ABSTRACTS

OPENING SESSION

Umbilical vein catheterization as an option for intravenous access in neonatal puppies

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Intravenous access is essential to manage critically ill neonatal puppies and is difficult to achieve. Intravenous access is achieved most commonly using intraosseous catheters but potential damage to the bone and growth plate is possible. In human neonatology, umbilical vein catheterization is the primary route of intravenous access in infants. This procedure has not been widely adopted in dogs. In hopes of using this procedure in neonatal puppies, the study objective was to determine the success rate of umbilical vein catheterization evaluated by gross dissection and imaging. Six neonatal puppies were used for the study (size range: Saint Bernard to Shih Tzu) and were either stillborn or died shortly after birth. A 26-gauge intravenous catheter was used to achieve vascular access by holding the umbilicus at a 45-degree angle from a dorsal plane through the abdomen. Entry from the ventral side of the umbilicus allowed a shallow puncture to enter the umbilical vein and easy sliding of the catheter into the vessel. A mixture of 4:1 latex:60% w/v barium sulfate suspension was administered into the catheter (total volume: Saint Bernard, 2 ml; Shih Tzu, 1 ml). Latex aided in vascular dissection and barium provided contrast during computed tomography that was performed using a 16-slice scanner (Aquilion LB, Toshiba/Canon America Medical Systems, Tustin, CA) using these parameters: sternal recumbency; slice thickness, 0.5 mm; reconstruction interval, 1.0 mm; 120 kVp; 50 mAs. Based on dissection and imaging, umbilical vein catheterization was successful in 4/6 puppies; 1/6 (injections) was into the umbilical artery and 1/6 was extravascular (Figure). Umbilical vein catheterization was successful 67% of the time and may be a worthwhile means for vascular access in critically ill neonatal puppies. Future studies using live puppies and maintaining catheter patency over time are warranted.

Keywords: Puppy, umbilicus, catheterization, imaging, neonatology

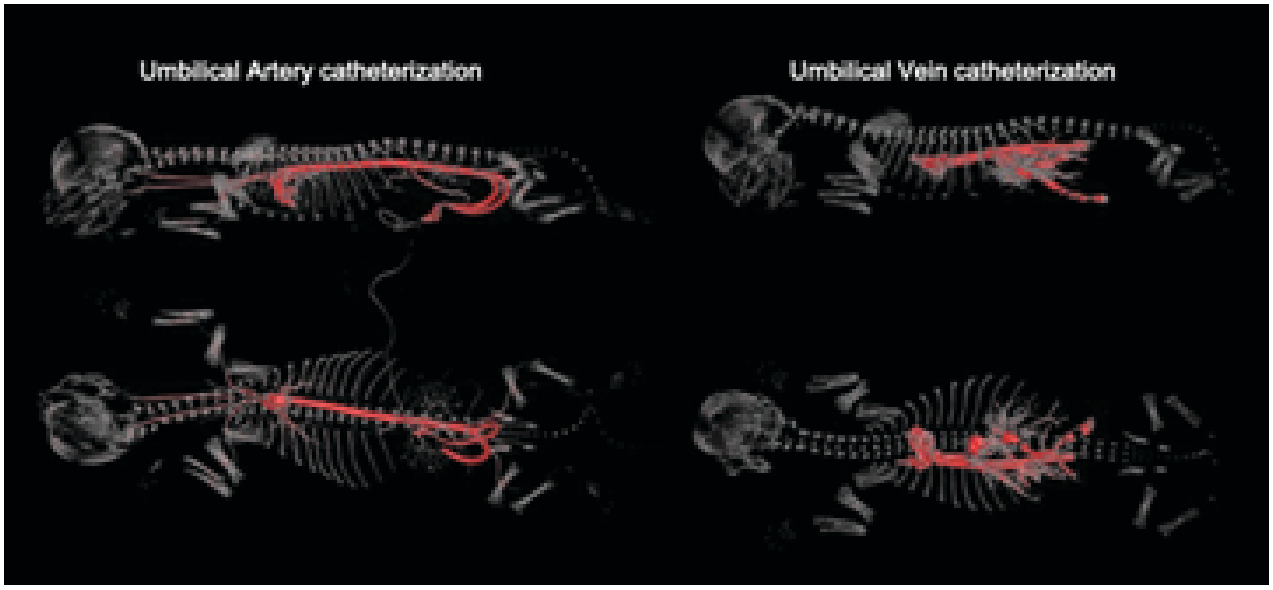

Figure. Catheter in umbilical artery and vein confirmed using computed tomography with barium contrast enhancement

Temporal associations of B-mode, power doppler, and ovarian steroid changes of the periovulatory follicle and corpus luteum during luteogenesis and luteolysis in jennies

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Assessing the relationships between B-mode and power doppler ultrasonography of the periovulatory donkey follicle and respective corpus luteum (CL) could prove useful in clinical practice to predict impending ovulation and determining CL viability. This study's objectives were to assess the associations between B-mode and power doppler ultrasonography and ovarian steroids of the periovulatory follicle and respective CL during luteogenesis and luteolysis in jennies. We hypothesized that information on changes in the granulosa are useful to detect impending ovulation and on blood flow are useful to differentiate an active versus inactive CL. One inter-ovulatory interval between 2 subsequent ovulations of 12 jennies (144 ± 22.5 kg; height 95.5 ± 113 cm) was used. Jennies were teased daily to a mature jack. B-mode ultrasonography was carried out until the detection of a periovulatory follicle (≥ 28 mm, endometrial edema, and estrous signs). Thereafter, jennies were monitored every 4 hours by B-mode and power doppler transrectal ultrasonography. Once presumed signs of impending ovulation (thickened and irregular follicular wall, hyper-echogenicity of granulosa layer in transrectal ultrasonography and softened follicle in transrectal palpation) were detected, jennies were reexamined at 1 hour intervals until ovulation. After ovulation, the CL was examined daily until the completion of luteolysis (progesterone < 1.5 ng/ml). Plasma estradiol and progesterone concentrations were assessed daily with chemiluminescence assays (Immulite 1000, Siemens, US). Data were analyzed using RM-ANOVA followed by Tukey`s test (steroid concentrations, follicle and corpus luteum parame-ters), Friedman test adjusted by Dunn's test (edema score and behavior), and Pearson's coeffi-cient correlations (thickness and echogenicity of the granulosa layer). Mouth-clapping, a species-specific estrous sign, was the first and the last sign to be detected (± 24 hours postovulation). The diameter of the ovulatory follicle was 34.6 ± 3.3 mm (31 - 38 mm). The echogenicity and the thickness of the granulosa layer increased ($p < 0.05$) from 36 to 1 hour before ovulation in 70% of jennies; strong correlations between thickness ($r = 0.70$), granulosa echogenicity ($r = 0.80$), and impend-ing ovulation were noted. Follicular wall blood flow increased ($p < 0.05$) from 72 to 24 hours before ovulation and estra-diol concentrations declined from 42 pg/ml at 72 hours to 31.6 pg/ml at 24 hours before ovulation. Vascularization of periovulatory follicle decreased ($p < 0.05$) from 62% (36 hours before ovulation) to 37% (1 hour before ovulation); 75% of the jennies had a homogenous CL echogenicity with a white hyperechogenic central lacuna. The maximum CL size represented 76% of the periovulatory follicle diameter. Vascularization of the CL and progesterone concentrations had a gradual rise, reaching the peak at 11 and 10 days after the ovulation, respectively ($p < 0.05$). Luteal echo-genicity increased ($p < 0.05$) 4 days after luteolysis as a consequence of corpus albicans formation). Vascularization of the CL started to decline ($p < 0.05$) 3 days before luteolysis and progesterone concentrations had a sharp reduction ($p < 0.05$) for 4 days before luteolysis. In conclusion, the structural changes of the periovulatory follicle detected on B-mode can be used to detec impending ovulation in donkeys; however, B-mode ultrasonography cannot be used to assess CL functionality. Conversely, power doppler can be used to differentiate a functional versus nonfunctional CL in jennies.

Keywords: Periovulatory period, luteogenesis, luteolysis, steroid

Suitability of noncycling recipient mares for in vitro produced equine embryos

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Suitable cycling recipient mares are not always available at an equine embryo transfer station (ETS). Noncycling recipients primed with estrogen followed by progesterone before transfer of in vivo embryos can have similar pregnancy rates as those that are cycling. However, in vitro produced (IVP) embryos may be less hardy and require a more precise window of synchrony with recipient mares compared to in vivo embryos.[1] To our knowledge, no data exist on the suitability of noncycling recipient mares when transferring IVP embryos. A retrospective study was conducted whereby data from a single ETS during the 2020 breeding season were analyzed and pregnancy rates were compared between cycling and noncycling recipients receiving IVP embryos. All embryos were derived from commercial donors and transferred at the ETS. Cycling recipients (CRs) were examined via transrectal ultrasonography until emergence of a dominant follicle, then evaluated daily until ovulation and subsequently received an IVP embryo 4 days postovulation. Noncycling recipients (NCRs) were determined to be anestrus or transitional by a lack of luteal tissue and the presence of small follicles based on transrectal ultrasonography and serum progesterone concentrations. Initially, NCRs were treated intramuscularly with 10 mg estradiol-17β in oil (E_2) on the first day, 6.6 mg on the second day, and 3.3 mg E_2 on the third day. The day after the last E_2 treatment, NCRs were treated intramuscularly with 200 mg progesterone in oil (P_4); once a day for 3 consecutive days. On the fourth day (at transfer), NCRs received intramuscularly 500 mg of P_4 and were supplemented with P_4 at least until their first pregnancy examination. Data were analyzed using Chi-Square or Fisher's Exact tests, and values considered significant at $p < 0.05$. During the study, 78 IVP embryos were shipped to the ETS. Of these, 60.3% (41/78) were transferred into CRs and 39.7% (31/78) were transferred into NCRs. Overall transfer rate for IVP embryos was 52.6% (41/78). Day 42 pregnancy and embryo loss rates were 30.8% (24/78) and

41.5% (17/78), respectively. There were no differences between CRs and NCRs in rates of initial transfer (55.3 versus 48.4%, p = 0.5485), pregnancy at day 42 (34.0 versus 25.8%, p = 0.1367) or embryo loss (38.5% versus 46.7%, p = 0.7449) rates. Results suggest that NCRs treated with E_2 and P_4 prior to transfer may be suitable recipients for IVP embryos.

Keywords: Embryo transfer, IVP embryos, recipients

Reference

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Serum prostaglandin E metabolite in diestrous and pregnant mares

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In the nonpregnant mare, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is luteolytic, and in early pregnancy prostaglandin E_2 is reported to be luteotrophic. However, there is a lack of information on circulating concentrations of prostaglandin metabolite (PGEM) during diestrus and pregnancy. We hypothesized that circulating concentrations of PGEM increase in pregnant mares during the expected time of pregnancy recognition day (D) D13 - D15 compared to diestrous mares. Our objective was to compare daily/hourly plasma PGEM concentrations and secretion profiles in pregnant and diestrous cycles using a randomized cross over design (n = 4 mares), with 1 cycle in between study periods. Transrectal ultrasonography was used to detect estrus, day of ovulation (D0), and pregnancy. Mares were bred to a fertile stallion during estrus. Blood was sampled on D0, 4, 8, 12, 18, and 20. A jugular catheter was used to obtain hourly blood samples from D13 through D16. Blood was placed in chilled EDTA tubes and immediately centrifuged at 4 °C. Plasma was separated, placed in cryovials, frozen in liquid nitrogen, and stored at -80 °C until assayed. PGEM was measured using commercial enzyme-linked immunosorbent assays (ELISA) (Cayman Chemical, Ann Arbor, MI) validated in our laboratory according to the manufacturer's instructions. Progesterone concentrations were determined every 6 hours from D13 to D16 (Siemen's Immulite, Los Angeles, CA). Both assays had an intra- and inter-assay coefficient of variation (CV's) of < 15.8%. Statistical analysis was performed on JMP® Pro 15 at $p < 0.05$ using Wilcoxon tests to compare differences between plasma PGEM in diestrus and pregnant cycles. Differences between days and times were compared individually by Student's *t*-test. One mare failed to become pregnant. Diestrous mares had higher ($p < 0.0001$) overall plasma PGEM concentrations from D0 to 20 compared to pregnant mares (mean ± SD) (30.7 ± 15 pg/ml and 17 ± 6 pg/ml, respectively) PGEM concentrations were also higher ($p < 0.0002$) in diestrous mares compared to pregnant mares for D13, 14, 15 and 16. The PGEM secretion profile was substantially different than that previously reported for PGFM. Pregnant mares had small peaks of PGEM that were different ($p < 0.05$) from diestrous mares on D13 and D14. This study is novel and demonstrated that plasma PGEM concentrations in diestrous mares are higher than in pregnant counterparts. However, a larger number of estrous cycles has to be studied to characterize the PGEM profile during early pregnancy. Further investigation of PGFM and PGEM in pregnancy is warranted to understand the importance of circulating concentrations and if the ratio or pattern of PGE:PGF may be altered during the expected period of luteolysis and maternal recognition of pregnancy.

