peripheral vein. In bitches with closed cervix, concentration was up to 391.7 pg/mL in uterine vein, and up to 219.4 pg/mL in peripheral vein.

Conclusions: The level of IL-2 did not differ significantly between the peripheral and uterine vein. This may be due to the chronical character of pyometra and as a result of peripheral and uterine blood mixing.

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104 | The concentration of IL-6 in peripheral and uterine blood in bitches with pyometra

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Introduction: Pyometra is a common reproductive disorder affecting female dogs. Intoxication leads to a strong immune reaction. As a consequence, interleukins are produced, which support the immune system. Interleukin 6 (IL-6) is an important mediator of fever and acute phase response.

Aim: The aim of the study was to compare the concentration of IL-6 in peripheral and uterine blood in bitches with pyometra.

Material and methods: Thirty-seven bitches with pyometra (11 with open cervix pyometra, 26 with closed cervix pyometra) and 6 healthy controls were enrolled in the study. At the same moment, during the ovariohysterectomy (control and test group), peripheral blood was collected from the cephalic antebrachial vein and uterine blood from the uterine vein. Serum was prepared by centrifugation (10 min, 4000 g), placed in micro centrifuge 1-mL tubes and stored at −71°C until use. IL-6 concentrations were determined using a ELISA Kit for Interleukin 6 (IL6) (Cloud-Clone Corp.SEA079Ca)

IL-6 concentrations were compared between paired samples (veins) using the Wilcoxon's signed-rank test and between unpaired samples (groups) using the Kruskal-Wallis test with Dunn's post-hoc test. A significance level was set at 0.05. The analysis was performed by the Statistica program.

Results: Average concentration of IL-6 in bitches affected by open cervix pyometra was 26 pg/mL 17.2–60.3 (5.2–105.8) in peripheral vein and 39.1 pg/mL 28.3–101 (6–285.4) 28.3–101 (6–285.4) in uterine vein. In bitches with closed cervix pyometra, concentration was 13.6 pg/mL3.6–24.4 (0–232.5) in peripheral vein vs. 30.1 pg/mL 18.8–55.1 (6.4–314.5) in uterine vein. In control group the average concentration was 0, 0–2.8 (0–5.2) in peripheral vein and 3.2 pg/mL 0–7.2 (0–11.6) in the uterine vein.

Conclusions: The level of IL-6 is significantly higher in bitches with ropomacic than in healthy bitches both in uterine and peripheral but it does not differ significantly between open and closed pyometra. The level of IL-6 is not significantly different between veins in healthy bitches but in bitches with pyometra (both open and closed), IL-6 level is significantly higher in the uterine vein. Higher concentrations of IL-6 in dogs with pyometra can be connected to generalized inflammatory reaction and production of acute phase protein, nevertheless this study needs further research.

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105 | The use of doxycycline for Mycoplasma treatment in early pregnancy in dogs – a preliminary study

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Introduction: *Mycoplasma spp.* is one of the components of the physiological bacterial flora of the vagina in dogs, however its overgrowth may cause early embryonic resorption during pregnancy. In females with a history of pregnancy loss in which *Mycoplasma spp.* strains were isolated, it is usually assumed that this pathogen was the source of problems. Treatment, however, is difficult and long-lasting, and also poses a threat to developing foetuses (teratogenic effect, D drug by the USA Food and Drug Administration (FDA))

Case description: Two females of different breeds (Border collie and German shepherd) in the proestrus phase of the ovarian cycle, who had a history of early embryo resorption in previous pregnancies were admitted to the Small Animal Clinic for clinical investigation before the next planned mating. Clinical and ultrasound examination (Mindray M6, microconvex probe 8Mhz) did not reveal any abnormality. A vaginal swab was performed for the exclusion of *Mycoplasma spp.*, *Herpesvirus* and *Brucella spp.* The presence of *Mycoplasma spp.* was confirmed by the PCR method. It was decided to perform artificial vaginal insemination and doxycycline treatment in a dose 5 mg/kg q12 h for 18 consecutive days (after LH peak) – corresponding to the time of embryonic nidation of the embryos into the vaginal mucosa. The total time of antibiotic therapy was 22 days in German shepherd and 24 days in Border collie. The ultrasound monitoring of pregnancy was performed weekly and the level of progesterone was measured, in which no abnormalities were observed. During delivery, a vaginal and placental swabs were taken. In both cases, a negative result was obtained. In puppies (6 German Shepherd, 8 Border collie), no clinical signs of disease were observed.

Conclusions: This preliminary study require further analysis and increase of the number of cases, however, it may suggest that until nidation, doxycycline does not have a toxic impact on dog embryos

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106 | Dog breeders profile in France – usual practices, maternal care perception and the stress and behavioral problem management in peripartum

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Introduction and aim: In France, dogs are an important part of society with a population appraised of 7.4 million. The exact demand for puppies in France is unknown. It is estimated to be of 800.000 per year with the majority of puppies being unregistered. The origin of the puppies is one important aspect to consider, since the conditions to raise dogs and animal welfare management are not the same in all places. In addition, puppies coming from well-established breeders seem to be more emotionally stable and have less behavioral problems as adult dogs (1). A possible explanation for this characteristic might be associated to maternal behavior and duration of the interaction amongst the bitch and the littermates during early age. Despite recent studies showing the importance of the quality of maternal care in dogs (2, 3, 4), its direct effects on behavioral problems later on in life are still unclear. To better explore the importance of maternal behavior and management of stress of the dam around parturition, a questionnaire was developed for breeders looking closely at the assessment criteria of maternal care and stress from dog breeders' perspective as well as the importance of the relationship of the dam toward the puppies soon after birth.

Materials and methods: An online survey consisting of 35 questions divided in 5 sections: breeder information, breeding program, whelping information, maternal behavior and stress management around parturition was submitted to almost 1200 breeders using professional email databases.

Results: 345 breeders answered the survey being representative of 119 dog breeds. In relation to the demographics of the responders, 58.8% define their activity as "non-professional", 86% report to raise up to two breeds and 74.4% have less than 5 litters/year. In relation to the changes regarding housing around parturition, amongst the options, 46.8% of the responders transfer the bitches to a maternity area, while in 29% the living area is maintained with the introduction of a whelping box. Estimation of whelping date is done using multiple criteria and the large majority of labor happens during the night (80.0%) under direct breeder's surveillance (91.5%). The majority of bitches (90.4%) fully embrace their maternal role soon after the birth of puppies. High frequency of nursing (86%) and licking (82%) the puppies is seen as an adequate maternal care by breeders. Excessive agitation is the main perceived (71%) sign of stress around parturition. To address the stress of the bitch during the peripartum period, remaining close to the bitch is the most common method used by 80.6% of breeders, whereas only 17.9% of respondents use natural calming products (pheromones, Bach flowers, and homeopathy). Inappropriate maternal behavior is described as likely in 67.1%, with an overrepresentation of primiparous bitches (96.1%).

Conclusions: The dog breeding industry in France is considered a familiar business with a close contact between animals and owners. Definition of maternal care has a common ground among breeders. Stress during the peripartum period is primarily addressed by more intensive human presence to reassure the bitch.

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107 | Interval between removal of 4.7 mg deslorelin implant and restoration of the function of sexual hormones in cats

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Introduction: Deslorelin implants have been widely used to produce a reversible sterilization in several species (1). A temporary suppression of reproductive activity is frequently needed in cat breeding establishments due to difficulties of managing feline reproduction. Long acting GnRH implants such as deslorelin have a prolonged duration in cats (12–15 months in tomcats and 18–22 months in queen) (2, 3) which is often too much for cat breeders who frequently come back asking for early implant. However, the interval between implant removal and resumption of fertility in cats has not been studied in details yet.

Material and methods: Thirteen privately owned cats (7 tomcats and 6 queens) with a presenting complaint of control of reproduction were administered a 4.7 mg deslorelin implant. Cats were divided in 4 different groups; tomcats implanted for 6 or 9 months (n = 4 and 3, respectively), queens implanted for 6 or 9 months (n = 4 and 4, respectively). Implants were placed in the periumbilical area and removed under light sedation at the end of the study period. All treated animals were evaluated weekly. Blood samples were taken every week and measurement of testicles, vaginal smears and penile spikes observation was performed in every visit. Testosterone levels were measured on serum samples of tomcats in order to determine testicular activity.

Results: Until now, 3 males and 2 females of the 6-month treatment and 2 males and 1 female of the 9-month treatment have resumed sexual hormones function after deslorelin treatment. Restoration of sexual hormones function was determined by presence of

testosterone, penile spikes, 40% of keratinization of vaginal smear of ≥ 40% and by appearance of estrus behavior. Results show that in average, cats treated with deslorelin implanted for 6 months restore sexual hormones activity in 29 ± 11 days while cats implanted for 9 months restored it in 27 ± 15 days (p = 0.355) independent of sex or season at removal.

Discussion: The effect of a 4.7 mg deslorelin in cats has been determined in both tomcats and queens with a block of sexual hormones activity (2, 3). Our results show that removal of the 4.7 mg deslorelin implant at a time when the implant is displaying its complete effects is followed by a resumption of sexual hormones functionality after about 4 weeks independent of sex, age or season. Further studies are needed to determine the exact time when activity of sexual hormones is restored after implant removal.

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108 | Canine semen quality and prostatic fluid composition during osaterone acetate (Ypozane®) treatment for benign prostatic hyperplasia

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Introduction: Benign prostatic hyperplasia (BPH) is a common prostatic condition of adult intact dogs for which androgenic hormones play a role (1). Steroidal antiandrogens such as osaterone acetate (OA) compete with androgen receptors at prostatic level with a specific inhibitory action on prostatic volume causing a decrease in prostatic size (2). Little is known about the effects of OA on semen quality and seminal plasma composition.

Materials and methods: Eight adult male dogs (>5 years of age) with BPH diagnosed through history, clinical exam and prostatic ultrasound were selected for the study. On the day of the diagnosis (D0) a) a fractionated semen sample was obtained using an artificial vagina; b) semen quality was assessed using the second fraction; c) the third fraction was centrifuged and the supernatant stored at −18°C for further examination; d) an oral OA (Ypozane ®) treatment (0.25–0.5 mg/Kg/7 days) was administered. Items (a), (b) and (c) were repeated on D60, D120 and D180. Electrolytes (Na, K, Zn, Cu, Cl, Mg), glucose, cholesterol and triglycerides were evaluated on the third fractions of each ejaculate using chemiluminescence (Immulite 1000, Siemens, Milano, Italy). ANOVA test was performed with statistical package SAS 9.4 (SAS institute, Inc. Cary, NC, USA) to test for differences of semen quality and biochemical(electrolytic composition across time.

Results: Prostatic volume of every dog decreased during treatment with maximum values at 60 days post treatment (mean reduction of 45.7 ± 5.8%, p < 0.05). Sperm concentration decreased during the first 2 months of treatment in 62.4% of dogs (p > 0.05) and increased over the next 4 months with maximum values at D180. The % of sperm tail abnormalities increased until D60 (from 10.6 ± 2.4 to 25.7 ± 2.4, p < 0.05) while other sperm defects did not change significantly. In prostatic fluid, Zinc concentration (µg/mL) increased during treatment (4897.2 ± 765.1) with higher values at D180 (9155.52 ± 1081.98) (p < 0.05) while remaining electrolytes, glucose, cholesterol or triglycerides did not show any difference.

Conclusion: Based on this data, the effect of OA treatment caused an increase in sperm tail abnormalities during the first 2 months which disappear during the following 4 months of treatment. Prostatic fluid Zn concentration increased during OA treatment with higher levels at the end of the treatment. This data correlates positively with the increase of sperm concentration at the end of the treatment suggesting that Zn may have a positive effect on canine sperm concentration similarly to what has been reported for humans (3).

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109 | A case report of a chronic vaginitis caused by a polyglycolic acid suture

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Clinical case: A ten-year-old Rhodesian Ridgeback was presented in consultation for a chronic vaginitis that appeared after an ovariohysterectomy. This bitch was spayed for a pyometra eight months ago in a private practice. The ovariohysterectomy was performed, PGA RESORBA® (polyglycolic acid with resolactone coating) was used for ligatures of ovarian pedicles and a uterine cervix. To ligate the uterus, PGA RESORBA® USP 2-0 (EP 3.0) was placed like a transfixing ligature through the cranial part of vagina and a transection was performed through the cervix. The uterus was removed

and the ovariohysterectomy was completed by the standard surgical protocol. Several weeks after the surgery a clear mucous discharge from the vulva was observed by the owner. A suspicion of a stump pyometra was pronounced and a surgical revision of an abdominal cavity was recommended by the private practice. The owner asked us at Small Animal Clinic for a clinical examination of her bitch and our recommendation if the bitch should undergo the surgery. The bitch was presented with a yellowish purulent odorless vulvar discharge lasting two weeks. The bitch was active and in a good general health. An ultrasonographic examination was performed and neither the ovaries nor the uterus were found. A vaginoscopy with a rigid endoscope was performed. Hysteroscope/TCI Endoscope Karl Storz with fiberoptic system was used. A vaginal mucosa was pink and clear. We visualized a dorsal median fold of vagina where a small amount of the purulent discharge was present. A source of the discharge was cranially from this place. An air insufflation was used for an opening of the vagina and a vaginal fornix was visualized. A remnant of the uterine cervix was present and a suture material was found between the cervix and a fornix bottom. The material was a polyfilament thread. We tried to remove the thread with an endoscopic forceps with double action jaws and third attempt was successful. We released and removed the suture which was very fragile. Than a lavage with a warm saline was performed. The fornix was inspected for suture residues and the examination was closed. No special care was required and the patient was released home. We contacted the owner three weeks later and no signs of any vaginitis were observed.

Discussion: The PGA RESORBA® is a polyfilament absorbable suture material with complete absorption in approximately 90 days. Metabolization of the PGA suture material within the tissue occurs by the uptake of water, thus reversing the synthesis. The monomeric glycolic acid is split enzymatically into CO_2 and H_2O by normal metabolism (1). The part of transfixing ligature passing through the vagina was not absorbed by metabolism and persisted. In the canine vagina is probably a lower humidity than in soft tissue so the PGA was not split and behaved like a foreign body. Based on this case the authors recommend to use only double circumferential ligature without suture through the vagina. Over the years, various types of vaginal foreign bodies have been described in bitches like grass awns (2, 3) or a piece of retained calvarium from a macerated fetus (4). To the authors' knowledge, this is the first reported case of a PGA foreign body in the vaginal fornix of a dog.

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110 | Prolonged whelping with birth of a vital puppy in a Labrador Retriever – A case report

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Clinical case: An eight-year-old Labrador Retriever bitch was presented to the clinic 46 hours after the delivery of one dead and 52 hours after birth of two vital puppies, respectively. The bitch was primiparous and the unwanted mating with an Australian Shepherd dog had happened 61 days before presentation. The owner reported that the signs of whelping had ceased with parturition of the third dead puppy. The reason for veterinary consultation was a sudden increase of the amount of vaginal discharge and recurrence of abdominal straining. Only few minutes after arrival at the clinic a vital puppy with its foetal membranes and a conspicuous amount of bloody discharge was delivered without any obstetrical help. Subsequently the dam was examined generally and gynaecological and a complete blood count, blood analyses as well as blood gas analysis was performed. All parameters were within normal range. An abdominal radiograph revealed no further foetuses remaining in the uterus. A vaginal cytology showed a moderate number of neutrophil granulocytes, erythrocytes and cells of epithelial origin. The new-born had a weight of 366 gram, showed no signs of malformation and started suckling milk immediately. In comparison, the body weights of the two previous born puppies, which were accompanying their mother to the clinic, were 594 and 596 grams. All three puppies developed well and were examined again 20 days later. The two first born puppies had reached body weights of 2600 and 3008 grams, the after-born had 2160, respectively. The bitch showed no signs of distress and good motherly behaviour at any time. The dogs were last seen by the authors at three months of age. The further development was without any special findings.

Discussion: To the authors knowledge a prolonged inter-whelping interval of 46 hours with spontaneous birth of a live puppy without any obstetrical help has not been described before. In dogs, normal whelping lasts up to 24 hours (1) and inter-whelping interval should not be longer than four hours (2). Dystocia is suspected when these timespans are exceeded and foetuses are expected to be dead (1). Problems in parturition can be caused by maternal or foetal reasons, combinations of both may also occur (3). In this case a foetal cause seems to be unlikely, as the retained puppy was born without obstetrical help after all. The most common maternal reason for dystocia

is uterine inertia, which can be primary or secondary. Since the dog was not under veterinary observation at the time of cessation of parturition, the cause for the dystocia will remain unclear. However, a temporary secondary uterine inertia is the most probable explanation for the phenomenon.

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111 | Fetal bones in the caudal vagina of a bitch 14-months post-spaying

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Introduction: Dystocia and stillbirth are common problems during parturition in bitches (1). However, prolonged fetal retention in the uterus in a bitch is not very common (2, 3) and usually produces metritis (2). A case of fetal retention in the vagina has not been published before.