Keywords: Equine, pregnancy, progesterone, prostaglandin

Cholesterol-loaded cyclodextrin improves cooling and fertility of donkey semen

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The donkey and mule show industry is an ever-growing industry. High-performance mules drive the stud fee values and increase their demand as a sire to breed mares and jennies. Cooled-shipped semen is the primary approach used by the industry. Skim milk-based (SKM) extenders are most used to cool and ship equid semen. However, donkey semen does not tolerate cooling with a SKM extender unless the seminal plasma is removed by centrifugation or 2% egg yolk is included as an additional cholesterol source. Neither approach is practical in ambulatory conditions; thus, alternatives must be identified. Inclusion of cholesterol-loaded cyclodextrin (CLC) in freezing extender improves post-thaw semen quality of donkeys; however, CLC has not been tested for cooling donkey semen. This study's objective was to compare semen parameters and fertility of cooled donkey semen extended in a commercially available SKM with and without CLC (SKM-CLC). We hypothesized that CLC enhances semen cooling and fertility of donkey semen. In the first experiment, 35 ejaculates from 7 mature jacks were split into SKM (BotuSemen, Botupharma) and SKM-CLC (BotuSpecial, Botupharma) groups and extended at 50 x 10^6 sperm/ml. After extension, samples were stored in a passive semen cooling container (BotuFlex, Botupharma) at 5 °C for 48 hours. Total motility (TM), progressive motility (PM), and percentage of sperm with rapid motility (RAP) were assessed with CASA (I.V.O.S. 12, Hamilton Thorne, Beverly, MA). Plasma membrane integrity (PMI), and mitochondrial membrane potential (MMP) were assessed with the combination of Yo-Pro® and MitoStatusRed with flow cytometry (LSR-Fortessa, Becton Dickinson, Mountain View, CA). Semen was assessed

before (time 0), 24, and 48 hours after cooling. In the second experiment, 2 estrous cycles of 15 mares were used for fertility assessment. Mares were examined every other day by transrectal ultrasonography (SonoScape A6®, China). Once a preovulatory follicle was detected (i.e. ≥ 35 mm in the presence of endometrial edema > 1, 0 absent and 3 max), ovulation was induced with 250 µg of histrelin acetate. At induction, semen from 1 jack was collected (n = 28), extended in either SKM or SKM-CLC, and cooled for 24 hours. Mares were randomly and equally assigned in a crossover for breeding with either extender 24 hours after induction of ovulation. Thereafter, mares were examined daily to detect intrauterine fluid accumulation and ovulation. Mares received oxytocin (20 units) to prevent intrauterine fluid accumulation. Pregnancy diagnosis was carried out on day 15 day after ovulation and mares received dinoprost (5 mg) intramuscularly to induce estrus. Data were analyzed with GraphPad Pris 8.0.1. (GraphPad, San Diego, CA). Semen parameters were analyzed with a Mixed model and Tukey's as posthoc. Pregnancy diagnosis was assessed with Fisher's Exact test. Significance was set at $p \leq 0.05$. There were no differences ($p > 0.05$) in TM, PM, RAP, PMI, and MMP for semen extended in either extender at time 0. There was a reduction in TM, PM, RAP, PMI, and MMP over time across groups; however, semen extended with SKM-CLC had superior ($p < 0.05$) TM, PM, RAP, PMI, and MMP than semen extended in SKM at 24 and 48 hours postcooling. Mares bred with semen extended in SKM had lower ($p < 0.05$) conception rate (13%, 2/15 cycles) than mares bred with SKM-CLC (47%, 7/15 cycles). Incorporating CLC to SKM extender improved semen parameters and fertility of cooled donkey semen

Keywords: Donkey, semen cryopreservation, extenders, chilling, cholesterol

Luteinizing hormone receptor activation stimulates endothelial adhesion of neoplastic canine T-lymphocytes

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Previous research from our laboratory has demonstrated that luteinizing hormone receptors (LHR) are expressed in neoplastic lymphocytes in canine lymph nodes and that activation of the LHR with human chorionic gonadotropin (hCG) increases LHR gene expression and cell proliferation in isolated neoplastic T-lymphocytes. The objective of the current study was to determine if hCG activation of LHR in neoplastic T-lymphocytes increase their adhesion to an endothelial cell monolayer. The hypothesis was that increasing hCG concentration induces a dose-dependent increase in neoplastic T-lymphocyte adhesion. Canine aortic endothelial cells (#Cn304-05, Cell Applications, Inc.) were cultured to form a monolayer. Endothelial cells were activated with tumor necrosis factor-alpha for 12 hours. Immortalized T-cell lines isolated from 3 dogs (CLC, EMA, CLK) with multi-centric lymphoma were cultured for 72 hours with increasing concentrations of hCG (from 4 - 4,000 IU/ml). Neoplastic T-lymphocytes were then fluorescently labeled (CytoSelect LeukoTracker, Cell Biolabs, Inc.) and added to the endothelial monolayer. After a 2-hour incubation, non-adherent cells were removed by washing. Images of adherent cells were digitally captured (#QIC-F-M-12-C, QImaging) at 400 x magnification using fluorescent microscopy (#DM4000B, Leica Microsystems). Adherent cells were then quantified on a fluorescence plate reader (Synergy 2, Biotek) using 50% gain. Four replicates of each T-cell line were used for each assay and the assays were repeated 3 times. Results (mean ± SEM) were expressed as a fold of baseline and compared between different hCG concentrations using a one-way analysis of variance (GraphPad Prism). Significance was defined as $p < 0.05$. Activation of LHR in neoplastic lymphocytes increased cell adhesion in a dose-dependent manner in all 3 cell lines (CLC: $p = 0.030$; EMA: $p = 0.016$; CLK: $p = 0.004$). Increases in hCG concentrations stimulated more neoplastic T-lymphocyte adhesion (Figure). This is the first study to demonstrate that activation of LHR in neoplastic canine lymphocytes increases endothelial cell adhesion. These results could explain why gonadectomized dogs with elevated circulating LH concentrations develop lymphoma at higher rates than intact dogs.

Keywords: Cancer, dog, human chorionic gonadotropin, lymphoma

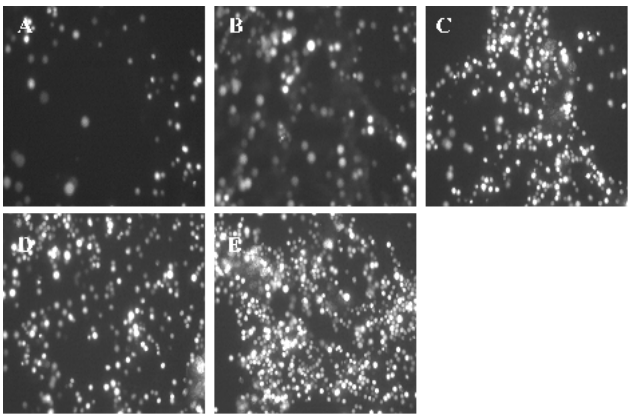

Figure. Neoplastic T-lymphocyte adhesion A: hCG 0 IU/ml; B: hCG 4 IU/ml; C: hCG 40 IU/ml; D: hCG 400 IU/ml; E: hCG 4000 IU/ml.

Characterization of reproductive parameters from Pennsylvania bull elk (*Cervus canadensis*)

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Pennsylvania (PA) elk (*Cervus canadensis*) are a population of free-ranging cervids that live in north central PA, and were initially reintroduced in 1913 as a measure to recover the elk population that was extirpated by uncontrolled hunting. Currently, the herd size of PA elk approaches ~ 1,400 individuals. During the 2019 calving season, a reduction in the number of calves was noticed. Gross anatomic inspection from the uteruses and ovaries harvested from selected cows revealed evidence suggestive of embryonic death whereas conventional sperm parameters (e.g. morphology, viability) analyzed from selected bulls were largely normal. This study was conducted to characterize certain sperm quality parameters from bulls (n = 24) harvested during the 2020 hunting season, to determine potential causes for reduced fertility. Testes and epididymides were harvested from hunted animals, immediately cooled at 5 °C and shipped from PA to Texas within 24 hours. Upon arrival, each testis and epididymis were isolated and weighed. Tissue wedges were obtained from testes and fixed in Davidson's fixative. Each caudal epididymis was flushed with 5 ml of INRA-96® extender and total sperm numbers (TSN; 10^9), total and progressive motility (TMOT, PMOT; %), curvilinear velocity (VCL; µm/s), plasma membrane/acrosomal intactness (VAI; %), DNA integrity ($COMP_{\alpha\text{-}t}$; %), and sperm morphology (Normal; %) were determined by CASA, flow cytometry and DIC microscopy, respectively. Overall, the sperm quality was consistent with parameters from other species (e.g. stallion, bull; Table). The % (mean ± SD) of morphologically normal sperm was low due to the incidence of proximal, distal droplets (13 ± 11, 43 ± 21; respectively), and bent midpieces (14 ± 11). Surprisingly, the % $COMP_{\alpha\text{-}t}$ was high (mean ± SD 45 ± 17; range: 3 - 71). Spearman's correlation analysis revealed no linear relationship ($p > 0.05$ [positive or negative]) among testes and epididymides sizes, sperm morphology, or $COMP_{\alpha\text{-}t}$ values. At this point, it can be speculated that the observed values of $COMP_{\alpha\text{-}t}$ (susceptibility of DNA to denaturation) in the wild PA elk population may partially explain the reduced fertility recently observed. Further studies are warranted to confirm this hypothesis and to determine a potential cause for such increased values of sperm DNA damage in the absence of other sperm quality abnormalities.

Keywords: Pennsylvania elk, *Cervus canadensis*, sperm quality, fertility, DNA integrity

Table. Sperm quality parameters from 24 PA elks obtained after epididymal harvest

Parameter	Testis	Epid.	TSN	TMOT	PMOT	VCL	VAI	$COMP_{\alpha\text{-}t}$	Nortmal
(Mean ± SD)	83 ±21	21 ± 5	0.6 ± 1	55 ± 20	34 ± 15	162 ± 51	87 ± 6	45 ± 17	34 ± 14

Expression and abundance of prostaglandins in healthy and fibrotic mare endometrium

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Inflammation and fibrosis of the endometrium is a major cause of infertility in the mare. A better understanding of the mechanisms of degenerative diseases of the endometrium can help to improve diagnosis and clinical outcomes in the mare. Prostaglandins have a wide range of roles including in tissue remodeling, inflammation, and fibrosis in the endometrium. We hypothesized that expression of prostaglandin-related genes in the endometrium and abundance of prostaglandins in low-volume uterine lavage is different in healthy mares compared to mares diagnosed with fibrosed endometrium. Our objective was to discern any changes in prostaglandin abundance and gene expression among healthy and fibrosed endometrium in order to better understand the mechanisms of endometrial inflammation and fibrosis. A total of 27 estrous mares were enrolled in this study. A uterine lavage using 250 ml of 0.9% NaCl solution was performed with a sterile bivona catheter, and the concentration of prostaglandins in the fluid was measured using enzyme-linked immunosorbent assays (ELISAs) for PGE_2 and $PGF_{2\alpha}$. An endometrial biopsy was collected and sectioned in half. One half of the endometrial biopsy was fixed in 10% formalin, sectioned, stained with hematoxylin & eosin and graded according to the Kenney-Doig system by a board-certified pathologist; the second half was snap frozen in liquid nitrogen and stored at -80 °C until analysis. Mares were assigned to either the healthy group (n = 15) if the endometrial biopsy score was I or IIA, or to the fibrosed endometrium group (n = 12) if the endometrium biopsy score was IIB or III. Total RNA was extracted from endometrial biopsies and real-time PCR was performed to evaluate the relative abundance of the following genes: PTGES, PTGS2, PTGFR, PTGER2, PTGER4, CBR1, and SLCO2A1. Mean threshold cycle (Cq) was determined and then normalized to the reference gene (GAPDH) (ΔCT). Statistical analyses were performed with JMP® software using $p < 0.05$. Data were

analyzed for normality and rank transformed when necessary. Student's *t*-test was used to compare differences between healthy and fibrosed endometrium groups. Healthy endometrium had higher expression of PTGES ($p < 0.03$) and SLCO2A1 ($p < 0.05$) compared to fibrosed ones. There was no difference ($p > 0.05$) in abundance of PGE_2 or $PGF_{2\alpha}$ in low-volume lavage between the 2 groups. In endometrial fibrosis, degenerative inflammatory conditions can increase cytokine production and disrupt normal cellular function that may impact pathways including prostaglandin synthesis and prostaglandin uptake/transport. SLCO2A1 is involved in the transport of PGE2 and $PGF_{2\alpha}$ that are present during pregnancy and luteolysis. A disruption of prostaglandin E-synthase could similarly affect prostaglandin concentrations, altering the PGE/PGF ratio and resulting in a deficient environment for development and maintenance of conceptus and corpus luteum.

Keywords: Endometrial biopsy, fibrosis, equine, prostaglandin

COMPETITION SESSION

Epididymal sperm granulomas are associated with antisperm antibodies in frozen-thawed donkey semen

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Prior to ejaculation, sperm is stored in the epididymis for a variable time; the blood-epididymis barrier regulates exchanges of nourishment and hormones while maintaining sperm isolation. The innate and adaptive immune system intervenes when sperm is extravasated in the interstitium. Although several conditions are known to affect the integrity of the blood-epididymis barrier (i.e. trauma, toxicants, parasites, and infections) in domesticated mammals, epididymal sperm granulomas and antisperm antibodies are a rare finding and have never been described in donkeys. This study aimed to describe and compare semen parameters (pre- and post-freezing) and antisperm antibodies of donkeys with epididymal sperm granuloma (Granuloma) and healthy controls (Control). Feral donkeys (n = 10) castrated in a concurrent study were enrolled. Three donkeys had unilateral granulomas, 2 donkeys had bilateral granulomas, whereas the remaining 5 were grossly normal. The granulomas were either single or multiple, firm, well-circumscribed, tan to red, and 1 - 5 mm in size. Upon incision, abundant, thick, tan to white-yellow fluid was recovered. Histopathology revealed epithelioid macrophages, multinucleated giant cells, and abundant sperm cell fragments with mineralized cellular debris. Semen was harvested for cryopreservation through retrograde flushing of the cauda epididymis. Sperm concentration and motility parameters (total motility, TM; progressive motility, PM) were assessed with an automated sperm analyzer; plasma membrane integrity (PMI), and mitochondrial membrane potential (HMMP) were assessed with flow cytometry pre- and post-freezing. Postfreezing semen was assessed through flow cytometry for the presence of antisperm antibodies (IgG and IgA). Statistical analysis was performed with the Wilcoxon matched-pairs signed-rank test. Significance was set at $p < 0.05$. The total sperm yield did not differ ($p > 0.05$) between groups (Control 11.0 ± 2.0, Granuloma 9.0 ± 0.4 x 10^9). TM did not change ($p > 0.05$) after freezing in the Granuloma group (TM prefreezing 29 ± 6%, postfreezing 18 ± 3%). After freezing, PM and PMI of donkeys with sperm granuloma were lower ($p < 0.05$) than healthy ones (PM Control 15 ± 2%, Granuloma 7 ± 2%; PMI Control 51 ± 4%, Granuloma 36 ±5 %). Pre- and post-freezing HMMP did not differ ($p > 0.05$) among groups. Three of the 5 donkeys with granuloma had a percentage of IgG- and IgA-bound sperm above the maximum value observed in control donkeys. Mean percentage of IgG- and IgA-bound sperm did not differ ($p > 0.05$) among groups (IgG-bound Control 2 ± 0.4%, Granuloma 16 ± 10%; IgA-bound Control 0.1 ± 0.1%, Granuloma 0.5 ± 0.4%). In conclusion, sperm granulomas only marginally affected sperm quality and resulted in IgG and IgA antisperm antibodies binding to sperm. It remains to be determined if sperm granuloma and antisperm antibodies affect fertility in donkeys.

Keywords: Antisperm antibodies, epididymis, blood-epididymis barrier, epididymitis

Laser ablation of the equine oviductal papilla as a novel contraceptive technique

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Reproductive control of wild horse populations has frustrated the bureau of land management for years. GnRH and Zona Pellucida vaccines have a limited duration of efficacy, intrauterine devices are unpredictably retained, and attempts to ovariectomize mares were met with public outcry in 2018. The development of a nonsurgical permanent sterilization technique has the potential to revolutionize management of wild horses and burros on public lands. The objectives of this study were to develop a safe and efficient sterilization technique, and to demonstrate technique effectiveness in reproductively healthy mares. We hypothesized that laser ablation of the oviductal papillae would be an effective method of permanent sterilization. Seven light breed reproductively healthy mares (5 - 21 years) were enrolled in the study after pregnancy confirmation at 14 days. Mares were given prostaglandin to induce abortion and within 14 days were sedated with xylazine hydrochloride for hysteroscopy. Examination of the endometrium and oviductal papillae was accomplished using a 103 cm flexible endoscope (Olympus GIF-160 Gastroscope, Center Valley, PA) attached to an Olympus EVIS EXERA CV-160 video processor. The endoscope

was guided through the cervix and the uterus insufflated with room air. In 5 mares, a 600 µm laser fiber was advanced through the biopsy channel, and using a diode laser (Dornier Medilas D, Dornier MedTech America, Inc., Kenneasaw, GA), set at 20 W, 2 - 6 direct contact pulses of 3 - 5 seconds in duration were delivered at the oviductal papilla. The endoscope was then guided up the contralateral uterine horn and the process repeated. Total energy delivered ranged from 700 to 1500 J. In 2 control mares, the oviductal papillae were visualized, but no laser ablation was performed. All mares received 5 mg dinoprost IM, and a uterine lavage was performed using 1 - 3 liters of lactated ringer's solution within 4 hours postprocedure. Transrectal ultrasonography was performed every 2 - 3 days until a 35 mm follicle was detected, and then mares were bred using a semen sample from a fertile stallion with a minimum of 500 x 10^9 progressively motile sperm. Mares were bred every 48 hours until ovulation, and pregnancy status was determined by transrectal ultrasonography 14 days postovulation. After examination, mares were treated intramuscularly with 5 mg of dinoprost to induce luteolysis and were rebred on 2 - 4 consecutive estrous cycles. Control mares conceived on 6 out of 9 cycles (67% pregnancy rate). Pregnancy rate was lower ($p = 0.003$) in the treatment group (5%, 1 out of 20 estrous cycles). The first mare that was laser ablated conceived on the 4th cycle, and repeat hysteroscopy determined that the left oviduct was not effectively ablated. The procedure was repeated, and the mare reenrolled in the breeding trial. The third mare enrolled in the study developed fever and tachycardia 6 hours after hysteroscopy and was diagnosed with peritonitis via abdominocentesis. The mare was treated with broad spectrum antibiotics and recovered uneventfully. The mare that developed postprocedure complications was believed to be due to equipment difficulties early in the development of the procedure, and the 3 final mares included in the trial had no inflammation on peritoneal fluid evaluation after oviductal ablation, and no change in physical exam parameters. In conclusion, when the laser ablation of the oviductal papillae was appropriately performed, scar tissue formation effectively prevented pregnancy for a minimum of 4 months postprocedure. Laser ablation of the oviductal papillae is a promising technique for permanent sterilization of the mare and a follow-up long-term fertility study is warranted.

Keywords: Antisperm antibodies, epididymis, blood-epididymis barrier, epididymitis

Mammary gland electrolytes and pH to detect impending parturition in jennies

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Assisted foaling is ideal for maximum foal survival, as it allows for early intervention to cope with simple dystocia or referral to appropriate tertiary veterinary care facilities. Unfortunately, most jennies foal unattended, and a high incidence of donkey foal mortality is a common problem in the breeding industry. The prediction of parturition is critical to ensure assisted delivery. Because equids have a prolonged pregnancy and foal during the night, continuous foaling monitoring is not feasible on small farms. Thus, serial assessment of mammary gland electrolytes and pH are used to circumvent this issue to detect impending parturition in mares but not in jennies. Major objective was to determine the usefulness of serial assessment of mammary gland electrolytes and correspondent pH to detect impending parturition in jennies. In addition, the relationships between maternal, fetal membranes, and foal birth weight were investigated. We hypothesized that serial assessment of mammary gland pH predicts foaling in jennies. Multiparous jennies (n = 37) were monitored daily starting from 350 to 355 days of pregnancy until foaling. The pH of mammary gland se-cretions was assessed daily with a hand-held device (LAQUA Twin pH Meter, Horiba, Irvine, CA). Aliquots of mammary secretions were frozen daily and then assessed retrospectively for electrolyte concentrations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) (Beckman Coulter, Switzerland) starting 5 days before foaling. Electrolyte concentrations and pH values were analyzed with a mixed-effect model. Student's t-test was performed to compare dependent variables (pregnancy length, birth weight, placental weight, and umbilical cord length) according to foal sex as an independent variable. Sensitivity, specificity, and negative predictive value (NPV) and positive predictive values (PPV) were evaluated using a cut-off value for pH ≤ 6.4 and Ca^{2+} >10 mmol/l. Most foalings (91.9%) were during the night. The overall pregnancy length was 374 ± 8.7 days (range 357 - 390 days). There were no differences ($p > 0.05$) in pregnacy length for colts (374 ± 2.1 range 357-385 days) and fillies (373 ± 2.3 range 358 - 390 days). Colts and fillies were 61.8 and 38.2%, respectively. Fetal membranes weighed 3.4 ± 0.1 kg (range 1.9 - 4.7 kg). Foals at birth weighed 31.1 ± 2.5 kg (range 26.5 - 37.5 kg), with no differences ($p > 0.05$) in birth weights for colts (31.1 ± 2; range 26.5 - 37.5 kg) and fillies (30.8 ± 2.2; range 26.5 - 34 kg). The ratio of foal birth weight with the dam's bodyweight was 9.7%, and the ratio with fetal membranes was 11%. There was a significant reduction in Na^+ and an increase in Ca^{2+}, Mg^{2+}, and K^+ concentrations leading to foaling. The pH of mammary secretions glands decreased during the 5 days preceding parturition. Additionally, 2 distinct profiles for pH reduction were recorded, with 32% of the jennies displaying a fast reduction in pH values (profile 1) and 65% presenting a slow reduction in pH (profile 2) from the mammary gland secretions; 3% foaled with high and alkaline pH (pH = 7.5). The pH had a 90% sensitivity for foaling within 24 hours, whereas the specificity was 70%, and the PPV and NPV values were 40 and 97%, respectively. Of interest, Ca^{2+} (> 10 mmol/l) had a sensitivity and specificity of 71 and 85%, respectively, whereas the PPV and NPV were 72 and 84%, respectively. In conclusion, daily measurements of the pH of mammary gland secretion can predict foaling in jennies, whereas Ca^{2+} is a useful marker to determine when parturition will not occur. Therefore, Ca^{2+} needs to be associated

with pH to predict donkey parturition. Jennies had a high placental efficiency, as demonstrated by the high placental, dam, and foal ratios.