Clinical case: A 4-years old bitch was referred to the veterinary clinic due to chronic vaginitis and severe pain in the perianal area since a couple of months. The bitch has undergone ovariohysterectomy 14 months before, short after she gave birth to four healthy puppies. Due to vaginitis, pain and general discomfort the bitch underwent many therapies with antibiotics and anti-inflammatory drugs, but short after therapies the symptoms were reappearing. During clinical examination, the bitch was aggressive due to severe pain in the perianal area. A bone structure was palpated in the vagina and the presence of os clitoris was suspected. The vaginal smear confirmed vaginitis. Blood tests revealed slight leukocytosis (20 G/L). Urine test was normal and the suspicion of urinary calculi was excluded. The X-Ray confirmed the presence of bone structures in the perianal area. The bitch was sedated, vaginal palpation was repeated and no os clitoris was found. Rectal examination excluded fecal fistula. The vaginal endoscopy was performed. In the caudal vagina, close to the cervix of the uterus bone fragments were noticed and were extracted. A sponge with adrenaline was placed in the vagina for 12 hours due to the bleeding. Antibiotic therapy (Enrofloxacin 5 mg/kg QD – we used this antibiotic due to previous history of treatment with first-line antibiotics and we suspected bacteria resistance to this antibiotics) together with anti-inflammatory drugs (Metacam 0.2 mg/kg QD) for one week. The bitch recovered soon after the bone extraction. The anatomical examination revealed the bone fragments corresponded to the cranial bones of previously mummified fetus.

Discussion: To the best of our knowledge this is the first described case of a bitch with the presence of fetal bones in the caudal vagina, 14-months post-spaying. This case shows how important is to proceed full clinical examination together with necessary additional tests after parturition as well as prior to the ovariohysterectomy.

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112 | Characterization of spontaneous autoimmune orchitis in dogs

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Introduction and aim: With a prevalence of up to 35% in dogs with reproductive problems, azoospermia is one of the most important reasons for male infertility. Published cases of dogs with azoospermia describe histopathological damage of the testicular tissue, including immune cell infiltration indicating autoimmune orchitis (1, 2, 3). However, the number of published cases is limited and there is no consent about the involved cell types in those two cases where characterization was performed. Namely, T- and B-cells were detected in the testicular tissue from one dog (2), whereas inflammation was described to be lymphoplasmacytic in the other case (3). In a previous study, we identified gross bilateral immune cell infiltrations in testicular biopsies of nine dogs with non-obstructive azoospermia, NOA (1). This was not the case in another dog with assumed obstructive azoospermia, OA. In this study, we aimed to characterize the immune cells in the respective canine testicular biopsies.

Materials and methods: Immunohistochemistry was performed using specific antibodies against CD3-, PAX5-, MAC387-, IgG- and IgM to proof the presence of T-lymphocytes, B-lymphocytes, macrophages and early and late plasma cells, respectively. In total, bilateral biopsies of nine dogs with NOA and samples from five healthy normospermic control dogs (CG) were stained and assessed. For each antibody, all immunopositive signals were counted in 20 random views at 400-fold magnification in each sample and the localization (interstitium, tubule, blood vessel) 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 numbers of immunopositive signals for CD3, PAX5, MAC387, IgG and IgM in NOA and CG by Mann Whitney test (exact, two tailed).

Results: In general, immunopositive signals against CD3, PAX4, MAC387, IgG and IgM were found in all NOA samples. Different to this, CG samples revealed no immunopositive signals against PAX5, IgG and IgM indicating absence of B-lymphocytes and plasma cells. Regarding the numbers of immunopositive signals, significantly more immune cells were found in the testicular samples of NOA dogs compared to CG. Not including immune cells present in the blood vessels, CD3, PAX5, IgG (each $p \leq 0.001$), MAC 387 and IgM (each $p < 0.05$) immunopositive cells differed significantly in NOA samples compared to CG. In CG immunopositive signals were restricted to the interstitium and blood vessels (MAC387 positive cells), whereas in azoospermic dogs about 1/3 of all identified immune cells were located in the tubuli (CD3-, IgG and IgM-positive cells only), the remaining 2/3 were found within the interstitium. CD3 and MAC387-immunopositive cells were the predominant populations in CG, whereas CD3, IgG and IgM-positive cells were predominant in NOA samples.

Conclusions: Presence of immune cells in healthy testicular tissue is low and restricted to T-cells in the interstitium and macrophages, the latter mainly within the blood vessels. Different to this, inflammation characterizing spontaneous autoimmune orchitis is of lymphoplasmacytic nature with T-lymphocytes and plasma cells being the predominant immune cell populations in NOA samples. Plasma and T-cells invade the testicular parenchyma, cross the basal membrane and blood testis barrier to finally reach the tubular lumen thereby disturbing spermatogenesis.

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113 | Correlation between sperm kinetics and Doppler velocimetry of domestic feline testicular artery

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Introduction and objective: Blood supply to the testes is performed by the testicular artery, and testicular ischemia is one of the main causes of infertility. Among all described methods for evaluation of testicular hemodynamics, Doppler ultrasound technology stands out as a rapid and non-invasive examination. However, literature regarding its use with domestic cat is still scarce. The aim of this study was to verify if there might be any correlation between the Doppler velocimetry of domestic feline testicular artery and sperm kinetics assessed by automated system (CASA system).

Materials and methods: Twenty-two adult mixed-breed tomcats were used in this experiment. The animals were sedated with dexmedetomidine (10 μg / kg) and ketamine (12 mg / kg), intramuscularly, then placed in dorsal decubitus position for shaving the scrotum and B-mode ultrasound analysis for testicular volume measurement using a 7 Mhz-linear probe (DC7, Mindray, China). A triplex Doppler analysis of the testicular artery from both testes was performed in the distal supratesticular and marginal regions, in accordance with the technique described by Trautwein et. al (2019) (1). Systolic peak velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI) and resistivity index (RI) were measured. Cats were then submitted to orchiectomy, and spermatozoa from the epididymal cauda were recovered and analyzed in the CASA system (Ivos II, Hamilton Thorne, USA), using setup for cats. The variables which were taken into account were motility; progressive motility; concentration; percentage of fast, medium, slow and static spermatozoa; average path velocity (VAP); straight-line velocity (VSL); curvilinear velocity (LCV); lateral head amplitude (ALH); beat-cross frequency (BCF); straightness (STR); linearity (LIN). The velocity index (SVI); movement index (SMI) and wobble (WOB) was also calculated. The kinetic variables were correlated with Doppler velocimetry using the Pearson correlation test, with Sigmaplot 12.0 software and a significance level of 5%.

Results: Mean Doppler velocimetry of distal supratesticular vs. marginal region was: PSV = 7.1 ± 2.1 vs. 11.1 ± 3.1 cm/s; EDV = 4.2 ± 1,3 vs. 7.3 ± 2.1 cm/s; PI = 0.52 ± 0.14 vs. 0.40 ± 0.11; RI = 0.41 ± 0.08 vs. 0.33 ± 0.07. The mean of the spermatic kinetics was motility = 48.5 ± 27.3%; progressive motility = 25.5 ± 15.9%; percentage of fast spermatozoa = 43.5 ± 26.2%; medium = 4.9 ± 2.8%; slow = 30.6 ± 18.1%; static = 20.8 ± 27.3%; VAP = 135.2 ± 45.8 μm/s; VSL = 108.9 ± 36.5 μm/s; VCL = 209.3 ± 69.0 μm/s; ALH = 6.6 ± 2.3 μm; BCF = 31.8 ± 10.4 Hz; SVI = 392.7 ± 128.0; SMI = 333.5 ± 59.16; WOB = 123.7 ± 9.1. There was a positive correlation between PSV of the supratesticular region with VAP (r = 0.330), VCL (r = 0.342) and SVI (r = 0.330); as well as between the PSV of the marginal region and VAP (r = 0.365), VSL (r = 0.336), VCL (r = 0.347) and SVI (r = 0.357). There was a correlation between PI and RI of the marginal region (r = 0.301 e r = 0.303) and static spermatozoa (r = −0.306 e r = −0.325).

Conclusion: It was concluded that there was a correlation between the Doppler velocimetry of the feline testicular artery and the spermatic kinetics, suggesting that cats with lower testicular blood flow have lower quality of spermatic kinetics.

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114 | Comparison of radiographic pelvimetric dimensions in Scottish Terriers of different countries

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Introduction and aim: Dystocia can be of maternal or fetal origin. Certain breed predispositions have been discussed concerning dystocia of maternal origin. Scottish Terrier (ST) is considered to be one of the predisposed breeds due to dorsoventral flattening of the pelvis (1). However, caesarean section (CS) rates vary highly among countries from 34% in US to 60% in UK and Denmark. The aim of the study was to investigate the incidence of dystocia in different countries and to determine whether correlations between physical traits and pelvic confirmation existed.

Materials and Methods: ST with a breeding history were recruited in Germany, Scandinavia (DK, S, FI) and Switzerland. Participating owners had to complete an online questionnaire for each bitch about details of the breeding history (including natural delivery, ND/CS; reason for dystocia). External parameters, as body weight, height at withers, length and width of the pelvis were measured. Additionally, radiographic projections of the pelvis were taken in two projections to evaluate pelvic conformation by the following pelvimetric parameters: Conjugata vera (CV), Diameter verticalis (DV), Conjugata diagonalis (CD), Diameter transversa (DT); furthermore CV/DT ratio and area of the pelvic inlet were calculated. Investigators were blinded to the dog's reproductive history. Statistical analysis (t-test, Mann Whitney) was performed to compare ND bitches with CS bitches.

Results and Discussion: Questionnaire data were obtained from 68, external dimensions and results of pelvimetry from 64 STs. The overall prevalence of OCS (CS required due to obstruction only) was 13%. A negative correlation between external and pelvimetric parameters was identified: large external measures were associated with smaller pelvimetric results. Bitches with reported OCS had significantly smaller CV/DT ratio than ND bitches. Although puppy body weight was higher in litters with CS, relative puppy weight (%) and litter size did not differ between ND and OCS. Significant differences between countries were identified. Countries with higher occurrence of dystocia generally had larger sized dogs with smaller pelvic measurements. An increased risk of delivering by CS was seen in bitches with a sire born by CS.

Conclusion: Different to an earlier study (2), no correlation between external traits and pelvimetric dimensions or risk for CS due to obstruction was found. However, as bitches with OCS had lower CV/DT ratio, i.e. more dorsoventral flattening, this parameter might be used to predict the risk for obstructive dystocia in STs and to select breeding bitches.

This study was supported by Agria and SKK Research Foundation.

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116 | The role of IGF-I, -II and IGF-I receptor for recrudescence of spermatogenesis following treatment with a GnRH agonist implant in dogs

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Introduction and aim: Insulin-like growth factor-I and -II (IGF-I) interact with testicular cells through the insulin-like growth factor-I receptor (IGF-IR) and have been shown to be critical for proliferation, development and function of Leydig- (LCs), Sertoli (SCs)- and germ cells in the testicular tissue of different species (1). This study aims to provide further knowledge about the role of the IGF system on canine testicular function during downregulation of endocrine and germinative testicular function due to treatment with a GnRH agonist and subsequent restart.

Materials and methods: Sexually mature male Beagle dogs (n = 16) were treated with a GnRH-agonist implant (GnRH-I; 18.5 mg azagly-nafarelin). Five months later, the implant was removed at downregulation and dogs were castrated at 3-week intervals (W0/3/6/9/12, n = 3–4 each). Untreated adult (CG, n = 5) and juvenile dogs (JG, n = 3) served as controls. Additionally, three dogs each were treated for 5 months with either an implant containing buserelin (PG) or deslorelin (SG) and castrated thereafter. RT-qPCR was performed using primers against the canine IGF-I and IGF-II. To test for the protein expression of IGF-I and the IGF-IR, immunohistochemistry (IHC) was performed, followed by computer-assisted analysis of the percent of immunopositive area (PIA) and staining intensity (mean grey scale, MGS) separately for the interstitial and tubular compartment with the program ImageJ FIJI. GraphPad Prism was used for statistical analysis studying either restart (dataset 1; W0-12 compared to CG) or downregulation (dataset 2, W0, PG, SG compared to JG/CG). $p < 0.05$ was considered significant.

Results: On mRNA level, significant differences could be identified for IGF-I for dataset 1 and 2 ($p < 0.05$ each). IGF-II mRNA expression revealed no significant differences on both data sets. On protein level, LCs, SCs, peritubular- and perivascular cells

stained positive for IGF-I and -IR. In the tubular compartment, significant differences in IGF-I expression were identified in dataset 1 for PIA ($p < 0.01$) and MGS ($p < 0.05$), with the highest MGS in W3 compared to CG and W0 ($p < 0.05$ each). In the interstitium, IGF-I expression differed also significantly (PIA $p < 0.05$; MGS $p < 0.01$) with highest PIA and MGS observed at W3. For data set 2, PIA and MGS ($p < 0.05$ each) differed significantly between groups in the tubular compartment, with PIA being significantly higher in JG compared to PG ($p < 0.05$). In the interstitium, PIA was significantly higher in JG compared to CG, SG, PG and W0 ($p < 0.01$–$p < 0.05$), but MGS did not differ. For IGF-IR and dataset 1, PIA ($p < 0.05$) and MGS ($p < 0.01$) differed significantly in the tubular compartment, with the strongest staining intensity at W9 and a significantly lower PIA in CG compared to W0 and 9. In the interstitium, PIA was lowest at W0 ($p < 0.05$). Strongest staining intensity of the interstitium was observed during early restart of spermatogenesis (W3, W6) with, however, no significant differences. For data set 2, PIA differed significantly for both compartments ($p < 0.01$ each) with highest PIA in the tubular compartment in PG compared to JG ($p < 0.05$) and CG ($p < 0.01$). Different to this, PIA in the interstitium was highest in CG compared to the W0, PG and JG ($p < 0.01$–$p < 0.05$). MGS differed only significantly in the tubular compartment ($p < 0.001$) with W0 and PG staining stronger than JG and CG ($p < 0.01$ each).

Conclusions: The results show that IGF-I and -IR are rapidly upregulated during restart of spermatogenesis after GnRH-I removal. Consequently, IGF-I, but not IGFII seem to play an important role during recovery of testicular function following downregulation with a GnRH-I.

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117 | Morphometric sperm head deviations in a poodle: a case report

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Introduction and aim: Pedigree champion dogs are valuable as potential sires to produce the next generation of champion dogs. Adequate fertility is, however, crucial for their long-term use as studs. Sub- and infertility can have various causes including abnormal semen parameters with increased percentage of morphologically abnormal spermatozoa or decreased total sperm counts etc., but also bacterial overgrowth (pure/high growth of Streptococcus canis, Escherichia coli var. hem., Mycoplasma canis/cynos); sometimes it can be even idiopathic. Here we aim to present a case of a male dog with altered sperm head morphometric deviations and to discuss possible consequences.

Materials and Methods – Clinical case: A three-year-old purebreed male poodle dog (P) was presented for examination as the first bitch he mated did not conceive. No ovulation timing had been performed in the bitch, but she got pregnant twice before with the same management, using different males. Clinical andrological examination including ultrasound was performed as well as semen collection and analysis. Bacterial culture was performed on a semen aliquot. Clinical andrological examination did not reveal any abnormality. Semen collection in the presence of an estrous bitch was successful and semen analysis was normal except for the fact that sperm heads seemed to be longer and smaller than normal. Bacteriological examination revealed high growth of Mycoplasma cynos in pure culture. Enrofloxacin treatment was initiated. To verify alterations of the sperm head's dimensions, sperm morphometry was performed using formol-citrate fixed sperm smears. Images were taken using 1000x magnification and spermatozoa of a healthy mature mixed-breed dog (C) prepared in the same way served as control. Dimensions (length, width and area) of 100 sperm heads of each dog were measured using GIMP 2.8 (https://www.gimp.org/) and the results in pixel were afterwards converted into actual µm. Statistical analysis was performed using GraphPad Prism 8.0.1 to test for differences in sperm head dimensions between the poodle and the control dog. Following testing for normal distribution, a t-test was applied for the width and Mann Whitney test for the length and area.

Results and Discussion: Length (P: 5.5 ± 0.2 µm; C: 5.1 ± 0.2 µm), width (P: 2.9 ± 0.2 µm; C: 3.4 ± 0.2 µm) and area (P: 17.7 ± 1.5 μm^2; C: 18.6 ± 1.2 μm^2) of the sperm differed significantly between both dogs ($p < 0.0001$ each). The sperm heads of the poodle were significantly longer, smaller and had a smaller area than the reference sperm heads. Different morphometry of canine sperm heads might be due to breed differences as previously described for British Bulldog (1), however, different to our case only sperm head length differed in earlier descriptions, but not width etc. The bacteriological sample that was collected after end of enrofloxacin treatment was sterile; semen analysis re-confirmed the sperm head deviations; two bitches mated conceived (8 and 7–8 pups in ultrasound).

Conclusion: The morphometric alterations of the poodle's sperm heads are bigger than normal diversity between breeds; however, clinically obvious morphometric sperm head alterations are not necessarily related to infertility. However, morphometric analysis should be performed on more breeds to gain further insights and identify deviations. Which role the pure growth of Mycoplasma cynos played for (in-)fertility, remains to be clarified. Anyway did high/pure growth not reveal in other alterations of the sperm parameters.

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118 | Long term effect of the GnRH antagonist acyline on corporal and radiographic development of domestic cats

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Introduction and aim: GnRH analogs use for contraception has been described, but there is little objectively obtained data to describe their influence on development. In a previous study, it was described the effect of a GnRH antagonist, acyline (NIH, Bethesda, MD, USA) vs. control cats (1). Data was described until puberty and no radiographic description was made. This study pretends enlarge the data of the same protocol until the cats finished growth and describe radiographic parameters.

Materials and Methods: Thirteen male and twelve female cats were included in this study. Treated group received acyline 33 μg/100 g sc within the first 24 hours of birth, and was repeated weekly until 3 months of age; control group remained untreated. Follow up was performed until they finish growth, which was defined when three consecutive body measurements were constant. Body weight (kg), withers height (cm), and body length (cm) measured from the most cranial aspect of the scapula to the most caudal aspect of the ischium, were recorded weekly. Lateral and front radiographs of the antebrachium were taken monthly, beginning at 4 weeks of age. Radial length was measured on the radiograph from the most proximal aspect of the radial head to the most distal aspect of the bone. Proximal and distal radial physis were considered closed when a radiolucent line was no longer visible. Measurement of the bone cortex and bone marrow was also performed. Age at end of growth in both groups was compared by a Student t test. Corporal measurements as radial length was calculated by repeated measures ANOVA. Pearson correlation was performed between body measurements and radial length.

Results: Age (weeks) at end of growth for acyline vs. control male cats was 31.5 ± 1.7 vs. 33.6 ± 1.1 ($p > 0.05$) and 36.0 ± 2.1 vs. 37.0 ± 1.0 ($p > 0.05$) in acyline vs. control female cats. The final estimated body weight, withers height and body length in acyline vs. control male cats were 3.49 vs. 3.04, 29.79 vs. 31.22 and 36.75 vs. 35.50 respectively ($p > 0.05$). The same parameters in female cats were 2.54 vs. 2.59, 28.84 vs. 28.05 and 34.78 vs. 32.28 ($p > 0.05$). The average rate of growth (weeks) for body weight, withers height and body length in male cats were 18.41 vs. 16.92, 8.38 vs. 7.80 and 8.59 vs. 8.52 ($p > 0.05$). The same parameters in female cats were 16.42 vs. 16.93, 7.71 vs. 7.11 and 8.23 vs. 7.98 ($p > 0.05$). The least squares mean estimated for radial length (cm), in acyline vs. control male cats was 8.48 vs. 8.23 respectively ($p < 0.05$). In female cats the radial length was higher between 8 and 28 weeks ($p < 0.05$). The thickness of radial bones cortex was lower in acyline vs. control females from week 32 onward ($p < 0.05$). No difference was detected in thickness of the radial bone marrow ($p > 0.05$) in all groups. All groups closed their proximal and distal physis within the normal ranges described for the species. Correlation coefficient between male radial length and body length, wither height and weight, were 0.93, 0.92 and 0.91 ($p < 0.01$) respectively. For females were 0.96, 0.93 and 0.93 ($p < 0.01$).

Conclusions: While no differences were detected in body measurements, acyline treated animals presented longer radial length than control animals, different to what was previously reported in gonadectomized cats (2). Although there is a high correlation between radial length and body measurements, it is not enough to detect the difference in the last ones because it is perhaps too small. The ages of physis closure agree with the normal values reported (3). Clinical relevance of the lower thickness of cortical bone in treated female cats requires further investigation.

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119 | Analysis of hematology, serum chemistry and reproductive function-related biomarkers in accordance with the age of large breed dogs

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Introduction and aim: The aim of this study was to evaluate the aspects of hematology, serum chemistry, and reproductive function-related biomarkers including growth hormone (GH) and insulin growth factor 1 (IGF-1) between old (8–12 years old, n = 7) and young (1–2 years old, n = 7) retriever dogs.

Materials and methods: The whole blood was collected from cephalic vein of each group of retriever dogs. The complete blood counts and serum chemistry panels was analyzed and ELISA analysis were performed in two groups. For ELISA analysis, concentration levels of GH and IGF-1, critically required for induction of sexual maturation, were analyzed. Data were analyzed by *t*-test using GraphPad Prism 5.0 with statistical significance accepted as $p < 0.05$. Values are shown as means ± standard error of mean.

Results: In results of blood analysis, there were significantly increased levels of total protein in old dogs compared with young dogs (old: 6.85 ± 0.17 vs. young: 6.41 ± 0.09, p < 0.05), while the concentration level of glucose was significantly higher in young dogs compared with old dogs (old: 87.57 ± 2.16 vs. young: 100.40 ± 2.66, p < 0.05). In results of ELISA analysis, the concentration levels of GH and IGF-1 showed significantly increased in young group (4.55 ± 0.82 and 104.70 ± 8.07, respectively) compared with old group (1.10 ± 0.09 and 68.60 ± 7.99, respectively, p < 0.05).

Conclusions: In conclusion, we demonstrated that significantly low levels of total protein and high levels of glucose, GH, and IGF-1 were observed in young dogs compared with old dogs, which is similar patterns in research for age-related diseases in human. In particular, the expression level of GH and IGF-1, well-known for regulating of sexual tissue in uterus/prostate/seminal vesicle as well as promoting fertility in both males and females, would extend our knowledge of the role of these factors in reproductive process dependent on ages in dogs.

This study was supported by Cooperative Research Program of RDA (CCAR, #PJ013958012019), and the authors would like to acknowledge the Research Institute for Veterinary Science and the BK21 plus program which provided insight and expertise that greatly assisted the research.

Key words: blood analysis, dog, enzyme-linked immunosorbent assay, growth hormone, insulin growth factor 1

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120 | Evaluation of hematology and serum chemistry parameters in small breed dogs associated with ovariohysterectomy

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Introduction and aim: The aim of this study was to evaluate the aspects of hematology and serum chemistry between intact female (n = 3) and spayed female (n = 4) healthy dogs especially in small breeds such as Pomeranian and Maltese which are well known as the most widely raised companion breeds in South Korea. We would like to discover whether the absence of female reproductive organ could affect the basic hematology and serum chemistry factors in healthy individuals.

Materials and methods: The whole blood was taken from the jugular vein of each female dogs under the consent of the client and collected into EDTA and SST tube. The complete blood counts and serum chemistry panels were analyzed. Data were analyzed by *t*-test using GraphPad Prism 5.0 with statistical significance accepted as p < 0.05. Values are shown as means ± standard error of mean.

Results: In results of blood analysis, there were significantly increased levels of MCHC in spayed female dogs compared with intact female dogs (spayed: 35.7 ± 0.26 vs. intact: 34.0 ± 0.28, p < 0.05), while all the other complete blood counts and serum chemistry panels did not show any significant differences.

Conclusions: In conclusion, we demonstrated that only MCHC indicator showed significantly high levels in spayed female small breed dogs compared with intact female small breed dogs, which could be extended through our further study to analyze the role of female reproductive organ in basic blood parameters. Besides, it will be necessary to secure larger sample pool of dogs, and a specific workup of comparing between breeds and age should also be included.

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122 | Sperm cryopreservation using conditioned medium derived from amniotic mesenchymal stem cells in dogs

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Introduction and aim: Cryopreservation technique have beneficial effects such as management of genetic diversity, safeguard against natural disaster, and transportation of frozen semen to multiple locations (1). However, cryoinjuries such as intracellular ice formation, imbalance in solute/electrolyte concentration, cell dehydration, and reduction unfrozen fraction occur (2). The aim of this study was to evaluate the effect of conditioned medium derived from amniotic mesenchymal stem cells (AMSCs-CM) of dogs in canine sperm cryopreservation.

Materials and methods: Amniotic mesenchymal stem cells (AMSCs) were established from amniotic tissues collected by Caesarean section, and its surface marker (CD44, CD90, and CD45) were checked by fluorescence-activated cell sorting (FACS). The conditioned medium was collected from the AMSCs cultured for 24 hours in serum-free Dulbecco's Modified Eagle Medium. The collected conditioned medium was centrifuged at 7000 g for 10 minutes, filtered using a 0.2-μm filter and stored at −30 °C until used. Five beagles were used for semen collection. The first buffer for sperm cryopreservation was prepared without CM (control) or with 5% AMSCs-CM. Sperm motility and viability were analyzed by computer assisted sperm

analyzer (CASA), and plasma membrane integrity was evaluated by hypo-osmotic swelling assay. All the experiments were replicated three times. GraphPad Prism was used for statistical analysis. The significance level was $p < 0.05$.

Results: The FACS analysis showed that CD44 and CD90 were positively expressed but not for CD45 in AMSCs. Unfortunately, the CASA results showed that there were no significant differences in motility, viability, linearity, straightness, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), and lateral displacement of sperm head (ALH) between the control and AMSCs-CM treated groups. Also, the hypo-osmotic swelling assay revealed that plasma membrane integrity of AMSCs-CM treated group was not significantly different from that of the control group.

Conclusions: In conclusion, we demonstrated that 5% addition of AMSCs-CM in first buffer did not show significant difference in terms of motility parameters and plasma membrane integrity of dog sperms after cryopreservation. The percentile of AMSCs-CM added in first buffer or the number of replication performed in this study might have been low. In further study, additional replication is necessary to estimate the variability associated with improvement of sperm quality and the amount of AMSCs-CM needs to be varied to improve this study. This study was supported by Cooperative Research Program of RDA (CCAR, #PJ013954022019), Research Institute for Veterinary Science, and the BK21 plus program.

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123 | Similarities in growth and reproduction parameters in three cloned dogs

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Introduction and aim: PPARα (peroxisome proliferator activated receptors) is a ligand-activated nuclear receptor that has a role in lipid and glucose metabolism; it is expressed in tissues with a high catabolism such as skeletal muscles, heart, kidney, liver, brown fat and in some vascular cells (1), Leydig cells and spermatocytes, especially in human testis (2). Three transgenic dogs overexpressing PPAR α in their muscles were reported in 2017 (3). This study was conducted to evaluate the similarities in reproduction parameters in these cloned dogs by measuring each dog's body growth, testis size and comparing their sperm parameters.

Materials and methods: Three cloned beagles (2 years old) overexpressing PPARα in a muscle specific manner were used in this study (AL1, AL2 and AL3); they were kept under the same conditions with the same diet. Body height was measured from the forefoot pads to the top of the scapula and length was measured from the anterior scapula to the posterior pubis. Testis length was measured with vernier calipers. Sperm collection was performed by manual stimulation on AL1 and AL3 only, as AL2 has unilateral cryptorchidism in the right testis in abdominal position. Semen was collected and transferred for sperm analysis with Computer-assisted sperm analyzer (CASA). Sperm motility, straightness, linearity and amplitude of lateral displacement (ALH) were evaluated.

Results: Dogs' growth parameters results are as follow: 69.3 cm, 74.9 cm and 64.1 cm respectively for each dog's length, 37.3 cm, 36.7 cm and 37.8 cm respectively for each dog's height, and 11.1 kg, 10.0 kg and 9.1 kg respectively for each dog's weight. The sizes of the three dogs' testis for the right and left longitudinal axis length were 3.1 cm and 3.3 cm for AL1, 3.2 cm for AL2, and 3.0 cm and 3.1 cm for AL3 respectively. As for the right and left transverse axis length the sizes were 1.6 cm and 1.7 cm for AL1, 1.7 cm for AL2 (descendent testis), and 1.9 cm and 1.7 cm for AL3. The CASA analysis for AL1 and AL3 sperm (200millions/mL concentration) showed a motility of 71.1% for both samples, 27.2% and 24.0% of linearity respectively, 47.7% and 45.9% of straightness respectively, and 4.7 and 4.9 μm of ALH respectively.

Conclusions: The cloned dogs showed similar testis sizes and growth parameters. In addition, sperm parameters (motility, linearity, straightness and ALH) were similar in CASA analysis. Unilateral cryptorchidism in one of the dogs could be the consequence of a mutation in the Insulin-like factor 3 gene which plays a role in testicular descent (4). We can conclude that cloned dogs have normal growth and some reproductive characteristics are not different between them. Further studies about a possible role of PPARα in the reproductive tract muscles should be conducted along with a comparison of semen quality and a study about the cryptorchid testis of AL2. This study was supported by NRF (#2018R1C1B6009536), Research Institute for Veterinary Science, and the BK21 plus program.

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124 | Leukemoid reaction in bitches with pyometra

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Introduction: Leukemoid reaction is a condition in which an increase of leukocytes count in peripheral blood is observed as a

result of infection, hemolysis or intoxication. The leukemoid reaction must be differentiated from hematopoietic cancer – leukemia. In the first one, there are no tumor cells in the peripheral blood, while the cells of different stages of the leukocytic line are present. The most frequent causes of leukemoid reaction development are pyometra, pyothorax and acute inflammation of the pancreas.

Due to the ongoing chronic inflammation of the endometrium, the production of granulocytes in bone marrow and spleen increases. The main triggering factor is the granulocyte growth factor (G-CSF), and the finding of its increase in plasma can be used in the diagnosis of leukemoid reactions. Significant leukocytosis is observed (total number of leukocytes greater than 50 000/μL) with neutrophilia and a clear left shift. In the white-blood cell count we can observe a large number of mature segmented neutrophils, slightly smaller number of banded neutrophils, as well as about 5% maximum of all leukocytes are metamyelocytes and myelocytes. While these extremely elevated leukocyte counts could lead to a misdiagnosis of leukemia, leukemoid reaction can be recognised because the leukogram reveals that the predominant leukocytes are segmented and earlier neutrophils.

Materials and methods: In the years 2018–2019, in 6 out of 26 females (average age 8.3 years old, different breeds) with diagnosed and treated pyometra, a concomitant occurrence of a leukemoid reaction was found. Each of the bitches underwent surgical treatment consisting of ovaries and uterus removal, broad-spectrum antibiotic therapy as well as supportive treatment consisting of symptomatic treatment.

Results: In all 6 females, the leukocytes count at diagnosis was above 59 000/μL (59 000–103 000/μL) and toxic granules and vacuolation were found in the neutrophil cytoplasm.

In 2 of this 6 bitches, thrombocytosis was also observed. In 2 bitches after ovariohysterectomy, anemia with Heinz bodies and eccentrocytes is developed. In one bitch, a full blood transfusion was necessary. In 3 patients, leukocyte count started to decrease only after the introduction of prednisolone at an anti-inflammatory dose (1 mg / kg). The decrease in the number of white blood cells, during the treatment was noticed after about 8 days, and the recovery of leukocytes count took from 3 to 9 weeks. All females with the observed leukemic reaction survived.

Conclusions: The phenomenon of leukemoid reaction requires further research and observation in a larger number of cases.

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125 | Incidence of microbial contamination of ejaculate in male dogs presented for infertility

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Introduction and Aim: Male dog fertility might be compromised by several factors, among which subclinical infections of genital tract, difficult to detect as often asymptomatic. Few data are present about incidence and identification of different microorganisms in canine semen (1, 2, 3). In human medicine, composition of sperm microbiota and identification of bacterial species in men of infertile couple is object of great interest in last years (4, 5). Microbial identification in semen is crucial for stud dog management and success of breeding. The aim of the present study is to evaluate the incidence of sperm microbial contamination in stud dog presented for infertility.

Material and Methods: In this study were included 29 male dogs, weighting between 5 to 70 kg, aged between 2 to 8 years. All dogs were presented because one or more failure in producing pregnancy when mated with healthy and correctly monitored bitches. Beside clinical evaluation of stud dogs and ultrasonographic examination of urogenital tract, sperm collection by manual stimulation, preceded by preputial rinse with warm saline, was obtained in sterile plastic container, not allowing contact of penile mucosa with the collection cup. Complete sperm analysis, including microbiological research for *Enterobacteriaceae*, *Pseudomonadaceae*, Streptococci, Staphylococci, Enterococci, *Mollicutes*, was then performed. For microbial analysis samples were seeded using a calibrated loop onto Blood agar Medium and MacConkey agar, which were incubated overnight, and enriched specific medium containing a pH indicator were used for *Mycoplasma* and *Ureaplasma*. The sample was considered positive when growth of an organism was greater than 10^5 CFU/mL for aerobic bacteria and greater than 10^3 Colour-Changing Units for Mollicutes (1, 2).

Results: Clinical evaluation and urogenital ultrasound demonstrated the presence of nodular testicular lesion in 5/29 and benign prostatic hyperplasia in 5/29 of the dogs analyzed.