Keywords: Predicting parturition, foaling, periparturition, donkey, pH, electrolytes

Clinical and physiological ultrasonography of normal and abnormal donkey pregnancies

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Ultrasonography of the fetoplacental unit is carried out to detect abnormalities and to stage pregnancy. Transrectal and transabdominal ultrasonography have well-established physiological parameters and clinical applications in horses, but these techniques have not been characterized or established in donkeys. Season and sex of the foal are known to affect pregnancy length in horses but their effects are not known in donkeys. Pregnancy loss in horses is associated with abnormal progestin profiles but is not studied in donkeys. Major objective was to establish clinical and physiological ultrasonographic parameters of jennies carrying and delivering normal pregnancies and jennies undergoing premature delivery of stillborn foals. Additionally, effects of season and sex of the foal on gestational length (GL) were assessed. We hypothesized that season and sex of the foal affect GL, and pregnancy loss results in abnormal ultrasonography parameters and progestin profiles. Multiparous jennies (n = 140) ranging 4 - 16 years in age were enrolled by 120 days of pregnancy. Jennies were artificially inseminated with fresh semen during the spring, summer, and fall, in a single calendar year, all on 1 farm. All jennies were submitted to transrectal ultrasonography (Well. D, Medical Electronics Co., Shenzhen, China) coupled with a 7.5 MHz linear transducer at 15 day intervals until delivery. A subset of jennies (n = 50) had transabdominal ultrasonography (Well. D, Medical Electronics Co.) coupled with a 3.5 MHz sectorial convex transducer, also performed at 15 day intervals until delivery. Parameters assessed during each evaluation included combined thickness of uterus and placenta (CTUP) and fetal parameters (eyeball diameter, thorax, heartbeat, and aortic diameter). Serum samples were collected from each jenny during each evaluation for the determination of progesterone concentrations by RIA. Foals were weighed after birth. Data were assessed for normality with Shapiro-Wilk's test, and then ANOVA and Tukey's (aortic diameter, heartbeat, and thorax) or Kruskal-Wallis followed by Dunn's (eyeball and CTUP). Mixed models were used to assess the effects of season and interactions with foal sex and GL. Statistical significance was set at $p < 0.05$. The incidence of late pregnancy loss was 3.5% (5/140 jennies). The GL was 365.4 ± 10.4 days (range; 345 - 390 days) for jennies carrying and delivering normal pregnancies and was 345 ± 32.3 days (range; 290 - 352 days) for the group experiencing pregnancy loss. Spring bred jennies had the longest ($p < 0.05$) GL (375 ± 8.7 days), followed by summer bred (360 ± 32.3 days) and then fall bred (358.6 ± 5.8 days). Colts had longer GL than fillies (363 ± 10.2 versus 358.5 ± 9.3 days). There was no effect of GL on the foal's birth weight. There were significant associations between GL with eye orbit diameter (r = 0.70), fetal thorax (r = 0.80), fetal aortic diameter (0.60) and CTUP (r = 0.60). Fetal heartbeat (r = -0.9) was negatively correlated with GL. CTUP significantly increased from 150 days of pregnancy to term. Two jennies with premature deliveries had CTUP outside normal ranges and placental separation consistent with ascending placentitis; before abortion, these jennies also had an increase in progesterone concentrations in comparison to other jennies. The remaining 3 jennies undergoing premature delivery did not experience these changes. In conclusion, the study established clinical, physiological, and ultrasonographic parameters for donkey pregnancy. The incidence of late pregnancy loss was 3.5%. Spring-bred had the longest GL in jennies and colt-bearing pregnancies resulted in the longer GL than fillies; 40% of the abnormal pregnancies had abnormal CTUP, placental separation, and abnormal progesterone profiles.

Keywords: Fetoplacental unit ultrasonography, pregnancy loss, CTUP

No adverse effect of air exposure on stallion sperm motility after 48 hours of cooled storage

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Stallion semen may be collected, extended, and cooled for up to 48 hours prior to insemination. It is common practice to remove all the air from the package of extended semen prior to cooled storage. The aim of this pilot study was to assess the effects of air exposure on sperm motility parameters during 48 hours of cooled storage. We hypothesized that air exposure is associated with decreased sperm motility after 48 hours of cooled storage. A total of 12 ejaculates were collected (4 ejaculates from each of 3 stallions) using an artificial vagina. Semen was evaluated and diluted with a commercial extender (INRA 96, IMV Technologies, Maple Grove, MN) to a concentration of 25×10^6 progressively motile sperm per ml. The extended semen was aliquoted into 3 treatment groups. Group A: 40 ml of extended semen was placed into a 50-ml all-plastic syringe with all air removed (Henke-Ject®, Air Tite-Products Co., Inc., Virginia Beach, VA). Group B: 20 ml of extended semen was placed into a 50-ml syringe with all air removed. Group C: 20 ml of extended semen was placed into a 50-ml syringe along with 20 ml of air. The loaded syringes were placed into passive cooling containers (Equine Express II™ Cooled Semen Shipper™ boxes, Nasco, Fort Atkinson, WI) along with a frozen ice pack (PolarPack®, Sonoco, Hayward, CA). An aliquot (1 ml) of

semen was removed after 24 and 48 hours of cooled storage and warmed for 10 minutes at 37°C prior to evaluation of sperm motility parameters using a computer assisted sperm analysis unit (SpermVision®, Minitube of America, Inc., Verona, WI). Data are presented as a mean ± SD. A mixed model was fit to each response variable separately (SAS Institute, Carey, NC). Treatment (40 ml, 20 ml, or 20 ml plus air) was included as a fixed effect. Sample ID was included as a random effect to account for repeat observations on each sample. Tukey adjusted pairwise comparisons were also performed. Data were considered different at $p < 0.05$. Total sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 71.9 ± 14.3, 73.3 ± 13.3 and 76.3 ± 12.5 %, respectively. Total sperm motility values after 48 hours of cooled storage for groups A, B, and C were 65.6 ± 14.1, 65.8 ± 17.3 and 70.9 ± 12.8%, respectively. Progressive sperm motility values for Groups A, B, and C after 24 hours of cooled storage were 66.7 ± 14.9, 67.8 ± 14.4 and 71.7 ± 14.3%, respectively. Finally, progressive sperm motility values for Groups A, B, and C after 48 hours of cooled storage were 60.3 ± 13.6, 60.8 ± 17.8 and 65.7 ± 14.3%, respectively. A difference ($p < 0.05$) in total and progressive motility was detected between Group A and Group C after 24 hours of cooled storage. There were no differences ($p > 0.05$) in total or progressive sperm motility values between aliquots of extended stallion semen in the presence or absence of air after 48 hours of cooled storage. These pilot data suggest that the necessity of removing all air during preparation of a cooled semen dose may not be as absolute as previously considered.

Keywords: Equine, cooled semen, air, sperm, motility

Comparison of nanoparticles and single-layer centrifugation for separation of dead from live stallion sperm

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Artificial insemination with fresh, cooled or frozen semen is commonly used in the equine breeding industry. Poor quality semen with a reduced number of live, motile sperm can lead to lower per cycle pregnancy rates. A study using boar sperm showed improved sperm motility when nanoparticles were used to separate dead from live sperm.[1] The objective was to determine if nanoparticles could separate dead from live stallion sperm. Our hypothesis was that iron-core nanoparticles bind to dead sperm and allow for subsequent separation from live sperm using a magnet. Experiment 1 compared 2 extenders (INRA 96 and TALP-E), 2 incubation temperatures (22 and 37°C) and 6 nanoparticle:sperm ratios (50, 100, 200, 400, 600, and 800 µl of nanoparticle working solution per 100 x 10^6 sperm) using magnetic nanoparticles (ST Genetics, Navasota, TX, US). A research model to mimic a poor-quality ejaculate was made by killing 50% of the sperm by submersion into liquid nitrogen. Experiment 2 compared sperm separation using single-layer centrifugation (SLC) with EquiPure™ (Nidacon International AB, Mölndal, Sweden) versus nanoparticle separation. In both experiments, total and progressive sperm motility, morphology, viability and acrosome status were evaluated. Statistical analysis was performed using one-way ANOVA (data presented as mean ± SD). Values were considered different at $p < 0.05$. Results of Experiment 1: Total and progressive sperm motility were not different between INRA 96 and TALP-E extenders or when incubated at either 22 or 37°C or when using 400 or 600 µl of nanoparticle solution per 100 x 10^9 sperm. Results for Experiment 2: Progressive sperm motility was higher ($p < 0.05$) after SLC (76 ± 9%) than after either nanoparticle treatment (59 ± 12%) or an untreated control (47 ± 5%). In addition, the percentage of viable and acrosome intact sperm was higher after SLC (61 ± 11%) than after nanoparticle treatment (43 ± 3%) or an untreated control (35 ± 3%). There was no statistical difference in sperm morphology among groups. In summary, under the current study conditions based on an induced sperm damage model, single-layer centrifugation performed better than nanoparticles for separating dead from live stallion sperm.

Keywords: Stallion, sperm, nanoparticles, single-layer centrifugation

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Comparing serum progesterone measurements by a point-of-care analyzer with a chemiluminescent immunoassay in bitch breeding management of the bitch

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Accurate serum progesterone measurements for timing bitches during breeding management is critical for reproductive practice. By monitoring the rise in progesterone during estrus, it is possible to predict the date of ovulation and the peak window of fertility, which is especially important as artificial insemination has become routine to facilitate breeding of animals that are geographically or temporally separated. Although progesterone is a highly conserved molecule across species, laboratory methods for measuring serum progesterone concentrations in the dog vary in accuracy and precision. To measure serum progesterone, chemiluminescent immunoassay (CLIA) has replaced radioimmunoassay as the current standard in the bitch, due to its high correlation and increased practicality. In January 2019, a colorimetric point-of-care (POC) immunoassay was released as an in-clinic diagnostic for quantitative canine

serum progesterone measurements in less than 30 minutes. This study provides an independent comparison of the POC (Catalyst One, IDEXX) to the current industry standard, CLIA (Immulite-2000, Siemens), used by most veterinary reference laboratories. To assess inter-assay imprecision of POC and agreement of the POC and CLIA results, 100 canine serum samples were analyzed on 3 analyzers (POC-1, POC-2, and CLIA), of which, 74 (POC-1) and 75 (POC-2) results were within POCs' reportable range of 0.2-20 ng/mL and included in the study. To assess intra-assay imprecision, pooled canine serum samples at low (L1), intermediate (L2), and high (L3) progesterone concentrations were analyzed 10 times each on POC-1 and CLIA. Relative to CLIA, POC values had good correlation (POC-1, r = 0.9366; POC-2, r = 0.9438, $p < 0.0001$) and significant positive proportional bias at values > 2 ng/ml. The POC inter-assay coefficients of variation (CVs) were 13.2% (0.2-2.9 ng/ml, 0.6-9.2 nmol/l, L1), 10.0% (3.0-9.9 ng/ml, 9.5-31.5 nmol/l, L2), 7.1% (10.0-20.0 ng/ml, 31.8-63.6 nmol/l, L3), and 11.2% (all samples). The intra-assay CVs for POC (L1, 15.3%; L2, 7.0%; L3, 4.7%) were higher than those for CLIA (L1, 5.89%; L2, 4.89%; L3, 3.44%). The POC had a more rapid increase in serial serum progesterone concentrations in ovulating bitches and had greater imprecision than CLIA. Therefore, caution should be used when interpreting the clinical significance of serum progesterone measurements by the POC as they relate to canine breeding management.

Keywords: Dog, commercial assays, catalyst, accuracy, imprecision

Kisspeptin-10 on in vitro migration of equine chorionic girdle trophoblast cells

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Chorionic girdle (CG) is a specialized component of the equine extraembryonic membranes, composed of rapidly proliferating uninucleated and terminally differentiated binucleated trophoblast cells (uTCs and bTCs, respectively). Gonadotropin-secreting bTCs invade the maternal endometrium to form key structures for pregnancy maintenance known as endometrial cups. Mechanisms that regulate bTC migration and invasion remain elusive. Kisspeptins (Kps), a family of small peptides with 10 (Kp-10) to 54 (Kp-54) amino acids, are highly expressed at the maternal-fetal interface during human and rodent placentation and may inhibit excessive TC invasion. Hence, we aimed to investigate the effect of the equine Kp decapeptide (eKp-10) on CG cell in vitro migration using gap closure and vesicle expansion assays. It was hypothesized that eKp-10 inhibit CG vesicle expansion and the closure of uTC/bTC monolayer gaps. Chorionic girdle was isolated from embryos collected transcervically at 33 - 34 days postovulation (n = 5 mares). Following mechanical dissociation, CG cells were cultured at 37°C in 8% CO2 on serum-supplemented (SM) or serum-free (SFM) medium containing Dulbecco's-modified Eagle's medium. Approximately 500 µm cell-free gaps were formed using silicone inserts (Ibidi®). Once ~ 90% confluency was achieved on both sides of the gap, cells were treated with 0 (control), 1, 10, and 100 µM of eKp-10 and photomicrographs were taken at 0, 6, 12, and 24 hours (n = 8 wells/group) with a Nikon inverted microscope. Concurrently, individualized CG vesicles were transferred from SFM to SM and treated with 0, 0.1, 10, and 100 µM of eKp-10 (n = 20 vesicles/group). Photomicrographs were taken at 0, 12, 24, 36, and 48 hours. Gap widths and vesicle areas were measured by observers blinded to the experimental design using Image J®. Data were assessed via mixed ANOVA and post-hoc Tukey's tests. Significance was set at $p < 0.05$ (JMP Pro 15). Gap closure was slower ($p = 0.04$) in wells treated with 100 µM of eKp-10 compared to control, whereas there was no difference ($p > 0.05$) in closure among control and groups treated with 1 µM or 10 µM of eKp-10). Vesicle expansion occurred in all treatment groups and there was an interaction ($p = 0.0008$) between time and treatment. Within each time point, compared to control, vesicle expansion rate was lower in 0.1 ($p = 0.002$) and 10 µM eKp-10 ($p = 0.03$) at 24 hours, and was also lower ($p = 0.007$) at 36 hours in 0.1 µM and eKp-10. Interestingly, compared to control, expansion rate was higher ($p = 0.04$) in groups treated with 100 µM eKp-10 at 48 hours. Therefore, eKp-10 may affect the migration of subpopulations of CG cells dynamically and in a concentration- and time-dependent manner. Further investigations of Kp expression in the equine maternal-fetal interface and potential role in endometrial cup formation are needed.