Semen was classified as poor in 15/29 dogs, with more represented abnormalities, sometimes combined, asthenozoospermia in 9/15, oligozoospermia in 8/15 and morphological defect in 8/15 (proximal and distal droplet). From the microbial analysis 17/29 samples were negative and 12/29 were positive. Microorganism isolated were 4/29 E. Coli, 3/29 *Ureaplasma*, 2/29 Streptococcus Canis, 1/29 *Mycoplasma*, 1/29 Proteus, 1/29 Pseudomonas Aeruginosa, with 5 of the 15 cases with poor semen quality resulted positive to microbial contamination.

Conclusions: In human medicine it has been demonstrated that the majority of patient subset providing semen for infertility evaluation was found to have bacterial contamination, with *Mycoplasma*, *Ureaplasma*, Escherichia coli and Chlamydia trachomatis, being more harmful to sperm function than others (4). Interestingly in our study 41% of the dog presented for infertility and 33% of the dogs with poor semen quality resulted positive to bacterial overgrowth, and microbial prevalence is similar to that described in humans. Thus, even if sperm bacterial contamination is not the only cause of infertility in stud dogs, specific bacteria, as *E. Coli*, are frequently isolated in stud dog with reproductive difficulties. For this reason veterinarian needs to include always research of bacteria during breeding soundness examination of dogs presented for infertility. Further studies need to be focused also on

the connection between microbial contamination of canine semen and specific genital diseases (i.e. orchitis and prostatitis) or urinary tract infections.

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127 | Changes in anti-Müllerian hormone concentrations in queens throughout the oestrous cycle

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Introduction and aim: Studies in cats have proved the usefulness of anti-Müllerian hormone (AMH) as a diagnostic tool to determine the castration status or to diagnose ovarian remnant syndrome in queens (1). The secretion pattern of AMH over the oestrous cycle of bitches has been described recently (2). The main findings were a significant increase in the AMH concentration from late anoestrus up to six days before ovulation and a significant decrease starting three days before ovulation. The aim of this study was to investigate the secretion pattern of AMH in queens throughout the oestrous cycle as it has not been described before.

Materials and methods: Serum samples have been collected in 5 healthy intact female European short hair cats (body weight 2.5–4 kgs, age 1–3 years) during normal oestrous cycles in late anoestrus, at several times during proestrus, oestrus, inter-oestrus and metoestrus. The behaviour of the cats has been examined daily for signs of heat to differentiate between oestrus and inter-oestrus. Progesterone values >2 ng/mL were diagnostic for metoestrus. The blood samples for anoestrus were collected in early January. In addition, AMH has been measured in blood samples of 5 neutered queens and 4 queens with pathohistological diagnosed ovarian remnant syndrome. Serum concentration of AMH was determined using a chemiluminescence immunoassay.

Results: All spayed females had AMH values under the lower limit of the test (<0.1 ng/mL). The cats with ovarian remnant syndrome had a median value of 0.56 ng/mL (minimum: 0.36 and maximum: 0.89). The cycling cats had AMH concentrations between 2.9 and above the upper limit of the test (22.96 ng/mL). High AMH values were measured before the onset of heat, in metoestrus, anoestrus and inter-oestrus. The measured AMH values in oestrus were significantly ($p < 0.05$) lower than in anoestrus ($p = 0.039$) metoestrus ($p = 0.039$) and inter-oestrus ($p = 0.06$). High inter-individual differences were recognized, and intra-individual variation was noticed within a heat cycle and between heat cycles.

Conclusions: This study shows that the used AMH assay can clearly distinguish between intact and spayed females and cats with ovarian remnant syndrome. The AMH secretion pattern over the oestrous cycle was comparable in all queens, but with high variation among and within the cats and between individual heat cycles within one cat. The identified AMH increased shortly before oestrus and the decreased during oestrus in this study is comparable to the AMH changes in bitches (2). The high value of AMH in anoestrus up to five weeks before the first heat seems to be a specificity in the queen. High AMH values have also been described in prepubertal queens up to 3 months of age (3). Further studies are necessary to emphasize the reasons for these high AMH concentrations in queens.

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130 | ProAKAP4 as a valuable marker to assess sperm quality in Dogs

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Introduction and Aim: Sperm proAKAP4 expression has been recently described as a pertinent and innovative approach to assess sperm quality (1, 2) and high concentrations of proAKAP4 have been associated with high fertility phenotype in bulls and with high fertility and litter size in pigs (2). Structurally, the proAKAP4 protein must be processed to release the functional protein A-kinase anchor protein 4 (AKAP4) that regulates motility, capacitation and fertilization. In this study, we then evaluated the relevance of the proAKAP4 concentrations as a sperm quality parameter in fresh Dog semen using the Dog 4MID® kit (4BioDx, France) and classical methods.

Material and Methods: Seventeen full ejaculates (urethral, spermatic and prostatic fractions) from 12 adult Dogs were collected. The spermatic fraction was analyzed by computer assisted semen analysis (IVOS version 12.0, Hamilton Thorne Research, Beverly, USA) (3). Sperm parameters included concentration, total and progressive motility as well as VAP (µm/s), VSL (µm/s), VCL (µm/s). 100 µL of each fractions were frozen at −20°C. Biochemical analyses including Western-blot, ELISA, flow cytometry, immunofluorescence and electron microscopy were performed on post-thawed semen. ProAKAP4 concentration was determined using the Dog 4MID® kit (4BioDx, Lille). Statistical analyses were performed using the

GraphPad Prism 8 software. The Spearman Rank Correlation test was used, and correlations were considered as significant with a $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Results & Discussion: As expected and using western blotting procedures, proAKAP4 is expressed in Dog semen and more especially in the spermatic fraction, not in the urethral or prostatic fractions. ProAKAP4 was strictly localized in the principle piece of the flagellum. The fibrous sheath was decorated by gold labeled proAKAP4 antibodies and no other structures of the spermatozoa were stained. Using the Dog 4MID® kit, the mean value of proAKAP4 concentration in the 17 ejaculates was of 26.41 ng/mL ± 14.17 ng/mL with variation between ejaculates from 12.46 to 61.44 ng/mL. Statistical analysis with the Spearman test shows in all the samples, a positive correlation between proAKAP4 concentrations with the total motility ($r = 0.6351$; $p = 0.0169$ (*)), the progressive motility ($r = 0.6366$; $p = 0.0166$ (*)) and the velocity parameters such as VAP ($r = 0.6967$; $p = 0.072$), VSL ($r = 0.6176$; $p = 0.0212$ (*)) and VCL ($r = 0.8374$; $p = 0.0004$ (***)). In contrast, the proAKAP4 concentration was still not significantly correlated with sperm concentration. Although proAKAP4 has been largely described in other mammals, this is the first study describing proAKAP4 expression, metabolism and comparison with sperm parameters in dogs. Our results are in accordance with what was previously described in bulls, stallions and humans. Further analyses will be performed using the 4MID® kit to investigate if proAKAP4 is also associated with fertility in Dogs.

Conclusions: Altogether we showed for the first time that proAKAP4 metabolism is also conserved in Dog sperm and that the Dog 4MID® kit could be a valuable tool to assess Dog sperm quality in research and practices.

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131 | Alternation of heart rate during interval exercise in genetically identical cloned beagles and non-cloned beagles

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Introduction and aim: The genetic influence on heart rate (HR) regulation during exercise is unknown in dogs. The purpose of this study was to examine the HR response to interval exercise in genetically identical cloned beagles and non-cloned beagles.

Materials and methods: The cloned beagles are produced by somatic cell nuclear transfer technology which was used in previous reports (1, 2). All beagles (4 cloned and 3 non-cloned) exercised on the treadmill two times a week (at 09:00–12:00) for five weeks. The ratio of the work (W) and rest (R) was set at 1:2 with progressively increased grades and speeds. The HR was monitored by a Polar H-10 HR censor throughout the periods of exercise. A rectal temperature and complete blood count were measured. Statistical analyses of all data were conducted by two-way ANOVA and Bonferroni post-tests using GraphPad Prism 5 ($p < 0.05$).

Results: HR values were changed with exercise intensity in all groups. Interestingly, the HR values of the non-cloned group were significantly different for each individual, but those of the cloned group were very similar. Before and after exercise, hematological parameters were within the reference ranges in both groups. Also, the exercise did not cause any abnormal physiological signs.

Conclusions: These results demonstrated that the HR values in cloned dogs indicates similar changes with exercise unlike non-cloned dogs. Further experiments will be conducted to investigate whether the HR response in dogs can be genetically affected. This study was supported by "CCAR (# PJ013959022019)" of RDA.

Keywords: Somatic cell nuclear transfer, Cloned Beagle, Interval Exercise, Heart Rate

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132 | Role of mesenchymal stem cell derived factors in the enhancement of parameters associated with sperm quality in dogs

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Introduction and Aim: Mesenchymal stem cells play an imperative role, both in reducing and repairing the cellular damage through the secretion of different biochemical agents (1). The aim of this study was to investigate the potential of canine adipose derived mesenchymal stem cells (Ad-MSCs) in protecting canine sperm against cryoinjury as a result of freezing process.

Material and Methods: Canine Ad-MSCs were selected on the basis of the comparison with canine skin fibroblasts for expression levels of different genes through real time PCR. Sperm rich fractions were collected from four healthy, and mature male beagles, weighing 10 to 15 kg and ranged between 4 to 6 years of age. Ejaculates having sperm count of ≥ 100×10^6/mL, ≥ 70% of motile, and ≥ 80% of viable sperms with normal morphology were pooled together. Following washing with the first buffer (24 g/L Tris aminomethane, 14 g/L

citric acid, 8 g/L fructose, and 0.15 g/L kanamycin sulfate in distilled water), semen was diluted using the second buffer (40% [v/v] egg yolk, 54% [v/v] first buffer, and 6% [v/v] glycerol), supplemented without Ad-MSCs (Control), with 2.5 × 10^6 Ad-MSCs/mL (Group 1) or with 5 × 10^6 Ad-MSCs/mL (Group 2). Eosin-nigrosin stain was used to analyze the viability, 1% formal citrate solution for normal acrosome and integrity of sperm plasma membrane was evaluated by hypo-osmotic swelling assay. All the experiments were replicated three times. SPSS 21.0 software (SPSS Inc., Chicago, USA) was used for statistical analysis. The significance level was $p < 0.05$.

Results: The results elucidated that canine Ad-MSCs had significantly higher gene expression of various proteins associated with cell repair including annexin-1, histone H-3, and high mobility group B proteins than skin fibroblasts ($p < 0.05$). The group 2 showed significantly higher percentages of motile sperm, and intact plasma membrane (53.7% vs. 49.6% & 44.3% and 45.0% vs. 42.7% & 41.0%, respectively). The percentages of live sperm and normal acrosome appeared to be non-significant among group 1 and 2, but significantly higher than the control (48.5%, 48.7% vs. 40.5% and 40.1%, 41.7% vs. 36.3%, respectively).

Conclusion: In conclusion, results demonstrated that the Ad-MSCs in comparison with the control can adequately preserve the viability of frozen-thawed sperm by reducing the plasma membrane damage. The post-thaw quality of cryopreserved canine sperm was significantly enhanced in the Group 2 than other groups. However, further study is required to properly understand the mechanisms responsible for this improvement. This study was supported by Cooperative Research Program of RDA (CCAR, #PJ013954012019).

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134 | Emergency C-section and pre-natal maternal corticosteroid therapy: Three cases in pre-term brachycephalic dogs

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Introduction: C-section performed before 62 days post LH surge is associated with a high risk of neonatal mortality due to fetal immaturity. Among other factors, increase of cortisol concentrations pre-partum plays an important role in final development of fetal pulmonary, renal, liver and gastro-intestinal systems (1). Pre-natal corticosteroid therapy has been used since 1972 in human medicine to prevent or, at least to reduce, respiratory complications in pre-term infants. This treatment is associated with a significant reduction of morbidity and mortality resulting from Neonatal Respiratory Distress Syndrome (2). Regazzi et al, Zaremba et al and Rider et al have confirmed the same results in dogs, calves and rabbits. Foetal lung development can be divided in five phases: embryonary, pseudoglandular, canalicular, saccular and alveolar. Pulmonary surfactant is secreted by pneumocytes II during the saccular phase. In 2009, Sipriani *et al* studied the development of the pulmonary structure throughout pregnancy in dogs. They reported that the saccular phase begins at the earliest 57 days post fertilization; thereby underlying the non-viability of younger puppies. Here we describe three cases of pre-term C-sections with pre-natal corticosteroid therapy.

Clinical cases: Two English Bulldogs and one Chihuahua underwent emergency C-sections. The three dogs had been transcervically inseminated once with fresh semen. They were followed during the estrus cycle, by vaginal smear and progesterone (P4) assay. Abdominal ultrasound with measurement of the inner chorionic cavity confirmed that the day of fertilization matched with the day of insemination (3). The two Bulldogs and the Chihuahua were presented for anorexia, dyspnea and exhaustion 56 days and 54 days post insemination respectively. Progesterone levels were around 10 ng/mL. The 3 dogs underwent the same surgical and anesthetic protocol. Two hours prior to surgery: perfusion with Hartmann + Glucose 5% solution, injection of prednisolone IM (0.5 mg/kg), metoclopramide SC (0.5 mg/kg) and amoxicillin clavulanic acid SC (8.75 mg/kg). Induction was achieved with dexmedetomidine (375 μg/m^2) and alfaxan IV (0.2 mg/kg) maintenance with Isoflurane. The linea alba was locally anesthetized with lidocaine. Methadone (0.1 mg/kg) was administered IV at the time of delivery and again upon waking. The first Bulldog gave birth to 8 puppies: 3 had a cleft palate and were euthanized. One died after 4 days from respiratory distress. The four remaining ones survived. The second Bulldog delivered 10 puppies: 1 water puppy and 1 with cleft palate that were euthanized, 2 mummies. Out of the 6 other puppies only one died at 15 days from respiratory distress. The Chihuahua gave birth to 3 puppies who survived without complication despite their high degree of immaturity (hairless) and were sent back home.

Discussion: Pre-natal corticosteroid therapy has been shown to improve neonatal viability in several studies. Betamethasone injected 2 days before surgery has been proposed as the treatment of choice. However, Vannuchi *et al* reported in 2012 a suppression of the fetal and maternal adrenal cortex as well as a premature labor with an administration of betamethasone at 0.5 mg/kg. Maternal treatment with prednisolone two hours before surgery should be investigated in order to measure its impact on the fetal and maternal cortisol levels in addition to its effect on surfactant production. Most studies on pre-natal corticosteroid therapy in dogs define the prematurity of the fetuses based on progesterone levels, the predicted LH surge and estimation of the ovulation 2 to 3 days post LH surge. In order to increase the accuracy of the gestational age, we combined progesterone levels, vaginal smear and US measurements. We report here three cases of emergency C-section with viable fetuses at 56 and 54 days post fertilization. We conclude that prednisolone injection 2 hours prior to surgery could represent an

interesting protocol to increase neonatal viability and could be further investigated.

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135 | Six years old French bulldog (78,XX; lack of *SRY* gene) with disorder of sex development and Sertoli cell tumor – a case report

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The 6-year-old French Bulldog was presented to Clinic of Department of Reproduction of Wroclaw University Environmental and Life Sciences for a routine spay. During pre-surgical examination an enlarged clitoris was found protruding from vulva with a palpable os clitoridis. Blood samples were taken before the surgery and hormone profile was as follows: Progesterone 1.08 ng/mL, Estradiol 16.32 pg/mL and Testosterone 0.05 ng/mL. The dog was referred for cytogenetic and molecular tests and 78,XX chromosomal constitution and lack of the *SRY* gene was observed. Thus, the patient was classified as a case of XX (*SRY*-negative) disorder of sex development (XX DSD).

During the surgery, it was found that in the area of the left ovary there is an oval formation of approximately 1.0 × 0.5 cm, slightly reminiscent of an atrophic testicle. On the right side, there was a typical *bursa ovarica*. Two uterine horns were normally shaped and had 15 cm length and 1 cm width. The uterine body was significantly enlarged (5 cm length and 2 cm width). No clear separation of the cervix was palpated. The whole structure was only slightly narrower towards the bladder. It was removed in 2/3 of its length. Histopathological examination revealed that the right gonad contained a typical ovarian structure with small follicles, tubules and cells similar to Call-Exner bodies, whereas the left gonad consisted of irregular seminiferous tubules and sustentacular cells inside, typical for Sertoli cell tumour (SCT). In the uterine horns a moderate cystic endometrial hyperplasia (CEH) was found. In the uterus body a mucous membrane with small number of glands, one large cyst and few smaller ones and the well-developed myometrium was observed.

In dogs showing signs of intersexuality, gonadohysterectomy is usually performed at the age younger than 2 years. The available literature indicates that XX DSD dogs may have abdominal testicles or ovotestis (5), thus the presented case seems to be exceptional due to the presence on one side the ovary and on the other the testicle. It has been suggested that testicular tissue located in the abdominal cavity is more prone to neoplasia. Cryptorchid testes are approximately 14 times more likely to develop a tumour than scrotal testicles (1, 4) and the most common neoplasia is the Sertoli cell tumour (SCT) (2, 3). In our case, the dog recovered well after the surgery and we do not expect that any disorders due to the intersexuality will develop in its foreseeable future life.

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136 | Possible estrogen-induced nonregenerative anemia in a dog: case report

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Clinical case: An unneutered 16-years-old mixed-breed female dog was presented to a veterinary clinic due to apathy and weakness. A physical examination revealed poor general condition
and multiple, large – up to 5 cm × 4 cm, necrotic mammary gland tumors. Additional diagnostic procedures were then conducted:
Chest X-ray: No abnormalities or signs of metastasis were observed.
Abdominal ultrasonography: Ultrasonography revealed enlarged uterus – shank up to 19 mm and horns up to 13 mm. Furthermore, multiple ovarian cysts of 30 mm, 15 mm and smaller were detected. Other organs were not changed in the ultrasound image.
Blood test: Complete blood count (CBC) and serum biochemistry were performed. Tests revealed leucocytosis resulting from neutrophilia, anemia and elevated AlAT. T4 and fT4 levels were normal.
Blood smear: Anemia with mildly hypochromic, microcytic RBCs and toxic neutrophils were observed.
SNAP 4Dx test (IDEXX): Negative for borreliosis, anaplasmosis, ehrlichiosis and heart-pulmonary dirofilariasis.
Treatment: As described by Sontas *et al.*, in case of estrogen-producing ovarian tumors or cysts surgical removal of the source may be curative with long-term intensive care, so castration combined with mastectomy was planned (1). Unfortunately the patient died during surgery.
Discussion: In this patient the differential diagnosis of nonregenerative anemia might include anemia of chronic disease and aplastic anemia. Anemia of chronic disease usually occurs during inflammation or

neoplasia. This kind of anemia is often described as normocytic and normochromic, but in long-standing microcytic and hypochromic erythrocytes might develop. In this case, the most likely infectious diseases were ruled out by SNAP 4Dx test (IDEXX). However, it might have been induced by mammary tumors (2). Meanwhile, anaplastic anemia is caused by bone marrow hypoplasia and is characterized by hematological abnormalities including anemia and leukocytosis or leucopenia. As reported by Sontas *et al*., it was confirmed that estrogen has myelotoxic effect, which might result in bone marrow hypoplasia (1). Although it is mainly observed after estrogen administration, it might also be seen in patients with ovarian tumors or cysts (3). Hormonally active ovarian cysts investigated by Knauf *et al*., were described to produce both estradiol and progesterone (4). Therefore it is likely that in this case multiple ovarian cysts led to early stage aplastic anemia, which is characterized by bone marrow granulocyte hyperplasia with neutrophilia. Examination of bone marrow aspirate would be useful to support the diagnosis, but due to financial reasons was not performed.

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137 | Relationship between placental characteristics and puppies' weight in toy and small sized dog breeds

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Introduction: Puppy mortality is reported to be a significant problem in the dog and can be above 20%, when no supervision at delivery, additional nursing or hand rearing are performed (1). Low birth weight identifies puppies at higher risk and might be caused by placental factors, such as the extension of the exchange area and the degree of vascularization (2). The relation between the newborn puppy and the features of its placenta has been poorly investigated in the dog.

Methods: Twenty bitches, 9 toy-sized (i.e. < 5Kg) and 11 small-sized (i.e. 5 to 10 Kg), were included in this study. During natural whelpings or c-sections, puppies were identified and their order of birth, sex and weight were recorded. Puppies weights were registered at birth (D0) and daily until Day 6 (D6). Placentas were weighed after trimming of extraplacental membranes and umbilical cord; a single picture was taken and assessed using the NIS-Elements Br Microscope Imaging Software. The Total Placental Area (TPA), the Transfer Zone Area (TZA) and the Marginal Hematoma Area (MEA) were calculated and their surface expressed in mm^2. Immunohistochemistry using monoclonal mouse anti CD31 antibody (Cat. n° sc-101861, Santa Cruz Biotechnology; TX, USA) was used to identify fetal and maternal vessels in the placental labyrinth zone. A vascularization index (VI) was determined for each placenta and the Total Vascular Area (TVA) was estimated. Puppies birth weight values were classified into quartiles, separately from small and toy breeds. The first quartile (Q1) represents the lowest 25% of registered values.

Results: Puppy's birth weight was positively correlated with placental weight ($p < 0.001$, $r = 0.689$). A positive correlation was found between puppy's birth weight and TPA ($p < 0.001$, $r = 0.786$), TZA ($p < 0.001$, $r = 0.772$), and TVA ($p < 0.001$, $r = 0.482$). Furthermore, a positive correlation was found between placental weight and TPA ($p < 0.001$, $r = 0.661$), TZA ($p < 0.001$, $r = 0.583$), and TVA ($p < 0.001$, $r = 0.333$).

In small-sized breeds, the placentas of Q1 puppies had a lower weight and a smaller TZA and TVA compared to not-Q1 ones ($p < 0.05$). In toy breeds no differences in placenta characteristics of Q1 and not-Q1 puppies were observed. The VI was higher in toy-sized compared to small-sized bitches placentas ($p < 0.01$). No effect of breed size, parity, litter size, or sex of the puppy was observed on early growth rate. As in humans (3), placental weight and the extension of the transfer zone correlates closely with placental total vascular area which can be considered a significant determinant of puppy's birthweight in normal pregnancies. In toy-sized breeds, the greater capillary density observed might support the development of fetuses proportionally bigger compared to small-breed ones.

Reference values for placental weight, total placental area, transfer zone area, total vascular area and index of vascularization were described in toy and small-sized dog breeds.

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138 | Colostrum absorption in one canine neonate: intestinal epithelium histochemical aspects

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Introduction and aim: Passive immunity transfer is an important mechanism that enhances neonatal immunological competence,

since the absorption of colostrum soon after birth promotes an increase in plasma antibodies (1). Vacuoles containing ingested colostrum have been demonstrated histologically in the cytoplasm of epithelial cells in intestinal villi of goats (2). Experimental findings suggest that immunoglobulins are absorbed by binding of IgG to receptors present in enterocytes (3). Absorption of colostrum in canine enterocytes has not been described histologically. A deeper understanding of the colostrum absorption kinetics can contribute to a better understanding of passive immunity failure pathogenesis and for the development of new therapeutic interventions. The aim of this study was to describe the morphology of colostrum absorptive vacuoles after different histologic staining techniques.

Materials and methods: Paraffin embedded intestine samples from one canine neonate were selected from a tissue bank. The neonate was a four-day-old German Spitz that died after bacterial sepsis at the breeding kennel and was necropsied in a previous study (4). Intestinal tissue sections, 4 μm thick, were stained with hematoxylin and eosin (HE), toluidine blue (TB) and schiff periodic acid (PAS), and examined under light microscope.

Results and discussion: Colostrum absorptive vacuoles were observed in the cytoplasm of intestinal epithelial cells and colostrum globules were visualized in the *lamina propria*, mainly in the mid-apical portion of the villi, in jejunum. In HE stain, the absorptive intracytoplasmic colostrum vacuoles and colostrum globules in *lamina propria* were large, occupying more than 50% of the cytoplasm in enterocytes, and stained as eosinophilic hyaline homogeneous material, which, when inside the *lamina propria*, were very similar to red blood cells. With TB, the vacuoles revealed a clear basophilic homogenous content, while goblet cells vacuoles stained purple. PAS stained the colostrum vacuoles and globules as a homogeneous bright red content, differing from the goblet cells that presented flocculent red material. These staining characteristics and morphology are identical to those described for colostrum absorption in goat kids (4) and lambs (5), with staining properties compatible to the glycoproteic constituents of colostrum. Further studies should be designed to describe colostrum absorption in canine neonates under different conditions, for example, in preterm vs. term neonates, and may contribute to the better understanding of canine passive immunity transfer and perinatal susceptibility to sepsis.

Conclusion: This is the first time that colostrum vacuoles are described intracytoplasmically in the intestinal epithelium and in the *lamina propria* in the jejunal mucosa of a four-days-old canine neonate. These findings demonstrate that colostrum absorption morphology in dogs resembles that described in other species.

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139 | Effect of medetomidine and dexmedetomidine administration at different dosages on cat semen quality using Urethral Catheterization after Pharmacological Induction (Ur.Ca.P.I.)

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Introduction and aim: Nowadays semen collection and evaluation for reproductive purpose in domestic cats is a well-known procedure in veterinary medicine. In clinical practice, the most used technique is electroejaculation, nevertheless, Ur.Ca.P.I. is currently another safe technique used (1–4). In literature two anesthetic protocols based on medetomidine administration are described (1–2). The D-isomer dexmedetomidine is the only pharmacologically active part of 1:1 racemic mixture of medetomidine and studies show that dexmedetomidine, at half dose of medetomidine, induces sedative, analgesic, cardio-respiratory and body temperature effect comparable with those of medetomidine (3). Considering this, the aim of the study is to evaluate the effects of dexmedetomidine at two different dosage on sperm quality compared to the previously protocol described with medetomidine (1–2).

Materials and methods: Twenty sexually mature healthy tomcats of different breeds, presented to our Unit to collect semen for artificial insemination, were enrolled for this study. Semen collection was performed twice with 24 hours in between. The tomcats were randomly divided into 2 main groups of 10 animals, receiving either a high (A) or a low (B) dosage of the drug used. The first five cats of each group were intramuscularly injected at day 1 with medetomidine and at day 2 with dexmedetomidine, the other five at day 1 with dexmedetomidine and day 2 with medetomidine.

The dosages used were 120 μg/kg of medetomidine (HMED) and 60 μg/kg of dexmedetomidine (HDEX) for group A, 50 μg/kg of medetomidine (LMED) and 25 μg/kg of dexmedetomidine (LDEX) for group B. The semen was evaluated for volume, concentration, total number of sperm cells, motility, movement score, normal sperm forms and vitality. Data obtained was analyzed with one-way ANOVA test and Kruskal-Wallis test depending on the type of data, normal or not. Parametric and nonparametric results are reported in table 1, respectively, as mean ± SD or median and interquartile range. The significance was set at p value <0.05.

Results: The statistical analysis showed no significant statistically differences between semen parameters in group A. Despite that literature reports that there is no difference in the sedative effect of the two α2-agonists (3), in the present study it was found that the level of sedation of HMED was better than of HDEX. In fact, using HDEX, it was necessary to administer isoflurane in some cases to permit urethral catheterization.

In group B there were no significant differences in semen parameters except for the value of the concentration which was higher in LMED. Moreover, pharmacological induction with low dosage of the two α2-agonists gave an insufficient level of sedation for urethral catheterization. There were significant differences in semen quality between group A and B: semen collected with the high dosage protocol presented better parameters regarding semen volume, total number of sperm cells and movement score.

TABLE 1. Semen quality after Ur.Ca.P.I. with 130 μg/kg of medetomidine (A-HMED), 60 μg/kg of dexmedetomidine (A-HDEX), 50 μg/kg of medetomidine (B-LMED) and 25 μg/kg of dexmedetomidine (B-LDEX)

	A-HMED	A-HDEX	B-LMED	B-LDEX
Semen volume (μl)	26.2 ± 14.09^{A}	20.3 ± 7.49^{A}	5.6 ± 3.62^{B}	5.5 ± 1.84^{B}
Concentration (x10^{6}/mL)	535 (228.75–945)$^{A-C}$	160 (11.375–972)$^{A-B}$	215 (157–248)^{C}	27.05 (0.040–110)^{B}
Total n° Spt (x10^{6}/vol)	10.72 (4.99–16.61)^{A}	4.40 (0.203–16.16)$^{A-C}$	0.85 (0.5435–1.15)$^{B-C}$	0.16 (0.0001–0.80)^{B}
Motility (%)	50 (42.5–60)^{A}	50 (39.75–58.75)^{A}	50 (40–57.5)^{A}	40 (0–50)^{A}
Movement score (0–5)	4 (4–4.75)^{A}	4 (3.25–4)^{A}	3 (2–3)^{B}	2 (0–2.75)^{B}
Normal sperm forms (%)	51.32 ± 17.34^{A}	48.42 ± 24.19^{A}	52.9 ± 11.44^{A}	37.8 ± 26.98^{A}
Vitality (%)	65 (56.25–67.5)^{A}	60 (56.25–65.75)^{A}	69 (65.25–71.50)^{A}	61 (0–65.5)^{A}

Different letters between groups indicate significant difference ($p < 0.05$). Parametric and nonparametric data are expressed, respectively, as mean ± SD or median.

Conclusion: In conclusion, both HMED and HDEX protocols permit a good quality semen collection, proving that dexmedetomidine is a good alternative to medetomidine for performing Ur.Ca.P.I technique. However, the HDEX protocol, could not always guarantee adequate sedation for urethral catheterization. The outcome of the LMED and LDEX protocols were unsatisfactory regarding both semen collection and level of sedation.

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144 | Unilateral intra-abdominal cryptorchidism and mammary gland collagenous hamartoma in a male dog: a case report

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Clinical case: Cryptorchidism is the most frequent congenital disorder in dog testes. Usually, the testis descend into the dog scrotum after 10 days of age. The incidence of this anomaly in dogs ranges from 0.8% to 9.8% and is more common in Toy and Miniature Breeds. In our work, a case is described of a 9 years old intact Golden Retriever male referred to the Clinical Hospital of the Faculty of Veterinary Medicine from Bucharest, Romania. At the moment of referral, the owner claimed the aspect of dog's multinodular mammary gland. The male showed exercise fatigue, normal rectal temperature and good appetite.

Clinical exam revealed a right M_{4-5} mammary gland affected area and a right testicle normally descended. A biopsy sample of that modified mammary tissue revealed a collagenous hamartoma. A left modified tesctile was discovered intraabdominaly, above the urinary bladder by ultrasound examination.

After the oncological and cardiological examination, cosmetic excision of the hamartoma and castration were performed. The cryptorchid testicle was larger than the other one because histopathological exam showed a Leydig cell tumor.

The healing process was *per secundam* and lasted about 2 months because some suture points splitted off 5 days post surgery.

Discussion: For this clinical case, our supposition for collagenous hamartoma occurrence in that area was because of humping behaviour regarding to intra-abdominal testicle. This mounting behaviour probably caused a constant skin irritation which led to hamartoma. From our knowledge, this is the first male dog in which these two conditions appeared.

Hamartoma are benign nodules that appear in different animals (bovine, small animals or exotic animals) and in different tissues and organs (kidney, lung, skin, periodontal ligament, vasculare, musculature, peripheral nerve, spinal cord, and hypothalamus). Although this benign tumor was reported in several anatomical, locations none was on mammary gland.

147 | Canine amniotic fluid as a new potential diagnostic tool for leptin and oxidative stress detection at birth

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Introduction and aim: Leptin is a hormone produced by adipocytes that mainly influences appetite and food intake with impact on obesity[1]. Furthermore, a leptin involvement on the regulation of reproductive function and fetal development has been demonstrated in several species[2]. During pregnancy and mostly at the transition from intra- to extra-uterine environment, the oxygen required for fetal and maternal metabolism increases and predisposes to oxidative stress[3]. The amniotic fluid is rich of antioxidant elements and, especially in the last weeks of pregnancy, plays a central role in preventing oxidative imbalance[4]. The first aim of this study was to measure leptin and antioxidant capacity in amniotic fluid collected at birth in canine species. Then amniotic outcomes were compared with those of maternal serum and both of them were related to clinical factors (maternal age and weight, litter size, neonatal weight and gender).

Materials and methods: Bitches age and weight ranged from 2 to 7 years (3.8 ± 1.8) and from 13 to 61.8 kg (34.9 ± 15.3), respectively. Maternal blood samples were collected at birth from ten healthy purebred bitches undergoing elective C-section. At extraction time, amniotic fluid was also collected from all pups. Both maternal serum and amniotic fluid were analyzed for leptin measurement by Canine leptin ELISA kit (EMD Millipore Corporation, Merck, Germany), and antioxidant capacity by Trolox equivalent antioxidant capacity (TEAC) assay[5]. Moreover, the concentration of thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation and oxidative stress was measured in the amniotic fluid[6].

Results: A total of 63 pups were delivered, 34 males and 29 females. All pups were born alive and 8 out of 63 died within seven days of life. Birthweight varied from 236 to 770 g (438.5 ± 140.5). The average leptin in amniotic fluid was 1.74 ± 0.2 ng/mL and those in maternal serum was 4.15 ± 2.1 ng/mL. Total antioxidant capacity (TEAC) and TBARS in amniotic fluid were 3.47 ± 0.5 μmoL Trolox equivalent/L and 2.78 ± 0.3 μM MDA equivalent/mL, respectively. Maternal serum TEAC was 6.04 ± 0.6 μmoL Trolox equivalent/L. Leptin in amniotic fluid and in maternal serum were significantly related ($p = 0.044$). Amniotic leptin was higher in male than female pups ($p = 0.001$). No other comparison resulted statistically significant.

Conclusions: This study provided preliminary data about leptin levels and antioxidant capacity in amniotic fluid of dogs. Amniotic fluid collected at birth could represent a modern non-invasive sample for early diagnosis in canine neonatal patients. However, further analysis involving a larger number of observations is needed before it can be applied in clinical practice.