Keywords: Mare, endometrial cups, binucleated, trophoblast cells, invasion.

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STUDENT CLINICAL CASE SESSION

Electroejaculation and breeding soundness examination on a clouded leopard

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In previous studies with clouded leopard electroejaculation, a variety of techniques have been performed, from varying probe sizes to varying stimulation procedures.[1-5] In 2018, the Smithsonian Conservative Biology Institute released a felid semen collection, evaluation, and freezing procedure that can be used in clouded leopards. This procedure uses a 1.6 or a 1.9 cm probe.[1] However, in this case, we used a 10 - 12 cm probe with 2 electrodes. This probe is commonly used in small ruminants, and therefore, more widely available to veterinarians than a probe specifically used for wild felids. The purpose of using the Pulsator IV electro-ejaculator with a ram probe was to determine if a more widely available probe could be used to collect semen from a clouded leopard or other species of similar anatomic size. A 3-year-old, intact male clouded leopard (*Neofelis nebulosa*) from the Nashville Zoo was presented to the University of Tennessee College of Veterinary Medicine Theriogenology service for a breeding soundness examination. On physical and reproductive ultrasonographic examinations, the clouded leopard appeared healthy with no physical abnormalities aside from an abnormally small left testis. Semen was collected with the Pulsator IV electro-ejaculator with a ram probe which is commonly used to collect semen in small ruminants. Approximately 0.2 - 0.3 ml of semen was collected using said device during this procedure. Collected semen was then examined for motility and morphology to determine the viability of the leopard's semen. Semen was determined to have 40% motility and 66% normal morphology. Thus, the breeding soundness exam concluded this leopard had viable semen and a good chance to impregnate a female, and that the Pulsator IV electro-ejaculator with a ram probe can be used to collect semen sample for evaluation in clouded leopards.

Keywords: Electroejaculator, ram probe, clouded leopard

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Preputial prolapse and injury in a brahman bull

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A 3-year-old brahman, bull presented with preputial prolapse secondary to multiple preputial injuries. Extensive swelling and edema engorged the prepuce in its entirety, with necrotic debris present along the ventral and left lateral aspects. The necrotic tissue was debrided and the prepuce was scrubbed with betadine before placing a pessary tube. Medical grade honey and petercillin were applied to the edematous preputial tissue with appropriate bandaging to hold the tube in place and promote reduction of edema. A support sling was applied, along with daily osmotic and hydrotherapy for prolapse reduction. Numerous tissue defects were allowed to heal by second intention before a reefing procedure was performed. *Bos indicus* bulls have a pendulous sheath, redundant preputial tissue, enlarged preputial orifices, and lack retractor prepuce muscles in homozygous polled bovids, predisposing them to preputial prolapse and injury.[1,2] Most preputial injuries occur at the ejaculatory lunge when epithelial and underlying elastic tissues of the bunched prepuce are damaged due to compressive forces between the bull's abdomen and the female's pelvis.[1] These tissue disruptions lead to edema, inflammation, further prolapse of preputial tissues, subsequent injury and fibrotic scar formation upon healing.[1,2] Whereas medical management is sufficient for returning tissues to the preputial cavity, surgical intervention is paramount for returning to breeding function.[1,4] A reefing (circumcision) procedure facilitates full extension of the penis and

prepuce to allow excision of ancillary and scarred preputial tissue,[3,4] ensuring 1.5 times the length of the free portion of the penis of preputial tissue is left to reduce the risk of phimosis and recurrent prolapse.[1,2] Prognosis following recovery from this procedure is good to excellent provided hemorrhage is minimized, urine flow is diverted away from the surgical sites, and extension of the penis remains achievable.[3,4]

Keywords: Prepuce, prolapse, injury, *Bos indicus*, reefing

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Atypical mammary mass in an intact geriatric female Labrador retriever

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Mammary pathology is a significant finding in any bitch. Published research suggests intact bitches are at a greater risk of developing mammary neoplasia than those ovariectomized before their first heat cycle.[1] A 12-year-old intact female black Labrador Retriever was examined in February 2021 for a rapidly growing mammary mass. The mammary mass was first noticed by the owner in July 2020 and grew slowly until December when it began to rapidly increase in size following standing estrus in late November. At presentation, a fluctuant mass (15 cm) extended from the cranial left abdominal mammary gland to the caudal abdominal mammary gland. The left inguinal lymph node was also enlarged and painful during palpation. All other lymph nodes palpated normally. The patient was over-conditioned, had age-related dental attrition, bilateral nuclear sclerosis, and a soft, free-moving subcutaneous mass medial to the right cranial abdominal mammary gland. All other physical exam findings were within normal limits. An ultrasonographic exam was performed on the mammary mass that revealed an accumulation of hypoechoic, heterogenic fluid with tags of tissue extending from the margins of the mass into the fluid filled center. Hair over the mass was clipped and skin was prepared for a fine needle aspirate which retrieved a hemopurulent, thin and nonfetid fluid. Few epithelial cells and red blood cells, moderate neutrophils, and no bacteria were evident on cytology. An aerobic bacterial culture of the fluid yielded no growth after 4 days. The mass was diagnosed as a sterile intramammary abscess. The patient was sedated with IV fentanyl and medetomidine then standard surgical preparation and anesthetic monitoring were used throughout the procedure. Stab incisions were made on the cranioventral and caudoventral aspects of the abscess. Compression was applied on the skin over the abscess to facilitate drainage. A gloved finger was used to digitally probe the abscess and debride tissue within the cavity. The cavity was lavaged repeatedly with dilute chlorhexidine solution and 2 Penrose drains were placed. The patient was reversed with intramuscular atipamezole. Recovery was uneventful and the patient was discharged on oral trimethoprim sulfa (960 mg twice daily for 2 weeks) and oral meloxicam (once daily for 5 days). The patient removed 1 drain tube 5 days after the procedure. Reevaluation was performed at 7 and 14 days with normal healing and minimal discharge. The second drain tube was removed 14 days after the procedure. At this abstract submission, the patient has not had recurrence of the mammary swelling. The cause of this sterile abscess is not known. Why the sterile intramammary abscess increased in size exponentially during diestrus is also unknown, though it may have to do with increased blood supply to the mammary glands during this stage of the estrous cycle. Due to financial constraints, concurrent mammary pathology could not be ruled-out histologically. Although the draining lymph node was enlarged, the pain and enlargement were likely due to reactivity from the abscess rather than neoplasia. Additional diagnostics were not performed. In addition, thoracic radiographs were not performed. This case illustrated that not all mammary masses are neoplastic in geriatric intact bitches.

Keywords: Canine, intramammary, sterile abscess, diestrus, geriatric

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Abnormal mobility in neonatal Labrador Retrievers

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A litter of 3-week-old Labrador Retrievers (n = 5) were evaluated for abnormal ambulation that was noted in 1 puppy. Upon presentation all puppies were evaluated for mobility on a blanket to improve traction. It was determined that the puppy in question had abnormal movement of the rear limbs compared to the remainder of the litter. In addition to this finding, a second puppy was determined to have an abnormally flat chest with decreased ambulation compared to littermates. The owner provided a daily weight chart and records of developmental milestones, such as opening eyes and first steps. Upon review

of this information, it was determined that the average daily gain of this litter was 3.37 ounces (95.5 grams) per day and that the 2 puppies exhibiting abnormalities had indeed met all developmental milestones within the same timeframe as littermates. Despite receiving no nutrition beyond milk supplied by the dam, all 5 puppies were considered obese. Of note, milk supply was plentiful, presumably due to the relatively small litter size. Prior to pursuing further diagnostics, the owner elected to complete at-home physical therapy and dietary management for both affected puppies and monitor for improvement. Physical therapy consisted of passive range of motion of both hind limbs and climbing over rolled towels for 15 minutes twice daily. After 1 week of physical therapy and dietary restriction, both puppies had improved overall mobility consistent with littermates and the puppy with altered hindlimb gait had a more consistent pattern of normal ambulation. Physical therapy was performed until 6 weeks of age with continued improvement of ambulation. At 13 weeks of age, both affected puppies successfully entered training to become working dogs. This case illustrated the importance of monitoring puppy weights and the detection of early abnormalities to prepare the animal for the best outcome

Keywords: Neonatal, canine, conformation, working dog

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Recurrent uterine torsion in an Arabian mare

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An 11-year-old multiparous 270 days pregnant Arabian mare was referred for a suspected uterine torsion. On arrival the mare was colicky, tachycardic, and normothermic. A 270 degree clockwise uterine torsion was diagnosed by transrectal palpation. She was anesthetized using a triple drip regimen and rolled 5 times using the flank plank technique. The mare recovered uneventfully. Two days after uterine torsion resolution the CTUP (6 mm) was within normal limits. The fetus was alive, and the mare was discharged. Ten days later, the mare returned with another colic episode. She was examined and a 90° clockwise torsion was identified. The mare was rolled again and recovered uneventfully. One day after resolution of the torsion, serum progesterone concentrations were normal (9.88 ng/ml) but serum estrogen concentrations (765.12 pg/ml) were lower than expected at 280 days of pregnancy. There was a concern about this as estrogens are a good indicator of fetal viability and tend to decline over the last trimester of pregnancy.[1] The mare delivered a healthy colt at home at 344 days. Uterine torsion in the mare is most common in mid to late pregnancy. Suspected causes include vigorous fetal movement, rolling, a large fetus in a relatively small volume of fetal fluid, lack of uterine tone, and a deep abdomen in larger breeds.[2] Transrectal palpation is the best way to diagnose uterine torsion and identify its direction that is essential for treatment. Repeated uterine torsions during the same pregnancy are rare. This case is important to the field of Theriogenology because uterine torsion in the mare is an emergency and presents with nonspecific clinical signs. Client education regarding the urgency of colic signs or discomfort in late pregnancy is essential. Early diagnosis and treatment improve outcome for the mare and foal. Close monitoring until foaling should be recommended.

Keywords: High risk, plank flank technique, endocrinology, colic

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Dystocia and diaphragmatic hernia in a Quarter Horse mare carrying twins

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Twin pregnancy is one of the major noninfectious causes of abortion and may jeopardize the health and welfare of the mare. A 16-year-old primiparous Quarter Horse mare was referred for dystocia due to abortion at ~ 10 months of pregnancy. She was depressed, tachycardic, tachypneic, and hypothermic. Rectum and vulva were edematous and malodorous; fetal membranes were protruding from the vulva. Due to signs of septic shock, she was treated with intravenous fluid and corticosteroids prior to attempting controlled vaginal delivery. The fetus was emphysematous and in anterior presentation with bilateral shoulder flexion and lateral deviation of the neck. Due to poor prognosis, humane euthanasia of the mare was performed. On necropsy, the uterus was friable and edematous with a partial thickness tear. Dysmature, autolyzed twins were removed from the uterus. An acute diaphragmatic hernia of the large colon was also diagnosed, which may have been a complication of straining efforts and the enlarged uterus. Incidence of double ovulation ranges from 8 - 21% in Quarter Horses, with an incidence of twin pregnancy ranging from 8 - 11%.[1] Spontaneous twin reduction occurs frequently by day 40 of pregnancy in

unilaterally fixed embryos; probability of twin maintenance increases substantially with bilateral fixation.[2] The standard practice is to attempt to reduce the pregnancy to 1 vesicle after pregnancy diagnosis at days 14 - 15, prior to embryonic fixation. In this case, pregnancy diagnosis was not performed after natural breeding. The prolonged duration of the dystocia and autolysis of the fetuses resulted in severe deterioration of the mare's health. The majority (64.5%) of twin pregnancies maintained past 42 days result in late term abortion.[3] This case illustrated the importance of close monitoring of follicular dynamics during breeding, early pregnancy diagnosis, and the need for client education on the importance of adequate breeding management for the welfare of the mare.