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148 | Impact of culture medium on in-vitro maturation and fertilization in the domestic cat

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Introduction: In-vitro fertilization (IVF) procedure is subjected to a constant optimization. In human and farm animals many culture media are already commercially available making the outcome comparable between different laboratories and researchers. In case of the domestic cat, many different media compositions have been presented by different authors and laboratories. In 2007 Herrick et al. designed and tested a feline-specific culture medium (FOCM) and achieved a high blastocyst rate of about 70% after in-vitro fertilization of in-vivo matured oocytes with fresh semen (1).

Aim: In this study we aim to explore the potential of FOCM on in-vitro maturation procedure and subsequent IVF with frozen-thawed epididymal sperm compared to our normally used TCM199 medium.

Methods: Ovaries of spayed females were obtained from the Berlin animal shelter and 382 oocytes were isolated by slicing. Good quality dark oocytes with 3–4 layers of cumulus cells were randomly divided into two groups (193 to FOCM; 189 to TCM199) and cultured individually in media drops of 20 μL. According to (2) maturation medium FOCM was supplemented with 1 ng/mL EGF and 1 IU/mL eCG whereas TCM199 was supplemented with 0.02 IU/mL FSH and 0.05 IU/mL LH. Oocytes were cultured for 24 h at 39°C, 5% CO2, and humidified atmosphere. IVF was performed with 1–4 × 10^6 sperm/mL, depending on the quality of sperm after thawing. Cleavage was evaluated after 36 h. Uncleaved oocytes were fixed, stained with propidium iodide and evaluated for nuclear maturation. Embryos were cultured for up to 7 days (expected blastocyst stage). Experiments were replicated 8 times. For comparison of IVM and IVF outcome a two-tailed contingency table was analyzed with Fisher's Exact Test.

Results: There was no difference in the maturation rate between both systems (51.8% in FOCM vs. 50.8% in TCM199), however, matured oocytes looked differently. In TCM199 cumulus was expanded and cells at the edge started to disperse, whereas in FOCM expanded

cumulus cells stayed more compactly associated. Cleavage rate was significantly higher in the TCM199 group (76% vs. 63%). There was only one embryo that reached the blastocyst stage in the TCM199 group and none in the FOCM group.

Conclusion: A feline-specific culture medium is not superior to our usually utilized TCM199 when performing in-vitro maturation and is even less effective in IVF outcome. Different hormones and concentrations utilized during in-vitro maturation produced comparable results. Therefore, other factors than medium composition, mainly the origin of material (in-vivo instead of in-vitro matured oocytes and fresh semen instead of frozen-thawed epididymal sperm) have probably led to the high success rates of Herrick et al. (1).

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149 | Cortisol impact on reproductive and behavioral parameters throughout the perinatal period: preliminary results in dogs

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Introduction and aim: The perinatal period ranges from pregnancy to the first months of life and represents the most critical phase of psycho-physical development in mammals[1,2]. Cortisol is a physiological well-being indicator used to assess the degree of stress in humans and animals[3,4]. Unsuitable environments that expose mother to stress can lead to cortisol increase with intrauterine growth restriction, premature delivery, low birth weight, and impairment of offspring behaviour[2]. The first aim of this study was to measure cortisol concentration in different biological materials, namely maternal blood, saliva, hair and milk, and neonatal saliva and hair. Then maternal and neonatal cortisol levels were compared between them and over time, and also correlated with the clinical and behavioral outcomes.

Materials and methods: Five healthy female German Shepherd show dogs delivering by vaginal parturition (age 4.4 ± 2.5 years; weight 28.2 ± 2.7 kg) were enrolled. All dogs were housed in the same breeding facility, fed with the same dry commercial diet, and exposed to the same environmental conditions. No changes to their routine occurred for research purposes during the survey's period. Maternal blood, saliva, and hair samples for cortisol titration were collected at estrus (T0), twenty-fourth day of pregnancy (T1), delivery (T2), weaning (T3), and two months after delivery (T4). At the same times from delivery (T2, T3, T4) both saliva and hair specimens were collected in pups, and milk in mothers. After extraction procedures, cortisol was measured in all the matrices by a commercial EIA kit (Salimetrics, State College, PA, USA). Clinical aspects were recorded (maternal age and body weight, parity, duration of pregnancy and delivery, litter size, neonatal viability and malformations, gender and body weight) and an environmental/behavioral test was administered to mothers[3].

Results: A total of 22 pups were born alive and survived until T4 but two of them were euthanized 24 h and 2 months after birth due to defects incompatible with life. Cortisol was detectable in all of the biological matrices both in mothers and pups. Bitches showed different cortisol concentrations among matrices ($p < 0.0001$) with the highest value in plasma (median 0.57 μg/dL) and decreasing levels in saliva (median 0.16 μg/dL), and hair (median 0.08 μg/dL). Cortisol in milk was almost undetectable (median 0 μg/dL). Similarly, cortisol in neonatal saliva (median 0.29) was higher than in hair (median 0.049 μg/dL) ($p < 0.0001$). Cortisol concentration showed no changes over time in any maternal matrix while in pups it was high at birth in saliva ($p = 0.0083$). Healthy pups showed lower saliva and hair cortisol levels than pups with malformations but without a statistical significance likely due to the low number of pups with malformations? No differences in maternal behaviour were recorded among bitches.

Conclusions: This study could help to deepen the knowledge about canine cortisol levels in new biological non-invasive matrices during the perinatal period both in mothers and pups, and its correlation with reproductive and behavioral aspects. The choice of a polyparous species allows speculations on the clinical significance of this potential marker, reducing the bias risk intrinsic to primates and human.

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150 | Sexual chromosome distribution, fertility and hormonal insights: Preliminary results in dogs

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Introduction and aim: In mammals, the standard meiotic model forecasts an almost equal and constant number of spermatozoa carrying the X and the Y chromosome (sex-ratio)[1]. Recent studies have speculated an involvement of pollution (endocrine-disruptor chemicals) on the endocrine system, semen quality, and sex-ratio[2]. Furthermore, fertility is negatively affected by obesity or over-weight. Indeed, high concentrations of leptin which is secreted by the adipose tissue, can damage spermatozoa[3,4]. The first aim of this study was to evaluate sex-ratio in the canine ejaculate. Then sex-ratio was correlated to

sperm parameters, and both of them to blood and spermatic concentrations of leptin, and to blood levels of testosterone.

Materials and methods: Five purebred male dogs (1 Am Bully, 1 Bouledogue, 1 Golden retriever, 2 Labrador retriever) intended for breeding underwent andrological examination every three months, for a total of 4 evaluations per dog, that is one per season throughout 2018. Moreover, in case of mating fertility was recorded by biological test (offspring outcome). Each examination included the analysis of ejaculate (spermiogram) together with blood and spermatic leptin (ELISA technique, EMD Millipore Corporation, Merck, Germany) and blood testosterone (ELFA technique, MiniVidas Biomerieux, France) measurement. Concurrently, sex-ratio was performed on semen samples all the time by DNA extraction and subsequent quantitative real-time PCR (detection system CFX96 Bio-Rad, Germany).

Results: Dogs weight ranged from 14 to 42.9 kg (32.05 ± 9.6) with BCS between 4/9 and 6/9, and age from 1.4 to 9.2 years (5.5 ± 3). Eight out of the nine mating resulted in 57 pups, 27 females and 30 males. The mean sperm concentration, motility rate, viability, and normal morphology rate in the ejaculates were $437.2 \times 10^6 \pm 239.1 \times 10^6$, 86.5 ± 5.9, 91.2 ± 5.9, and 80.6 ± 7.8, respectively. Leptin concentration varied from 2 to 31 ng/mL (7.2 ± 6.9) in the blood, and from 1.1 to 1.9 ng/mL (1.1 ± 0.2) in the ejaculate. Blood testosterone ranged 0.08 to 12.4 ng/mL (3.3 ± 3.2). The percentage of the X-spermatozoa was 56% (ds 2.9) and of the Y-spermatozoa was 44% (ds 2.9). No relations were found between sex-ratio and sperm parameters or offspring gender distribution. Blood leptin was directly proportional to both age (p = 0.015) and BCS (p = 0.048). Sperm quality was negatively influenced by spermatic leptin (p = 0.037) and positively by blood leptin (p = 0.017).

Conclusions: The limited sample of this research requires caution in interpreting the results that should be considered as preliminary data. However, further studies about the distribution of sex chromosomes in ejaculate and its interactions with leptin and fertility could open up new frontiers in the field of canine andrology.

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151 | Working with frozen canine semen in the small animal practice: Is semen safe in nitrogen vapor?

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Introduction and aim: The damage of frozen semen due to wrong storage, handling and transfer is an underestimated cause of pregnancy failure after artificial insemination with frozen semen in the dog. Most of the points lined out here may well be known by theriogenologists, who were specifically trained and routinely deal with frozen semen and embryos of several species. Since there is an increasing demand for using frozen semen in small animals, many general practitioners have adopted working with frozen semen in their practice. The newcomer in this field, however, may not be aware that the only safe place for frozen semen is liquid nitrogen, because it has a constant temperature of −196°C. Whenever frozen semen is shipped in Dry shippers, transferred or searched for, the frozen semen will be moved around in vapor. The presence of vapor alone, however, does not prevent the frozen semen from harm, since the temperature in vapor has a very wide range, depending on the closeness and amount of the liquid nitrogen it derives from. Old studies using bovine frozen semen could demonstrate, that recrystallisation, which is a short thawing and refreezing of ice crystals between the spermatozoa occurs in straws that reach an inside-temperature of −130°C. The re-crystallization of ice crystals can damage the neighboring spermatozoa. The temperature of −130°C within a straw may well be reached when semen is handled in vapor. The purpose of this study was to measure the temperature of nitrogen vapor in Wet-tanks with different nitrogen levels and in Dry shippers with different weights.

Materials and methods: Two MVE-SC-4/3 Dry shippers (DT) and 6 MVE-XS20 Wet-tanks (WT) were used for the measurements using a thermocouple instrument. (Oaton, TempJKT meter-USA). The temperature of Dry shippers in degrees of Celsius (C) were measured at different weights (reflecting the amount of liquid nitrogen absorbed in their inner walls as specified by the manufacturer) on the bottom (B) and at 12 cm from the opening (T). The vapor temperature of six Wet-tanks with different levels of liquid nitrogen (cm LN) were measured also 12 cm from the opening of the tank.

Results: WT1:10 cm LN: −54.C, WT2:10 cm LN: −54.8C, WT3:15 cm LN: −67.3C, WT4:17 cm LN: −69.2C, WT5: 25 cm LN: −84.2C, WT6: 30 cm LN: −89.0C. DT1: 8.15 kg: −196.0C (B), −138.1 C (T), 7.0 kg: −163.2C (B), −110.0C (T), 5.5 kg: −99.8C (B), −45.0C (T), DT2: 7.5 kg: −190.4C (B), −138.2C(T), 6.4 kg: −171.3C (B), −132.1C (T), 5.2 kg: −130.1C (B), −71.5C (T).

Conclusions: A lower nitrogen level in a Wet-tank results in a lower vapor temperature in its neck. If the tank is used frequently, the nitrogen level should be at least at 30–35 cm and/or cover the upper goblets. Dry shippers should be shipped and received at full or nearly full weight irrespective of shipping distance. It is advised to weigh each Dry shipper before shipping and upon arrival or to measure its internal temperature to monitor possible problems. Any manipulation of frozen semen in vapor should not exceed 5–8 seconds.

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152 | Dystocia in dachshunds – a case study

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Parturition is the most critical moment during the whole pregnancy and labour difficulties are frequent reasons for consultations in veterinary practice. Maternal or fetal factors that hinder the natural progression of fetal expulsion lead to a serious condition called dystocia. When it occurs, should be treated as a life-threatening condition for both the mother and the fetus (3).

The available literature provides general information on the incidence and most common reasons of dystocia in bitches (1, 2). Unfortunately, the data on individual breed's predisposition, as well as fetal or maternal factors leading to labour difficulties is still sparse. This fact prompted us to make such an analysis based on the clinical records collected at our clinic.

We analyzed 119 cases of obstetrical assistance in pedigree dachshund bitches presented at our clinic in the last 35 years. All the investigated females were privately owned, were intentionally mated with a pedigree dachshund male (except 2 cases) and brought in due to a prolonged labour. The youngest recorded female was 14 months and the oldest 10 years, with the majority (71%) being 3–5 years old. We found that the most common reason (51.2%) of dystocia was the uterine inertia, mostly primary – 48 cases (40.3%), with too weak and infrequent contractions, and with or without reaching the 2nd stage of labour; the secondary inertia – only 13 cases (10.9%), with myometrial fatigue resulting from persistent uterine contractions and remained despite the relief of the obstruction; followed by the single pup pregnancy (19.3%). The remaining 29.5% of dystocia cases were distributed among fetomaternal disproportions (14.4%), fetal maldispositions (5, 9%), and other causes like uterine torsion, uterine rupture, prolonged pregnancy and monsters (9.2%).

In the 34 (28.6%) of the investigated cases the conservative approach such as medical (oxytocin) treatment (17 cases), manual aid (10 cases) or both medical and manual (7 cases) was successful to deliver all the puppies. However, in the remaining 85 bitches (71.4%) the Caesarian section (CS) had to be performed. In 24 cases CS was done after the medical and/or manual attempts, which turned out to be ineffective to deliver the litter. In total there were born 395 pups, with a mean number of puppies per pregnancy of 3.3. We noted a high incidence of dead fetuses at the moment of delivery: 141 (35.7%). The high number of dead puppies was most probably related to the delayed obstetrical aid provided, as in over a half of the investigated cases 64 (53, 8%) the labour was ongoing for at least 12 hours before the owners decided to seek veterinary assistance.

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153 | Computed tomography of the accessory glands in clinically normal intact tomcats. Preliminary results

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Introduction and aim: The accessory glands of the tomcat consist of the ampullary, the prostate and the bulbourethral glands. The ampullary glands are located at the terminal part of the deferent duct forming the ampulla of the deferent duct (1). The prostate is located dorsal to the beginning of the urethra at the caudal edge of the pubic symphysis. The prostate consists of the compact part, large and globular, almost surrounding the urethra and the disseminated part, vestigial, consisting only of a few scattered lobules in the urethral wall (1, 2, 3, 4). Bulbourethral glands lie dorsolaterally on the pelvic urethra, at the level of the ischiatic arch, and cranial to the anal sacs (1, 4). Very few reports can be found in the literature concerning the pathology and the imaging of the feline prostate and, up to the authors knowledge, no report concerning the pathology and imaging of the bulbourethral gland. The purpose of this study is to present the first imaging data of the accessory glands in clinically normal intact tomcats.

Materials and methods: Six healthy male intact cats, with no previous hormonal treatments, of a mean age 15.5 (9–24) months and a mean body weight of 5.3 (4–6.7) kg were included in the study. All cats were sedated with intramuscular administration of dexmedetomidine (0.01 mg/kg), butorphanol (0.2 mg/kg), ketamine (2 mg/kg) and their caudal abdominal and pelvic area were scanned with computed tomography (CT, Syngo CT VC30-easy IQ 16-slides). Windows were obtained at "Baby-abdomen" protocol, Kernel H30s, slice high resolution (HR) 0.6. All animals were also scanned 30 seconds after the intravenous administration of iohexol (Omnipaque, GE) as a contrast media at a dose of 600 mg/kg, bolus. Axial, sagittal and coronal planes were obtained, and maximum dimensions of the prostatic and bulbourethral lobes were recorded.

Results: Prostate and bulbourethral glands were better visualized in axial and coronal planes, as ovoid to spherical structures. Axial and coronal height and width of left and right lobes of the prostate are presented in table 1, and those of the bulbourethral gland in table 2. Ampullary glands were not clearly visualized.

TABLE 1. CT dimensions of the prostate gland

	Axial plane		Coronal plane	
	Height (cm±SD)	Width (cm±SD)	Width (cm±SD)	Length (cm±SD)
Left lobe	1.06 ± 0.24	0.51 ± 0.08	0.54 ± 0.05	0.76 ± 0.1
Right lobe	1.13 ± 0.26	0.54 ± 0.03	0.56 ± 0.05	0.75 ± 0.12

TABLE 2. CT dimensions of the bulbourethral gland

	Axial plane		Coronal plane	
	Height (cm ± SD)	Width (cm ± SD)	Width (cm ± SD)	Length (cm ± SD)
Left lobe	1.15 ± 0.16	0.95 ± 0.13	0.75 ± 0.02	0.84 ± 0.11
Right lobe	1.08 ± 0.18	1.01 ± 0.2	0.75 ± 0.08	0.85 ± 0.12

Conclusions: CT can be used for imaging the prostate and bulbourethral glands of the intact tomcat. These preliminary data can be the basis for further future research.

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154 | Investigation on IgM and IgE as possible cause of hypoluteoidism in dogs

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Introduction and aim: A reasonable causality for insufficient luteal function is so far not verified. An autoimmune antibody response to P_4 was considered as one possible factor (1). The aim of the present investigation was to go more intensely into this topic.

Material and methods: Fifty intact, clinically healthy bitches of different breeds were included. Out of the 38 dogs used for breeding, 23 had an undisturbed pregnancy, nine showed embryonic resorption, six did not become pregnant and 12 were not mated. Five ovariohysterectomised (OHE) Beagle bitches were submitted to P_4 treatment and six intact male dogs of different breeds served as negative control. In the breeding bitches mating was performed in the time span of day 1 to 3 after ovulation (ov.) according to vaginoscopical and cytological criteria as well as peripheral P_4 concentration. In all intact bitches blood samples were collected (1) day 24–30, (2) day 31–40, (3) day 41–50, and (4) day 51–56 after ov. The OHE bitches received Utrogest® soft capsules (100 mg three times daily) over a period of four weeks. Blood sampling was performed before the start of treatment (0), and four (1) and eight weeks (2) thereafter. A single blood sample was collected from each male dog. In all bitches blood serum was analysed for P_4 and IgM and IgE, the two immunoglobulins were also measured in blood samples of male dogs. Analysis of P_4 was performed using the Immulite® system, IgM and IgE were determined as described before (2).

Results: P_4 concentrations (ng/mL) in the four periods were (1) 20.2 ± 6.2, (2) 15.9 ± 6.1, (3) 9.4 ± 4.1, (4) 6.1 ± 3.0 in undisturbed pregnancies and (1) 22.4 ± 4.6, (2) 18.4 ± 10.7, (3) 8.7 ± 5.7, (4) 4.7 ± 3.9 in bitches showing embryonic resorption. Five bitches of this group had P_4 values of 2.2–6.0 in period 3. In nonpregnant bitches (mated and not mated) average P_4 values were at similar levels in all four periods: (1) 20.3 ± 6.0, (2) 12.9 ± 6.9, (3) 7.4 ± 6.1, (4) 3.9 ± 3.4. In the Utrogest® treated OHE bitches mean P_4 values four weeks after treatment were 11.5 ± 6.0. Mean IgM-levels over the four periods were for normal pregnancy 0.41 ± 0.02, resorption 0.33 ± 0.02, nonpregnant bitches 0.62 ± 0.03. Corresponding mean IgE-levels were 0.19 ± 0.01, 0.19 ± 0.01 and 0.05 ± 0.02. IgM- and IgE-levels in OHE bitches and intact male dogs are shown in table 1.

TABLE 1. IgM and IgE levels in ovariohysterectomized bitches and intact male dogs

bitch no.	IgM_0	IgM_1	IgM_2	IgE_0	IgE_1	IgE_2	dog no.	IgM	IgE
1	0.17	0.17	0.17	0.03	0.05	0.00	1	0.47	0.00
2	0.84	0.74	0.06	0.07	0.22	0.40	2	0.19	0.16
3	0.47	0.32	0.38	0.02	0.09	0.20	3	0.80	0.03
4	0.12	0.03	0.07	0.26	0.24	0.49	4	0.74	0.40
5	0.00	0.00	0.00	0.00	0.00	0.00	5	0.32	0.56
							6	0.11	0.00

Conclusion: P_4 concentrations in normal pregnancy, embryonic resorption and nonpregnant luteal phase showed the typical secretion pattern with no differences within the four periods. Due to the low mean IgM level found in embryonic resorption, a causal connection with luteal function seems doubtful. The same is implied by the equal mean levels of IgE found both in normal pregnancy and resorption. Furthermore P_4 treatment of OHE bitches did not cause any increase in IgM. However the increasing levels seen in three of the five OHE bitches after P_4 treatment may indicate an immunoreaction to the exogenous hormone. Independent of P_4, IgM and IgE values in male dogs were within ranges of intact and OHE bitches.

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155 | Oral melatonin supplementation in female domestic cats, a double blinded prospective study

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Introduction and aim: Multiple studies have described prolonged interoestrous intervals with the administration of melatonin in the domestic cat. The majority of the studies concern subcutaneous melatonin implants (1). Very little is known about oral melatonin supplementation: 2 studies describe the effect of oral administration 2 h before sunset of 4 mg and 30 mg respectively (2, 3). These studies mainly involve European Shorthair queens and there is little data on other breeds. This study aims to assess the efficiency of 0.9 mg (M1), 5 mg (M2) and 10 mg (M3) oral administration of melatonin on female domestic cats owned by French breeders, in order to determine what would be the lowest efficient dosage.

Materials and methods: Recruited cats had to be at least 1 year old, with a normal clinical examination. All breeders lived in France. Cats with medical contraceptive treatments within 6 months prior to inclusion, and those lactating were excluded. Dates of inclusion were limited from February to August, and queens had to exhibit at least 1 previous oestrus during the breeding season, to avoid any bias due to seasonal anoestrus. The duration of normal oestrous and interoestrous periods prior to treatment were assessed. Treatment started at the end of oestrus or in interoestrous, determined by vaginal cytology and clinical behaviour, and cats were divided in groups M1 M2 and M3 in a randomly double blinded manner. They were given a daily standardized size tablet 2 to 3 hours before sunset, for 2 months. Clinical examination, body weight, vaginal smear and progesterone were assessed at days 0, 7, 30 and 60 to detect any signs of oestrus or ovulation. The protocol ended whenever animals showed an increased progesterone level (>2 ng/mL) 7–10 days after the inclusion, when owners did or could not give daily treatment, or whenever an oestrous behaviour was observed before the end of the 2 months treatment. Statistical significance was evaluated using Student test. p values of <0.05 were considered statistically significant.

Results: 46 privately owned queens from 14 breeders representing 13 different breeds were included in this study. All females lived in multiple cat households. Of the 11 cats from group M1, all returned to oestrus within 8 days after the beginning of treatment. Mean interoestrous interval in M1 group prior to treatment was 13.22 ± 8 days [4–30] and was 9.14 ± 4.5 days [4–15] during treatment. Surprisingly, daily oral 0.9 mg melatonin had a tendency to decrease the length of interoestrous, but not significantly (p = 0.07). Due to strict inclusion and exclusion criteria, 4/11 cats from group M2 and 3/14 cats from group M3 reached the end of the 2 months protocol without showing any signs of oestrus. They had significantly longer interoestrous intervals (p = 0.004 and p = 0.02 respectively) than prior to treatment. Among queens who did not reach the end of the treatment, 5 ovulated in group M2, 3 in group M3. Two cats expressed an oestrous behaviour within 10 days after the start of the treatment in group M2, 3 in group M3. Four were excluded in group M2 and 6 in group M3 for the following reasons: the inability to give treatment 2 h before sunset, the difficulties to give the treatment orally and the incapacity to detect oestrous behaviour. One cat was excluded due to *Malassezia* dermatitis, and another due to the reoccurrence of epilepsy.

Conclusion: This is the first study assessing the efficiency of melatonin in 13 different breeds of cats, with 3 different doses. These results indicate that both 5 mg and 10 mg may be able to postpone oestrus up to 2 months although not in all cats, and the main drawback is the time limitation of administration. Further studies are

required to make the oral administration of melatonin easier for the owners. It shows, as was also described in the case of subcutaneous 18 mg melatonin implants (1), that some females may still exhibit an oestrous behaviour within 10 days after an oral administration of 5 mg and 10 mg melatonin.

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156 | Involvement of IGF-1, 25-hydroxyvitamin D_3 (25-OHD_3) in the pathogenesis of benign prostatic hyperplasia in the dog?

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Introduction and aims: Benign prostatic hyperplasia (BPH) is an age dependent primarily not inflammatory enlargement of the accessory gland. It is the most frequent prostatic disease in the intact dog, in most cases showing subclinical course (1). As canine prostate specific arginine esterase (CPSE) makes > 90% of the proteins in canine prostatic secretion it serves as useful marker of BPH in dogs (2). Besides androgen dependency factors which may cause BPH in dogs are largely unknown (3). The impact of IGF-1 on proliferation of many tissues is considered regarding BPH in men (4). Moreover, the prevalence of vitamin D deficiency in human BPH patients may represent a causal association between BPH and vitamin D status (5). The aim of the present study was to investigate serum IGF-1 and vitamin D status represented by serum 25-OHD_3 in male dogs with normal and hyperplastic prostate gland.

Material and methods: Eighteen Labrador Retrievers/LR and 20 Rhodesian Ridgebacks/RR were assigned to the age groups 18–24 mo (n = 12), 25–48 mo (n = 13), and 49–72 mo (n = 13). Prostate gland status was determined by rectal palpation, B-mode ultrasound, calculation of the prostate gland volume (length × width × height × 0.523), semen analysis regarding hemospermia and was classified according to blood plasma CPSE concentrations ≤ 60 ng/mL: healthy – group 1 (n = 28, 18 LR, 10 RR), and ≥61 ng/mL: BPH – group 2 (n = 10, all RR) (6). CPSE analysis was performed by ELISA Odelis® CPSE (Virbac, Bad Oldesloe), IGF-1 was measured in blood serum by RIA (Fa. Beckman Coulter, Prague), and 25(OH)-vitamin D in blood serum and prostatic secretion using direct day ELISA K2108 (Immundiagnostik, Bensheim). Statistical analysis included Wilcoxon's signed rank test and Spearman's rank correlation test.

Results: Prostatic volume was 35.7 ± 25.5 cm^3 in group 1 and 81.3 ± 38.1 cm^3 in group 2 ($p < 0.05$). Significant differences were detected between LR and RR in all age groups combined with a significantly more pronounced age related increase in prostatic volume in RR. Hemospermia was observed in 4 LR and 18 RR. Serum IGF-1 concentrations were 475.8 ± 121.7 and 415.6 ± 79.1 ng/mL and serum 25-OHD_3 concentrations were 89.2 ± 37.0 and 70.5 ± 36.0 ng/mL in groups 1 and 2, respectively. In both breeds, serum IGF-1 concentrations showed a consistent level in all age groups. In RR, 25-OHD_3 serum concentrations decreased markedly between age groups 25–48 mo and 49–72 mo (100.8 ± 44.5 and 55.5 ± 44.2 ng/mL, $p = 0.08$). In prostatic secretion, 25-OHD_3 was only detectable in RR (healthy n = 4: 63.2 ± 50.2 ng/mL; BPH n = 7: 44.4 ± 31.6 ng/mL). Positive correlations existed between prostate gland volume and CPSE (total: $r = 0.51$, $p < 0.001$; RR: $r = 0.51$, $p < 0.05$), age (total: $r = 0.63$, $p < 0.0001$; LR: $r = 0.71$, $p < 0.001$; RR: $r = 0.59$, $p < 0.01$) and breed ($r = 0.67$, $p < 0.001$), between IGF-1 and CPSE ($r = 0.64$, $p < 0.05$) and between 25-OHD_3 in prostatic secretion and prostate gland volume ($r = 0.55$, $p < 0.05$).

Conclusion: These results indicate a predisposition for BPH in RR, which had been described in a previous study (7). The positive correlation of IGF-1 with CPSE, the accepted marker for canine BPH, may imply the involvement of IGF-1 in the pathogenesis of the disease. The relationships found in RR between age, BPH and low 25-OHD_3 concentrations in blood serum and prostatic secretion may indicate an endogenous or nutritional 25-OHD_3 deficiency as causal background of BPH.

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157 | Anti-Müllerian hormone concentration and its correlation with ovarian follicle numbers during cat pregnancy

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Introduction and aim: Anti-Müllerian Hormone (AMH) is produced by preantral and early antral follicles and inhibits excess recruitment

of primordial follicles (1). It is a regulator of growth in tissues of Mullerian origin, such as endometrial, ovarian, cervical and breast tissues. In humans, AMH is highly expressed in endometriosis glands and stroma (2), it acts as a negative regulator of cell cycle and viability by inducing apoptosis (3, 4). Due to these features local functions during pregnancy might be supposed. Our aim was thus to determine the physiological serum and ovarian AMH concentrations during normal cat pregnancies and to investigate for relations with ovarian follicle and corpora lutea numbers as a first step.

Materials and methods: A total of 27 cats with a mean age of 1.13 ± 0.30 were divided into 5 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 group V (n = 6, non-pregnant interoestrous controls). All cats underwent ovariohysterectomy following blood sampling. Immediately after the operation, the uteri and fetuses were investigated for pathological lesions and the left ovaries were fixed in 10% neutral formalin for follicle counting. Right ovaries and blood sera were stored at −80°C until analyzed. AMH concentrations in serum and ovaries were analysed by using a feline-specific commercial AMH ELISA kit (ABIN3073421; antibodies-online, Aachen, G); before measurement, ovaries were homogenized and the tissue centrifuged at 1550 g. Follicles of the left ovaries were counted in five consecutive areas of 17.000.000 μm^2 of four sections at magnification × 400 and categorized as primordial, primary, secondary, early antral and antral follicles according to their size and histological features; the number of corpora lutea was counted.

Results: No pathologies were detected in any tissues, fetuses were even distributed between uterine horns. *Serum AMH* for group I, II, III, IV and V was 0.72 ± 0.22 ng/mL, 2.95 ± 1.21 ng/mL, 2.83 ± 1.33 ng/mL, 1.34 ± 0.98 ng/mL and 0.72 ± 0.44 ng/mL, respectively; serum AMH was higher in group II compared to I and V ($p < 0.05$). *Ovarian AMH* for group I, II, III, IV and V was 461.6 ± 50.4 ng/g, 297.2 ± 163.0 ng/g, 481.8 ± 344.7 ng/g, 219.9 ± 120.4 ng/g and 520.9 ± 239.9 ng/g. Ovarian AMH was lower in group IV compared to V ($p < 0.05$). In the non-pregnant controls (V), there were positive correlations between ovarian AMH and antral follicles ($r = 0.931$, $p < 0.05$), and total antral follicles ($r = 0.858$, $p < 0.05$) respectively. However, in pregnant cats, these correlations were not determined. Instead, in group I, there was a positive correlation between serum AMH and corpus luteum count ($r = 1.000$, $p < 0.05$), and in (IV) between ovarian AMH and corpus luteum count ($r = 0.939$, $p < 0.05$). In group II, a positive correlation between serum AMH and ovarian AMH ($r = 0.737$, $p < 0.05$) was determined. Interestingly, negative correlations were measured between serum AMH and secondary follicles (III $r = -0.985$, $p < 0.05$) and between ovarian AMH and secondary follicle count (IV, $r = -0.845$, $p < 0.05$).

Conclusions: Serum AMH increased between day 30 to 50 (II and III), then decreased towards parturition, eventually related to the corpora lutea function, which has to be proven. The positive correlation between the ovarian AMH and serum AMH (II) was supposedly due to follicular development that may occur during this stage. This study arises the question for physiological, probably regulatory, ovarian functions during feline pregnancy.

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158 | Vaginal tumor together with ovarian cysts, uterine tumor, cystic endometrial hyperplasia, and mammary gland tumor in 14-years old bitch

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Introduction: Vaginal tumors, after mammary gland tumors are the most common female reproductive tract neoplasia in the bitch (1). Ovarian cysts as well as cystic endometrial hyperplasia (CEH) are very common in intact bitches (2). However, uterine neoplasias are rare. The presence of vaginal tumor together with ovarian cysts, uterine tumor, CEH and mammary gland tumor in a bitch is a rare finding.

Clinical case: A 14-years old crossbreed bitch was referred to the veterinary clinic due to abundant sanguineous vaginal discharge, which owners associated with the heat. The bitch had naturally delivered a litter of seven puppies at the age of 1 year with absence of dystocia. The bitch had heat twice a year with regular time intervals. The last two heats were quite long and with abundant vaginal discharge. The owner noticed a mass in the vaginal area. The clinical examination showed the presence of a vaginal tumor in the upper vault of the vagina and the presence of mammary mass on the right mammary chain (size of 1 × 2 cm). The owner did not want to perform biopsy of the mammary mass. Cytology of a vaginal smear was compatible with the pro-estrus. Blood tests showed no abnormalities, apart from increased ALT (117 U/L; normal range: 3–60 U/L) and a high level of estradiol (215.9 pg/mL; normal range: 20–60 pg/mL). Ultrasound examination revealed many cysts on both ovaries, a big mass on the left uterine horn (about 12 × 7 cm) and thickening of the uterine wall. Ovariohysterectomy together with vaginal tumor removal and semi-mastectomy (removal of the whole right mammary chain) was planned. Due to the estimated prolonged time of the surgery, mastectomy was planned to be performed a month later. The surgery was successful. Antibiotic therapy (amoxicillin with clavulanic acid 12.5 mg/kg BID during 7 days) together with anti-inflammatory drugs (meloxicam 0.2 mg/kg during 5 days) was prescribed to the

bitch. The bitch recovered gradually. The histopathological examination confirmed the presence of: ovarian cysts on both ovaries, cystic endometrial hyperplasia together with mild endometritis within the uterus, uterine leiomyoma (within the uterine mass), and vaginal fibroma.