Keywords: Twins, abortion, dystocia, diaphragmatic hernia, breeding management

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Vulvar discharge associated with exogenous estrogen exposure in a spayed Weimaraner bitch

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Vulvar discharge in spayed bitches is often associated with infections, chemical irritation, foreign bodies, anatomical defects, neoplasia, or an ovarian remnant.[1] Rarely, the discharge is associated with exposure to exogenous hormones.[2] A 4-year-old spayed Weimaraner bitch was presented for evaluation of inappetence and intermittent sanguineous vulvar discharge. The patient had elevated rectal temperature and respiration rate. Physical examination indicated presence of vulvar edema and a sanguineous vulvar discharge. Vaginal cytology revealed mainly parabasal cells, occasional intermediate cells, and abundant neutrophils and red blood cells. Ultrasonographic findings were suggestive of an enlarged, fluid-filled uterine stump, and a complete blood count (CBC) indicated leukocytosis, neutrophilia, and monocytosis. A uterine stump pyometra due to ovarian remnant syndrome was suspected and celiotomy performed. The uterine stump appeared grossly cystic and thickened. Histopathological evaluation of the removed uterine stump and ovarian pedicles revealed cystic endometrial hyperplasia and no ovarian tissue. Fifteen days after surgery, the patient presented again with a sanguineous vulvar discharge. Vaginal cytology revealed predominantly superficial cells, indicating estrogen influence.[3] Differential diagnoses included ectopic ovarian tissue, exogenous estrogen exposure, or an adrenal tumor. Further questioning of the owner revealed long-term use of a topical estrogen cream by a member of the household. Serial examinations were performed, and the cytology remained uniform, with predominantly superficial cells, indicating continued estrogen influence.[3] Progesterone and anti-Müllerian hormone concentrations were determined to rule out ectopic ovarian tissue.[4,5] Both tests came back negative. The absence of any clinical signs of adrenal disease coupled with the history of topical estrogen cream use in the household suggested that the patient's clinical signs were likely due to exogenous estrogen exposure. Several recommendations were made to prevent the exposure. Follow-up vaginal cytology and CBC evaluations were also recommended to monitor future estrogen exposure and possible adverse effects on the patient's health.

Keywords: Dog, cystic endometrial hyperplasia, exogenous estrogen, vaginal cytology

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Phimosis and preputial abscessation with draining tract in an Angus bull

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A 22-month-old, Angus bull presented for preputial laceration and phimosis with secondary cellulitis and ventral swelling cranial to the scrotum. The bull received 2 doses of ceftiofur crystalline free acid, 1 dose of transdermal Banamine, and a 5 day course of penicillin. A preputial abscess was suspected. Preputial lacerations commonly occur during breeding generally due to tissue rupture secondary to compressive force.[1] Preputial laceration and subsequent prolapse is more common in *Bos indicus* breeds due to do their redundant preputial tissue and pendulous sheaths. However, *Bos taurus* bulls are often capable of fully retracting preputial injuries leading to subsequent abscessation.[1] Prognosis is poor to guarded for return to breeding soundness.[2,3] The bull was treated daily with hydrotherapy focused on the swelling. Epsom salt poultice was applied over the swelling and a sweat wrap was applied using a support sling. After 6 days of treatment, the abscess ruptured along a draining tract that terminated near the preputial orifice. Hydrotherapy, Epsom salt

poultice application, and the support sling were continued. The draining tract was lavaged daily through a catheter with a 10 liter carboy of normal saline containing 0.5% betadine solution. Silver sulfadiazine was infused along the draining tract daily. The draining tract improved briefly but the abscess recurred and ruptured a second time. The silver sulfadiazine infusions were discontinued and the lavage frequency was reduced to every other day until the draining tract could no longer be accessed. After 33 days in clinic, the bull was discharged. The bull returned 6 weeks after discharge for evaluation and breeding soundness examination. On electroejaculation, erection, protrusion, and ejaculation were successful. His semen was of good quality with 70% motility and 83% normal morphology. This case demonstrated successful medical management of a condition that commonly has a poor clinical outcome.

Keywords: Cattle, preputial laceration, retropreputial abscess

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Spermatic cord torsion in an Arabian stallion

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Spermatic cord torsions greater than 180 degrees compromise the vascular supply to the affected testis and are a medical emergency in stallions.[1] A 5-year-old Arabian stallion presented for an episode of acute colic signs. Diagnostic work-up including physical examination, bloodwork, abdominal ultrasonography and transrectal examination did not reveal a cause for colic signs. No sign of pain or discomfort was noted on or after admission. The left testis felt enlarged on palpation and a pronounced tubal structure was noted in the left inguinal canal during transrectal palpation. However, the scrotal structures were in the correct orientation on palpation and ultrasonography. Scrotal ultrasonographic examination had an increased left spermatic cord diameter (6.5 cm) compared to right spermatic cord (3 cm). Doppler demonstrated a reduction in blood flow through the left spermatic cord and testis compared to contralateral structures. Although suggestive, the ultrasonographic findings did not confirm spermatic cord torsion (complete occlusion of the cord). Therefore, it was elected to continue monitoring the stallion's condition. On the following day, scrotal ultrasonography demonstrated changes to the left testicular parenchyma with minimal to no blood flow through the left spermatic cord. Spermatic cord torsion of ≥ 360-degrees was diagnosed based on these findings and a closed hemicastration was performed. Although potentially at higher risk for contralateral torsion,[2] the owner elected hemicastration to preserve the breeding potential of the stallion. The stallion had an uneventful recovery and was discharged 5 days following presentation. Ischemic (coagulative) necrosis was observed on histopathological analysis of the testicular parenchyma. Although 360-degree spermatic cord torsions occur infrequently, torsion should always be considered for stallions presenting with symptoms of colic. It is important to note that colic signs and pain can subside due to the necrotic/ischemic changes in ≥ 360-degree torsions.

Keywords: Stallion, spermatic cord torsion, hemicastration

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STUDENT RESEARCH SESSION

Incidence of and factors affecting congenital defects in miniature Dachshunds

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A congenital defect is a malformation that is present and identifiable at birth. Congenital defects may result from genetic and/or environmental influences. In a review of several dog breeds, the most common defects reported were cleft palate and hydrocephalus.[1] In this review, Dachshunds had a relatively low frequency of congenital defects compared to other breeds.[1] However, the specific congenital defects in Dachshunds were not stated. As such, the current study aimed to investigate the incidence of congenital defects in miniature Dachshunds, along with the potential influences of neonatal sex, maternal age, and sire-dam consanguinity. Descriptive statistics with an odds ratio was performed on the data. One hundred and nineteen puppies from 33 litters from a single kennel were examined at birth. Fifty-nine were male and 60 were female pups. The mean ± SD litter size was 3.6 ± 1.3 pups. There were 3 stillborn pups (2.5%) including a schistosomus reflexus.[2] Additional congenital defects included severe retrognathism with class II distocclusion (n = 2; 1.6%), hydrocephalus with occipital dysplasia (n = 1; 0.8%), and complete cleft palate (n = 1; 0.8%). One puppy with retrognathism also had congenital hindlimb rigidity that resolved spontaneously over the first 3 weeks of life. The overall frequency of congenital defects was 4.2%. Within this kennel, the odds of having a congenital defect were 1.6 times larger in male than female pups. The incidence of congenital defects appeared (p = 0.334) to increase with maternal age (≤ 2 years old: 1 out of 56 pups [1.8%]; 3 - 5 years old: 2 out of 41 pups [4.9%]; ≥ 6 years old: 2 out of 22 pups [9.1%]) but this was not significant with this sample size using a Chi square. None of the bitches with malformed pups were primiparous. One litter of consanguineal mating between half siblings did not result in malformed pups; however,1 litter with a consanguineal mating between mother and son and another litter between great grandmother and son resulted in 2 malformed pups (1 per litter). These results confirmed previous findings that Dachshunds have a relatively low frequency of congenital defects.[1] Both cleft palate and hydrocephalus had been reported in Dachshunds.[1,3] In addition, the results from this study indicated that advancing maternal age may increase the incidence of congenital defects in dogs but more research with a larger sample size is needed to confirm this observation.

Keywords: Dachshund, congenital, defects, factors

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CLINICAL CASE SESSION

Proteomic analysis of sperm with impaired acrosomal exocytosis from a subfertile stallion

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Stallion subfertility due to impaired acrosomal exocytosis has been reported in Thoroughbred stallions that carry the genotype A/A-A/A for 2 SNPs in exon 5 of the FKBP6 gene (ECA13). These animals are subfertile despite having otherwise normal sperm quality and good breeding management. Mass spectrometry-based technologies are a powerful tool to investigate the potential causes of unexplained subfertility in the stallion and might further improve our understanding of the molecular processes that take place during fertilization. Herein, we describe a preliminary experiment conducted to compare the sperm proteome from a fertile stallion (percycle pregnancy rate = 60%) and a subfertile stallion that carries the susceptibility genotype for IAE (percycle pregnancy rate = 30%). Fresh semen from each stallion was processed to induce spontaneous acrosomal exocytosis (AE) using a lactate-only-containing modified Whitten's medium (Lac-MW). At 0, 4, and 6 hours of incubation, sperm aliquots were analyzed for sperm viability (VIAB) and the rate of AE in viable sperm (AE/VIAB) via flow cytometry (FITC-PSA and fixable live/dead red stain). Also, at each period, the sperm proteomes from each stallion were analyzed via data-independent acquisition mass spectrometry. Student's t-test was used to assess differences between experimental groups. During incubation in Lac-MW, VIAB was similar ($p > 0.05$) between both stallions and was not affected by incubation time. AE/VIAB increased ($p < 0.05$) over time (0 hour: 3%, 4 hours: 32%, and 6 hours: 56%) for the fertile stallion, but not ($p > 0.05$) for the subfertile stallion (0 hour: 3%, 4 hours: 5%, and 6 hours: 5%). Mass spectrometry analysis detected a total of 2,252 proteins in sperm (false discovery rate < 1.0%). Of these, 144 proteins exhibited differences in relative quantity between the fertile and subfertile stallion (log2 fold change; $p < 0.01$). Data analysis using the PANTHER protein class annotation system revealed that most of the proteins with lower abundance (▼) in the subfertile stallion belonged to the calcium-binding protein ($p = 3.36 \times 10^{-5}$), and the metabolite interconversion enzyme ($p = 1.11 \times 10^{-7}$) groups, whereas proteins with higher abundance (▲) included those of the chaperonin ($p = 8.25 \times 10^{-4}$), protease ($p = 5.21 \times 10^{-4}$) and histone ($p = 3.22 \times 10^{-11}$) groups. Among these, proteins of interest that were identified and are known to be related to sperm capacitation/acrosomal exocytosis and stallion fertility included: ADAM7 (▼), Annexin-A2, -A4, and -A5 (▼), Calpain-5 (▼), Kallikrein-1E2(▼), and CRISP-2 (▼). Current experiments are focused on determining the potential relation between the FKBP6 gene genotype and the changes observed on the sperm proteome of more IAE-affected stallions.

Keywords: Stallion subfertility, acrosomal exocytosis, FKBP6, proteome

Thoraco-omphalopagus conjoined twins in a Standardbred mare

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An eight-year-old Standardbred mare presented to The Ohio State University College of Veterinary Medicine in dystocia of 30 minutes duration. No breeding records were provided at presentation. Pregnancy had been confirmed in this mare at 14 days postovulation with 1 embryonic vesicle visible. On vaginal palpation, the fetus was in posterior presentation, dorsosacral position with hindlimbs extended and there were no signs of fetal viability. General anesthesia was induced to allow for better manipulation of the fetus for extraction. During controlled vaginal delivery, 2 hind limbs were identified and a fetotomy was performed at the level of the tibiotarsal joint. Following removal of 2 hind limbs, another hind limb was identified on palpation and removed via fetotomy at the level of mid-femur. Following removal of 3 hind limbs, 2 distinct pelvises were identified on palpation. Retropulsion of 1 pelvis was attempted, however no progress could be made to separate the suspected twins. Caesarean section was recommended, but humane euthanasia was elected due to financial constraints. Postmortem examination revealed a thoraco-omphalopagus conjoined twin following a ventral midline approach to the uterus. Prior to necropsy of the conjoined twin, a computed tomography scan was performed. The calvarium of the conjoined twin was fused at the level of the facial crest caudal to the orbit. Two independent vertebral columns with separate spinal cords were present connecting at a single sternum. One thoracic limb was present on

either side of the joined sternum. There was another fused thoracic limb that formed a bipedal hoof below the metacarpophalangeal joint with the metacarpal bones fused. There were 2 prepuces and 2 anuses present with meconium present in both anuses. Two tails were also present. There was an umbilical hernia present with small intestines protruding through the hernia. Respiratory and digestive tracts were fused at the larynx with a single trachea and esophagus. There was a single pair of lungs along with a single heart with 2 descending aortas. The single esophagus entered into a single stomach. The jejunum was divided into 2 ~ 90 cm oral to the ileocecocolic junctions. Caudal to that point, there were 2 ilea, ceca, and large intestines present with fecal balls in both small colons. Thoraco-omphalopagus conjoined twins have been reported in other species, including humans. To our knowledge, this is the first reported case of thoraco-omphalopagus conjoined twins in the horse.