Discussion: Leiomyoma is the most frequent uterine tumor in bitches, thought it has low prevalence. However, fibroma is not the most frequently diagnosed vaginal tumor. The presence of ovarian cysts as well as CEH is quite common in intact bitches. However, presence of multiple reproductive tract disorders is uncommon and the case of a bitch with a vaginal tumor together with ovarian cysts, uterine tumor, CEH and mammary gland mass is worth being described. This case confirms that there are strong relationships between these comorbidities, as previously described (2).

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160 | Anastrozole treatment of idiopathic hyperestrogenism in a 5 year-old castrated male dog

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Introduction: Hyperestrogenism in male dogs can occur due the presence of functional testicular neoplasms or due to estrogen administration (1), but in castrated male dog is a rare finding. However, apart from the ovaries, testicles, adrenal cortex, placenta, liver, muscle and fat tissue may also be a source of estrogens (1).

Clinical case: A 5 years-old crossbreed male dog was referred to the veterinary clinic due to severe perianal pain and inflammation of the mucous membrane of the anus. The dog was taken from the shelter and was castrated at the age of 1 year. It was presented with defecation problems and at the same time the dog was very attractive to other males. Before visiting our clinic, the dog had been treated several times with antibiotics and anti-inflammatory drugs, the ultrasound examination showed no abnormalities, the parasitological examination of feces was normal and perianal glands were also normal at the clinical examination. When the dog came to our clinic the perianal area was inflamed and erythematous. Blood test results revealed increased liver enzymes activity (ALT: 320 U/L – normal range <60; AST: 68 U/L – normal range <45; AP: 178 U/L – normal range: <155) as well as very high estradiol (224 pg/mL – normal range 20–35 pg/mL). The other blood parameters were within the normal range. An ultrasound examination was done and no ovarian tissues were found. A GnRH stimulation test was performed. Two blood samples were collected: before and one hour after an intramuscular injection of buserelin (Receptal, MSD; 2.2 mg/kg). High estrogen levels after stimulation suggested an active estrogen production. The dog was prescribed anti-estrogenic treatment with anastrozole (Arimidex, 0.05 mg/kg QID). The dog started to recover gradually. One month after the onset of the treatment the dog had no perianal pain, the perianal inflammation disappeared, and the dog was less attractive to other male dogs.

Discussion: In the present case, the dog presented hyperestrogenism with visible clinical symptoms. We could not find a source of estrogens but GnRH stimulation test proved active estrogen production in this patient. We suspect that adrenal glands might be the source of estrogens. However, further investigation is necessary. Excessive estrogens production may lead to occurrence of multiple side-effects, among which suppression of bone marrow is the most dangerous. Therefore, medical treatment is necessary. Anastrozole is a novel drug indicated in the treatment of breast cancer in women. It selectively inhibits the aromatase enzyme, which is responsible for converting androgens (produced by women in the adrenal glands) to estrogens. Therefore, it significantly lowers serum estradiol concentrations. Up to now, tamoxifen, a selective estrogen receptor modulator, was used as an anti-estrogenic therapy in dogs. However, this drug has both estrogenic and anti-estrogenic effects. It seems that anastrozole is a safer drug of choice in treatment of hyperestrogenism in dogs than tamoxifen. This is the first described case of anastrozole treatment of hyperestrogenism in a male dog.

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161 | Towards good quality feline embryos: improving embryo culture by supplementation with growth factors

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Introduction and aim: Twenty-five of thirty-six species, or almost 70% of the cat family, are included in the IUCN Red List (IUCN-International Union for Conservation of Nature). The success rate for *in vitro* embryo development in cats is still lower as compared to other species and the *in vivo* development. One of the main differences between the *in vitro* and *in vivo* conditions is that *in vivo* a number of well balanced growth factors operate embryonic development. Many laboratories try to mimic the *in vivo* condition by the variation of culture media composition, but growth factors are not often used for these purposes in felids. IGF-1 (Insulin-like growth factor 1) has been studied in different experimental models and it has been shown to improve *in vitro* embryo development in a lot of mammalian species. In cats adding IGF-1 to culture media improved the blastocyst formation rate in single culture system to a level

similar to group culture. Granulocyte-macrophage colony stimulating factor (GM-CSF) also has been implicated on growth and development of the preimplantation embryo in mammals, but the effects of this factor have never been studied in felids. The aim of this study was to evaluate the effects of growth factors, IGF-1 and GM-CSF in single and group culture systems for domestic cat embryos.

Materials and methods: Cumulus oocyte complexes (COCs) were collected from ovaries from local veterinary clinics. COCs were matured and fertilized in vitro using protocol for IVM/IVF, developed by IZW. Zygotes were randomly assigned to one of the following treatments: 1) control group without any growth factors, single culture, 2) IGF-1, 10 ng/mL single culture, 3) IGF-1, 20 ng/mL single culture, 4) GM-CSF, 2 ng/mL single culture, 5) combination of GM-CSF and IGF-1 (10 ng/mL), single culture. The same experimental groups were used also for group embryo culture. Cat embryos were cultured singly or in groups of 3 per drop in 20 μL of culture medium under mineral oil, at 5.5% CO2 and T = 39°C. During in vitro culture, the embryos were analyzed for development to morula (day 5) and blastocyst (day 7) stages using light microscopy. All embryos were fixed when they stopped their development and were analyzed for nuclei counting using PI staining.

Results: In single culture like in group culture growth factors didn't improve morula or blastocyst formation in domestic cat, it was similar for all groups of treatment. We found effects only in total cell number in embryos, IGF-1 (20 ng/mL) and GM-CSF increased the total cell number in embryos at day 7 of culture, 41 ± 4.6 and 40 ± 4.8 respectively compared with control group in single culture.

Conclusions: We didn't find any differences between single and group cultivation without growth factors in domestic cats. Positive influence of growth factors was seen in single culture system, whereas no effects were found when embryos were cultured in groups. This supports the hypothesis that embryos secrete factors into the culture medium, which may have similar effects as growth factors.

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163 | Semen quality of dog compared with haemocytometer and dog-side ONGO sperm analyser

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Introduction and aim: Assessment of semen quality is one of the most important examination procedures in male stud dogs. Although the gold standard of sperm number counting is the haemocytometer, it does not offer information about total and progressive motility of sperm cells (1, 2). The CASA is an accurate method for measurement of sperm number and motility however its expenses limit the use in everyday clinical practice (3). Aim of this study was to compare in practice the semen quality examined with haemocytometer and ONGO sperm analyser.

Materials and methods: Second sperm fraction of thirteen stud dogs was collected manually into double wall glass, the wall filled with 37°C water. Sample was divided in two aliquots, the progressive motility was estimated with light microscopy and subjectively scored 1–5 (low to high). Then 10 μL semen was added to 390 μL Hank's solution and sperm number was counted in a haemocytometer. The other aliquot of sperm sample was diluted with TRIS-Egg solution and examined with an ONGO sperm analyser (Microfluidlabs, Budapest, Hungary). Statistical analyses were performed using the SigmaPlot 12.0 statistic computer program.

Results: Examination with ONGO took 3x 1.5–2 min. Minimum dilution was 1:2 and maximum dilution 1:5. The average, diluted concentration was 135.3 ± 30.5 M/mL, re-calculated concentration 438.6 ± 129.7 M/mL. The sperm number counted in the haemocytometer was 409.4 ± 146.8 M/mL, did not differ from ONGO count ($p = 0.87$). The average total motility, measured with ONGO was 94.3%, the progressive motility was 34.4 ± 11.2%. Sperm sample motility scores with light microscope ranged between 4 and 5 to and progressive motility scores between 3 and 5. Progressive motility was different with ONGO and subjective scoring at the level of significance ($p < 0.05$).

Conclusions: Sperm samples of stud dogs need to be diluted to 100–200 M/mL for most accurate measurement. The difference between ONGO and haemocytometer counted cell number was 6.6% which was comparable to other methods. Total motility was similar between ONGO and subjective estimation but progressive motility was subjectively overestimated. The estimation of cell number and total motility by a well-trained examiner gave similar results with both methods, however, progressive motility was more accurately determined by ONGO sperm analyser.

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164 | Slow freezing or vitrification: which method is better to cryopreserve canine ovarian tissue?

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Introduction and aim: Cryopreservation of gonadal tissues is an assisted reproductive technique that allows the preservation of

the genetic material from any animal species that die unexpectedly or that will be submitted to elective or therapeutic gonadectomy (1). However, research on cryopreservation of gonadal tissue is still scarce, especially for the canine family. Considering the similarity between the reproductive physiology of domestic dogs and their wild counterparts, the establishment of an efficient cryopreservation protocol is of pivotal importance in the conservation of endangered wild canid species. Therefore, the aim of this study was to evaluate which cryopreservation technique (vitrification or slow freezing) is more suitable for canine ovarian tissue. For that purpose, we compared follicle quality and apoptosis rates after thawing.

Materials and methods: Ovaries obtained from 14 bitches were sectioned into small fragments of approximately 5 × 2 × 2 mm in length, width and thickness, respectively, and then were randomly allocated into three groups: vitrification, slow freezing and control (fresh ovarian pieces). Vitrification was performed according to (2): the ovarian cortex fragments were immersed in PBS containing 1M DMSO at room temperature (RT) for 60 sec and then transferred to cryotubes containing PBS-DMSO at 0°C for 5 min. DAP 123 (1M acetamide, 2M DMSO and 3M propylene glycol) was then added to each cryotube and kept at 0 ° C for another 5 min. Subsequently, cryotubes were stored in LN2. For warming, the cryotubes were kept at RT for 60 seconds and then diluted with PBS containing 0.25M sucrose preheated to 37° C. Slow freezing was performed according to (3): ovarian fragments were equilibrated in cryotubes containing 1 mL of HF-10 medium, 1.5M DMSO, 10% SAH and 0.1M sucrose for 30 minutes at 4°C and then were frozen using a programmable freezer, being cooled to −7°C at rate of −2°C/min, until seeding. Then, the cooling rate decreased to −0.3°C/min until it reached −30°C, when the cryotubes were immersed directly into LN2. For thawing, the cryotubes were exposed at RT for 2 min and then placed in a water bath at 38°C for 2 min. Tissue damage was evaluated by conventional histology (H&E stain) and also by immunohistochemistry (cleaved caspase-3) in order to determine the degree of apoptosis cells. Data were analysed by Kruskal-Wallis test followed by Dunn′s multiple comparison test, with $p < 0.05$.

Results: Histological examination was carried out in 2004 ovarian follicles and it revealed that more than 50% of the primordial follicles were degenerated in both cryopreserved groups vs. 35% of the control group ($p < 0.005$). In addition, there was a difference in cryotolerance depending on the follicle type and cryopreservation method: while slow freezing method presented lower degeneration rates of primary follicles compared to vitrification ($p = 0.0003$), the latter presented less damage in mature follicles compared to the former ($p < 0.0001$). Immunohistochemical evaluation indicated that 84.21% of the follicles evaluated in the slow freezing group did not express caspase-3, that is, they were not in apoptosis. No significant difference was found concerning apoptotic cells between slow freezing and control group, but there was a significant difference between vitrification group and control.

Conclusions: Taking these results together, it seems that slow freezing was able to better preserve the ovarian cortex compared to vitrification and can be considered up to now the method of choice to cryopreserve canine ovarian tissue. However, advances in slow freezing protocol are necessary in order to improve its ability to maintain follicle integrity, especially in those at immature stage of development.

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165 | Transabdominal ultrasonography of the accessory glands in clinically normal intact tomcats. Preliminary results

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Introduction and aim: The accessory glands of the tomcat consist of the ampullary, the prostate and the bulbourethral glands. The ampullary glands are located at the terminal part of the deferent duct forming the ampulla of the deferent duct (1). The prostate is located dorsal to the beginning of the urethra at the caudal edge of the pubic symphysis (1). The prostatic body does not completely surround the urethra. The disseminate part consists of a few scattered lobules in the urethral wall. (1, 2). Bulbourethral glands lie dorsolaterally on the urethra, at the level of the ischiatic arch, and cranial to the anal sacs. Very few reports can be found in the literature concerning the pathology and the imaging of the feline prostate (3–11) and, up to the authors knowledge, no report concerning the pathology and imaging of the bulbourethral gland. The purpose of this study is to present the ultrasonographic findings of the normal accessory glands in clinically healthy intact tomcats.

Materials and methods: Twelve healthy intact male cats (n = 12) of a mean age 3.7 (10–0.8) years and a mean body weight of 4.86 (4–6) kg, with no previous hormonal treatments were included in the study. None of the cat was sedated, while the hair of the caudal abdominal and pelvic area was clipped. Acoustic gel was liberally applied to the skin. Ultrasonographic examination of the prostate and the bulbourethral glands was performed with the animals in dorsal recumbency. A linear transducer of variable frequency (5–14 MHz, L6–12 RS, GE) with a Logiq F8 and a Vivid iq (GE) ultrasound machines was used. Urethra was used as anatomical landmarks. Maximum dimensions of the accessory glands were recorded.

Results: The body of the prostate gland was visualized in longitudinal planes in 11 out of 12 animals. The disseminate part and bulbourethral glands could not be identified in any of the 12 animals included in the study. Prostate was imaged as an ovoid structure, surrounding the urethra. In 6 cats the prostate was hypoechoic and

in 5 cats isoechoic compared to its surrounding tissues. Longitudinal maximum length and width of left and right prostatic lobes as well as the total maximum longitudinal width of the gland are presented in table 1.

TABLE 1. Ultrasound dimensions of the prostate gland

	Length (cm ± SD)	Width (cm ± SD)	Total width (cm ± SD)
Left lobe	0.94 ± 0.10	0.51 ± 0.08	1.12 ± 0.17
Right lobe	0.99 ± 0.14	0.51 ± 0.06	

Conclusions: Transabdominal ultrasonographic examination of accessory glands of the tomcat is a challenging procedure. The prostate might be difficult to identify, because of its small size, the similarity to the surrounding tissues' echogenicity, and its caudal position. Bulbourethral glands could not be examined transabdominally, most probably because of their anatomic position, over the pelvic arc, at the caudal part of pelvic urethra.

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168 | Luteinizing hormone receptor expression in canine lymphoma

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Introduction and aim: Luteinizing hormone (LH) is secreted by the anterior pituitary gland in response to stimulation from gonadotropin releasing hormone (GnRH). LH receptors (LHR) are present in reproductive tissues (gonads, placenta) as well as in many non-reproductive tissues (skin, thyroid, urinary bladder).[1] Previous research in our laboratory has shown that LHR are also present in normal and neoplastic canine lymph nodes and lymphocytes.[2] In circulating canine lymphocytes, gonad removal (spaying/neutering) increases the percentage of cells positive for LHR compared to intact dogs.[2] It is not known if gonad removal increases the percentage of cells positive for LHR in neoplastic canine lymph nodes. It is also not know if the type of lymphoma (B-cell or T-cell) influences LHR expression. We hypothesized that LHR expression would differ with reproductive status and lymphoma phenotype. The aim of this study was to determine LHR expression (percentage of LHR positive cells and LHR staining intensity) in dogs with known lymphoma phenotypes.

Materials and methods: Lymphoma tissue samples (n = 20) were formalin-fixed, paraffin embedded and sectioned onto charged slides. Slides were subjected to routine immunohistochemical techniques. All slides were deparaffinized, rehydrated, subjected to heat-induced epitope retrieval. Endogenous peroxidase activity was inactivated with 3% hydrogen peroxide and nonspecific binding was blocked protein block serum-free. A rabbit polyclonal anti-human LHR antibody (1:100 dilution) or a rabbit negative control antibody were applied to slides. Slides were reacted with one step horseradish peroxidase-conjugated polymer anti-rabbit IgG followed by NovaRed peroxidase substrate. Slides were counter-stained with hematoxylin, dehydrated, and mounted. The percentage of cells positive for LHR and the staining intensity (scored 0–3) were determined at 400X magnification by a single observer (AV). Groups were compared using a two-tailed Students t test. Data were expressed as mean ± standard deviation and significance was defined as $p < 0.05$.

Results: All samples contained cells positive for LHR but the percentage of cells expressing LHR and the staining intensity varied. The percentage of cells positive for LHR did not differ between male intact dogs (7.0% ± 4.2%) and male neutered dogs (9.8% ± 5.7%; $p = 0.33$). However, the intensity of LHR was higher in intact male dogs (2.6 ± 0.9) compared to male neutered dogs (1.9 ± 0.4; $p = 0.04$). The percentage of cells positive for LHR did not differ between B-cell lymphomas (9.4% ± 5.8%) and T-cell lymphomas (8.3% ± 4.5%; $p = 0.69$). In addition, the intensity of LHR did not differ in B-cell lymphomas (2.0 ± 0.6) and T-cell lymphomas (2.3 ± 0.7; $p = 0.43$).

Conclusion: This is the first study to evaluate the influence of reproductive status and tumor phenotype on LHR expression in canine lymphoma. Current research is investigating the effects of LHR activation using canine lymphoma cell lines. Future research efforts will provide evidence to support using a complementary treatment for canine lymphoma by downregulating LH with a commercially available canine GnRH agonist.

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