Keywords: Mare, dystocia, twins, thoraco-omphalopagus

Anaphylactic reaction following intrauterine administration of misoprostol in a mare

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Deep horn intrauterine application of misoprostol, a synthetic prostaglandin E1 (PGE1), has been demonstrated to improve fertility in mares suspected of oviductal dysfunction.[1] Adverse reactions in horses included mild abdominal discomfort and soft feces after oral misoprostol and in women anaphylactic reactions were observed after oral and vaginal treatment. An 18-year-old Friesian mare weighing ~ 650 kg was presented for unexplained infertility of 3 years duration. Misoprostol (600 µg, Greenstone LLC, Peapack, NJ) dissolved in sterile water was deposited at the tip of each uterine horn.[1] Ten minutes after treatment the mare collapsed in the stall. Her mucous membranes appeared dark red with a prolonged capillary refill time of 4 seconds and she demonstrated tachycardia of 100 beats per minute, cool extremities, tachypnea of 60 breaths per minute, and was minimally responsive. Dexamethasone (50 mg) and flunixin meglumine (750 mg) were given intravenously in addition to 6 mg epinephrine. A 14-gauge intravenous catheter was placed and 5 liters of lactated Ringer's saline (LRS) was given. While the mare was laterally recumbent, a cuffed intrauterine catheter was inserted and the uterus lavaged with 9 liters of LRS in 3 liter aliquots. Fifteen minutes after the onset of treatment, the mare's heart rate and respiratory rate improved and she was able to achieve sternal recumbency and stand with encouragement. However, 15 minutes later she again became tachycardic and tachypneic and collapsed a second time. An additional 6 mg of epinephrine was given intravenously in conjunction with continuous bolus fluids and the uterus was again lavaged with 9 liters of LRS. Ten minutes after the second epinephrine the mare improved and regained ability to stand. Her vital signs gradually normalized, she passed normal manure and began to graze. Over the course of the 1.5-hour treatment window the mare received intravenously 50 mg dexamethasone, 750 mg flunixin meglumine, 12 liters of LRS, 2 doses (6 mg each) epinephrine, and twice 9 liters of uterine lavage with LRS. Following recovery, no further ill effects of the incident were noted. The mare was bred with fresh cooled semen 2 weeks later but failed to become pregnant. She had no previously reported medication allergies or history of drug reaction. This is the first reported adverse event of its kind associated with intrauterine PGE_1

Keywords: Misoprostol, intrauterine, adverse reaction, anaphylaxis

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Next generation sequencing in deciding to discontinue antibiotic treatment in a stallion

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A 10-year-old Morgan stallion presented for test cooling of semen with a history of mares not conceiving from cooled shipped semen. Five weeks prior, the stallion was collected for semen shipment. The semen was greyish in color and had 60% progressively motile sperm (PMS), mild teratozoospermia, 58% morphologically normal sperm, and 4.48 x 10^9 total number of sperm. Penis was washed thoroughly before collection to minimize debris in the semen sample from the penis as a possible cause of semen discoloration. Semen was collected using a Missouri artificial vagina, and the semen sample was again greyish in color and had 3.4 x 10^9 total number of sperm, 60% PMS, and 66% morphologically normal sperm. Cytological examination of the semen sample with Diff-Quik stain had no leukocytes or germ cells. Large numbers of branching rods were observed on cytology and an aerobic culture was performed. A fastidious and slow-growing *Actinomyces* species was identified by culture. Antibiotic susceptibility was not possible due to the bacteria's slow-growing nature. *Actinomyces* has been reported to infect the testes and accessory sex glands in humans. Empirical antibiotic selection was based on published reports and oral doxycycline (10 mg/kg) was prescribed for 8 weeks. Stallion was presented again 43 days later. Semen appeared normal with improved number of total sperm (7.45 x 10^9), 60% PMS, and 90% normal morphology. A culture was performed, and the sample was submitted for next generation sequencing (NGS) of 16S rRNA for bacteria and ITS for fungi. The culture was negative, and NGS had 59% *Klebsiella oxytoca*, 18% *Petrimonas* sp., 7% *Streptococcus uberis*, 3% *Corynebacterium kroppenstedii*, 2% *Luteococcus* sp., and 2% *Proteiniphilum* sp. for bacteria and 91%

of ITS reads were *Mucor circinelloides* and 8% *Pleospora herbarum*. Traditional culture method is of limited use to identify when antibiotic treatments can be discontinued due to antibiotics' inhibitory effects on culture results. The NGS results had several mixed bacteria and fungi that are more resistant to antibiotics. *Actinomyces* species was not identified, which we interpreted as a positive sign. The improved spermiogram, negative culture, and lack of *Actinomyces* detected by NGS were indications that antibiotic treatment could be discontinued. Ideally, NGS should have been performed prior to the start of antibiotic treatment to be used as a baseline, and further studies are indicated to determine thresholds of potentially pathogenic bacteria on fertility. Availability of a commercial clinical NGS laboratory service for the veterinary industry is a potentially groundbreaking advancement. One potential use of this technology may be to identify when long-term antibiotic treatments can be safely discontinued.

Keywords: Stallion, *Actinomyces* species, next generation sequencing

Thromboembolic disorder in a postcesarean section bitch

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Pregnancy and the postpartum period substantially increase the risk for venous thromboembolism (VTE) in women, a disease that leads to pulmonary embolism and deep venous thrombosis. The most important risk factor contributing to a thrombotic event is the hypercoagulable state that occurs during a normal pregnancy, with the highest risk occurring the first 6 weeks postpartum. Risk factors among women are age (> 35-years-old), cesarean delivery, hypertension, heart disease, obesity, and the presence of infection postpartum. To the authors' knowledge, this condition has not been reported in dogs. A 3-year-old female intact primiparous Labrador Retriever presented as an emergency due to fever (104.3 °F), lethargy, and anorexia. History included dystocia due to secondary uterine inertia with fetal distress (stillborn fetus) that was resolved by emergency cesarean section 4 days prior to presentation. On initial evaluation, the patient was quiet and responsive, obese with a body condition score of 8/9, and had a moderate amount of lochia with no foul-odor. Bloodwork revealed a normocytic, normochromic, regenerative anemia (hematocrit of 27%, absolute reticulocytes 99.6 x 10^3/µl), leukocytosis with a neutrophilia characterized by a left shift and monocytosis (WBC 43.6 x 10^3/µl, reference interval [RI] 5.7 - 14.2; segmented neutrophils 35.8 x 10^3/µl, RI 2.7 - 9.4; band neutrophils 0.4 x 10^3/µl, RI 0.0 - 0.1; monocytes 2.6 x 10^3/µl, RI 0.1 - 1.3), hyperproteinemia (8.0 g/dl, RI 5.9 - 7.8) and hypoalbuminemia (1.8 g/dl, RI 3.2 - 4.1). Considering the clinical condition, medical intervention and hospitalization for further diagnostic testing were pursued. Medical management consisted of intravenous plasma-lyte A fluids (60 ml/kg/day), ampicillin/sulbactam (Unasyn, 30 mg/kg every 8 hours), and maropitant citrate (Cerenia®, 1 mg/kg every 24 hours). A disseminated intravascular coagulation panel indicated significantly increased D-dimers (4965 ng/ml, RI 0 - 575), decreased antithrombin III activity (50%, RI 65 - 145), and an increased aPTT (19.6 seconds, RI 8.5 -15.5). Abdominal ultrasonography revealed a diffusely, severely mottled spleen with innumerable hypoechoic regions of acute, multifocal infarction, suggesting the presence of splenic infarcts and no evidence of peritonitis. That evening, the patient developed labored breathing with a respiratory rate of 36 breaths per minute and pulse oxygenation of 93% (RI > 95%). An arterial blood gas revealed an alveolar-arterial oxygen gradient of 30 mmHg (RI 10 - 25). These findings were concerning for pulmonary thromboembolism. The patient improved after 24 hours of supplemental oxygen, and the supplementation was discontinued. Subsequent echocardiogram revealed no evidence of pulmonary hypertension. The patient was discharged 48 hours after hospitalization with oral amoxicillin/clavulanate (13.75 mg/kg every 12 hours for 14 days). Considering the clinical manifestation and increased risk reported with cesarean delivery and peripartum obesity in women, it was presumed that the pregnancy-related hypercoagulable state and postpartum period led to the development of a VTE in the postcesarean section bitch.

Keywords: Mare, pregnancy, placenta, cervix, ultrasonography

Funding: Cesarean section, hypercoagulable, pulmonary embolism, venous thromboembolism

Polled intersex syndrome in a Finnish Landrace lamb

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An apparently healthy, 4-month-old female Finnish Landrace lamb was presented for the presence of female and male external genitalia. The lamb was polled and a twin to a normal female. On initial examination the lamb had a short anogenital distance of 3.5 cm and a normal appearing vulva with a mildly enlarged clitoris. Left gonad was descended and palpable in the inguinal area, whereas the other gonad was suspected to be retained intra-abdominally. Left inguinal ring was severely dilated and the descended gonad could be moved freely intra- and extra-abdominally. Abdominal ultrasonography revealed a retained intra-abdominal right gonad that appeared testicular in origin with a hyperechoic mediastinum testis and a less echogenic parenchyma as seen in a normal testis. A fluid filled uterus was also identified, but ovaries were unable to be identified. Lamb underwent a midline exploratory laparotomy with a bilateral castration/hysterectomy. Uterus was identified and the uterine horns were followed to the location of the ovaries where testicular appearing structures with a pampiniform plexus

and epididymis were identified. The testicular tissue was attached to the broad ligament as the uterus and ovaries would be. Both testes were ligated and removed, the uterus was removed via a routine hysterectomy. Incision was closed in a routine fashion, the lamb recovered from anesthesia with no complications. Lamb was euthanized at a later time. Uterus and gonads were submitted for histopathology. Histopathology revealed that gonads contained hypoplastic testicular tissue. Seminiferous tubules were diffusely hypoplastic with a complete lack of spermatogenesis. Epididymis was hypoplastic with compressed ducts. There was marked congestion in the uterus with the lumen diffusely filled with erythrocytes consistent with intraluminal hemorrhage and the endometrial lamina propria was expanded by edema. No ovarian tissue was identified in the sections of the testis that were evaluated. However, due to the difficulty associated with identifying ovarian tissue, the presence of ovotesticular tissue could not be ruled out. Blood was submitted to the Texas A&M College of Veterinary Medicine, Molecular Cytogenetics Laboratory for karyotyping. The results revealed a genetically female sheep with a normal sheep karyotype (54 XX). On polymerase chain reaction, the lamb was negative for the presence of the Y-linked SRY gene and positive for the X-linked androgen receptor gene. No chromosomal abnormalities were observed. Intersex conditions in goats and in sheep are believed to be linked with the polled gene. Affected animals are genetically female (XX), SRY negative and believed to be homozygous for the polled gene. In this case the lamb is an intersex, consistent with polled intersex syndrome sex reversal but, may be a true hermaphrodite.

Keywords: Sheep, polled, intersex, sex reversal, chromosome, hermaphrodite

Reduction of equine monozygotic twins using craniocervical dislocation via colpotomy

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Twin management is a very important facet of equine reproduction management. Compared to dizygotic twins, monozygotic twins are a relatively rare occurrence in mares. Management of monozygotic twins is considerably more complex when it comes to preserving the safety and welfare of the mare and developing pregnancy. Of the options of twin reduction after day 50 of pregnancy, craniocervical dislocation (CCD) has been reported to be a superior choice.[1] We have modified the surgical approach for a CCD from the reported flank laparotomy to colpotomy. In our hands, successful reduction of dizogotic twins by CCD via a colpotomy approach has been 71% (n = 29). To-date we have managed 2 cases of monozygotic derived twins with a 50% success rate. Our hypothesis for the current case was that CCD via colpotomy is a successful technique for monozygotic twin management given its successful application on our first case of monozygotic twin reduction. An embryo recipient mare that received 1 embryo was presented at 65 days of pregnancy for the reduction of 1 fetus after diagnosed to have monozygotic twins via ultrasonography by the referring veterinarian. The referring veterinarian was actively monitoring the unilateral twin pregnancy for natural reduction. Once the pregnancy reached 60 days with no reduction, the case was referred to us for CCD. After confirmation of monozygotic twins (i.e. 2 separate amnions were observed within 1 allantoic sac), a CCD was performed on 1 fetus via a colpotomy approach. Unfortunately, both fetal heartbeats were lost on detection by the referring veterinarian and presumably aborted between 4 and 6 weeks after CCD procedure. Other cases of monozygotic twins have been reported in recipient mares that received a single embryo.[2-4] Several management techniques were employed in each of these cases with varying outcomes. CCD via colpotomy is a novel approach and was chosen in this case due to the age of the fetuses and a desire to reduce the most craniad that we presumed would have less chorionic attachment and lower development capacity as the pregnancy progressed.[1] The importance of this case is in demonstrating that CCD via colpotomy can be a useful method for management of monozygotic twins; however, prognosis for successful development of the remaining fetus is guarded due to the orientation of the twin fetuses enclosed within a single allantochorion.

Keywords: Twins, monozygotic twins, twin management, embryo recipient, embryo transfer

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Chronic seminal vesiculitis and blocked ampullae in a stallion

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Seminal vesiculitis is a rare condition in the stallion; however, it can result in blocked ampullae. A 12-year-old Gypsy Vanner stallion with a previous successful breeding history was presented for persistent polyspermia, manifesting as an abnormal grey

ejaculate. Semen collected at presentation had pus with no sperm. Testes felt normal but were mildly enlarged with firm epididymides. Ultrasonography findings were normal. Transrectal palpation of the ampullae and seminal vesicles (SV) elicited a painful response. Transrectal ultrasonography revealed fluid-filled ampullae and SV with thickened walls (1.5 cm). Stallion demonstrated normal libido during 3 collections and did not exhibit pain. Cultures were obtained from the unwashed urethral fossa, the distal urethra before and after ejaculation, and from the gel fraction. *Streptococcus equi* subspecies *zooepidemicus* was isolated, confirming the presumptive diagnosis of seminal vesiculitis. Microscopic examination of fresh ejaculate and Diff-Quik stained smears revealed azoospermia, high number of neutrophils, and bacteria in both fractions of the ejaculate in all 3 collections. Transrectal massage of the ampullae and oxytocin treatment immediately prior to the third collection did not alter the ejaculate. The diagnosis of blocked ampullae was confirmed by azoospermia and low concentration of alkaline phosphatase (30 IU/L) in the filtered fraction. Semen was collected 3 times a day and the stallion was treated orally twice a day with trimethoprim/sulfamethoxazole (TMS; 30 mg/kg) for 3 weeks at the owner's facility. Stallion was reexamined at the hospital. There were no changes to ampulla and SV. The first collection had > 70 ml heterogenous gel fraction and concentrated sperm-rich fraction (917 x 10^6 sperm/ml, 14.5 x 10^9 total) with marked number of detached heads and dead sperm (< 1% motility). Sequential collections had decreasing volume of gel, increasing number of sperm (up to 83.2 x 10^9), and an increasing trend in the motility rate in the ejaculate. Due to lack of complete response to TMS, local treatment of the SV was initiated using video endoscopy (5.9 mm x 110 cm). The local treatment consisted of vigorous lavage (500 ml lactate Ringer's saline per gland) followed by local infusion of ampicillin (2 gram in 18 ml) initially for 2 days followed by a compounded penicillin gel (5 MIU in 20 ml) every other day for a total of 3 treatments. Purulent material within the seminal vesicles markedly decreased throughout treatment. Stallion was discharged with instructions to have twice daily collections and continued TMS treatment at the owner's facility before readmission for follow-up. This case highlighted the importance of managing seminal vesiculitis aggressively, and the possible sequela of blocked ampullae due to local inflammation and accumulation of pus and sperm.

Keywords: Stallion, seminal vesiculitis, blocked ampullae

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EQUINE SESSION

Low volume uterine lavage: advantages for use in problem mares

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Infectious endometritis is a leading cause of subfertility in broodmares. The standard sample collection technique for diagnosis of bacterial and fungal endometritis is a guarded swab for uterine culture and a guarded swab or brush for uterine cytology. However, these methods only sample a very small area of the uterus and may not always yield microbial growth in mares with infectious endometritis. Low volume lavage (LVL) is an alternate diagnostic technique that samples the entire uterine lumen. Aim of this study was to compare the results of microbial culture and cytologic evaluation from samples collected using a guarded swab and brush versus samples subsequently collected using a LAL technique in mares suspected with infectious endometritis. Reproductive records from 27 mares managed at Colorado State University were analyzed retrospectively. Transrectal ultrasonography examination and a standard uterine swab/brush were performed prior to LVL procedure. Low volume lavage was performed using 250 ml of sterile lactated Ringer's solution and the effluent fluid was then transferred into a pair of 50 ml conical tubes for centrifugation. Microbial culture was performed on swabs and LVL pellets using Spectrum™ 4-Part (Colorado) microbial agar plates (Vetlab Supply, Palmetto Bay, FL). Glass slides for cytologic evaluation were stained with a modified Wright-Giemsa stain (MWI Animal Health™, Boise, ID) and evaluated under 400 and 1000 x microscopy. Percentages of mares with microbial growth of a uterine pathogen and presence of white blood cells on cytologic evaluation were compared by Chi Square analysis; data were considered different at $p < 0.05$. Initial examination results determined that all mares were in estrus at reproductive evaluation based on the presence of mild to moderate uterine edema and a dominant follicle. An increased volume (> 2 cm depth) of uterine fluid was noted in 13 of the 27 mares (48.1%) and increased echogenicity of uterine fluid was noted in 12 of the 27 mares (44.4%) on initial transrectal ultrasonography examination. Cytologic evaluation of the guarded brush sample revealed the presence of white blood cells and/or the presence of increased background debris in 12 of the 27 (44.4%) mares. Microbial growth of 1 or more pathogenic bacteria was detected in the culture of guarded swabs collected from 4 of 27 mares (14.8%). Overall, 21 of 27 mares (77.8%), had 1 or more factors that were suggestive of endometritis. Culture from LVL pellets yielded growth of pathogenic bacterial in 15 of the 27 samples (55.6%) that was higher ($p = 0.002$) than guarded uterine swabs. Cytology samples from LVL pellets had white blood cells in 24 out of the 27 mares (88.9%) that was higher ($p = 0.005$) than guarded uterine brushes. In summary, the LVL technique is a valuable procedure in the detection of uterine inflammation and microbial infection in problem mares.

Keywords: Mare, uterus, low volume lavage, problem mare, endometritis

Use of progesterone and estradiol-17ß prior to transvaginal aspiration of oocytes

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This pilot study assessed the impact of progesterone and estradiol-17ß treatment on ovarian follicle number. Objective was to determine whether 150 mg/ml progesterone + 5 mg/ml estradiol 17ß (BioRelease P&E; BET Pharmacy, Lexington, KY) treatment can impact mare ovarian follicle numbers to increase the odds of having more ovarian follicles on a predicted day. To increase foal production, transvaginal aspiration (TVA) of mare oocytes is followed by in vitro maturation, fertilization (via intracytoplasmic sperm injection), and embryo culture. In efforts to aspirate several oocytes during 1 TVA, common practice is to repeat ultrasonographic examinations of the ovaries on multiple sequential days and schedule the procedure on the day when the mare is expected to have her 'peak' number of suitable follicles. Such a high maintenance management routine increases mare owner costs on a per foal basis. It also allows only ~ 1 - 2 days' advance notice to the practitioner performing the TVA procedure, due to the variability in follicular growth dynamics. Currently, there are no accepted treatment regimens for advanced scheduling/planning of oocyte collection via TVA in the mare. A validated estrous cycle management protocol would allow practitioners and mare owners to

plan multiple days in advance and also increasing the number of follicles that could be aspirated. Furthermore, it would be highly beneficial to the equine industry and scheduling TVA procedures. We hypothesized that a single injection of P&E result in synchronization and optimization of the number of follicles to occur at least 9 - 10 days after treatment. Eight mares underwent initial ovarian follicles counting (i.e. follicular mapping) via transrectal ultrasonography and were given 10 ml of BioRelease P&E intramuscularly on day 0; transrectal palpation and ultrasonographic examination, and follicular mapping was repeated for each mare 3 times (i.e., on days 3 and 6, in addition to days 9, 10, or 11, based on mare availability). Serum was collected on the days of ultrasonographic examinations and frozen for determining concentrations of anti-Mullerian hormone (AMH), luteinizing hormone (LH), and follicle stimulating hormone (FSH). Data on follicle count data from this study were combined with previously obtained pilot data, for a total of 24 mares. Data on follicle count were analyzed by unpaired two-tailed Student's *t*-test and AMH concentrations by a multiple comparison test. Follicle counts increased ($p = 0.024$) by a mean of 4.13 follicles/mare between the first and last follicular mapping sessions. AMH concentrations on day 3 (0.8473 ng/ml ± 0.1699, mean ± SEM) were significantly higher compared to day 6 (0.7033 ng/ml ± 0.1737, mean ± SEM).

Keywords: Mare, progesterone, estradiol, follicle, transvaginal aspiration, oocyte

Prepartum amniotic rupture in a Thoroughbred mare

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Prepartum amniotic rupture has been described in humans.[1] In mares, there are reports of prepartum placental disruption associated with hydropsical conditions. This report describes a full thickness prepartum amniotic tear in a Thoroughbred mare, initially diagnosed by transrectal ultrasonography at 236 days prepartum. A 7-year-old Thoroughbred mare was pregnant with her first foal. Transrectal ultrasonography (15, 17, 28, 42, 60, 89, 119, 148, and 183 days) findings were normal and the mare apparently had a filly. Transrectal ultrasonography evaluation on 211 days revealed a normal amnion and a combined thickness of the uterus and placenta (CTUP) of 0.6 cm. At 236 days, transrectal ultrasonography was performed as part of routine screening and no amnion could be identified. Allantoic fluid was moderately and uniformly increased in echogenicity and the fetus was visualized with no amnion separating the fetus from the allantoic membrane. No precocious mammary gland development was noted, but substantial placental edema was observed in the chorioallantois and endometrium and CTUP was 2 cm. Serum amyloid A was slightly elevated (32 µg/ml) with normal white blood cell count (7.6×10^3), and fibrinogen (200 mg/dl). Total estrogens were 1,040.04 pg/ml and progesterone concentrations were 4.51 ng/ml. Treatment for placentitis (once every 24 hours for altrenogest [0.044 mg/kg], 57 mg of firocoxib and 500 mg of flunixin meglumine, and once every 12 hours for pentoxifylline [8.5 mg/kg] and doxycycline [10 mg/kg]) was initiated. Serial transrectal and transabdominal ultrasonography were performed and the chorioallantois continued to thicken and was edematous. Amnion was intermittently viewed in transrectal ultrasonography, but in an abnormal and wrinkled appearance. At all times, fetal heartbeat was within 80 - 100 bpm. At 313 days of pregnancy, the mare was admitted for monitoring, and altrenogest, firocoxib and pentoxyfiline treatment continued. In addition, once every 24 hours, 2,660 mg of aspirin was given. At day 317, the mare foaled. No amnion was visible after observing the mare's water breaking, foaling required manual assistance, and the amnion was not noted. Fetus was born alive but euthanized due to 90° contracture of both front fetlocks. Fetus and fetal membranes were submitted for histopathology analysis and revealed an amniotic rent measuring 10 cm with rolled edged and a diffusely thickened amnion. Allantois was thickened with areas of adenomatous hyperplasia and embedded hair shafts. To our knowledge this is the first reported case of prepartum amniotic rupture diagnosed by transrectal ultrasonography in the mare

Keywords: Mare, amniotic, rupture, prepartum

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Evaluation of passively acquired rabies antibody titers and immune responses in healthy foals vaccinated against rabies at 4 or 6 months of age

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Passively acquired antibodies provide protection against disease in foals prior to completion of a primary immunization series. Postvaccination immunologic protection against rabies is expected with titers ≥ 0.5 IU/ml, but information on the lifespan of passively acquired antibodies and influence on immune response is lacking. In the spring of 2020, the American Association of Equine Practitioners updated vaccination guideline recommendations to begin rabies vaccination at 4 to 6 months of age instead of initiating at 6 months of age. The update made initiation of foal vaccination recommendations consistent for all 5 core equine diseases (Eastern equine encephalomyelitis [EEE], Western equine encephalomyelitis [WEE], West Nile virus [WNV], tetanus, and rabies). The aim was to evaluate the rate of passively acquired rabies antibody decline and compare the immune response to vaccination when initiated at 4 or 6 months of age. Forty-nine foals, in 3 farms, born to mares vaccinated against EEE, WEE, WNV, tetanus, rabies, equine influenza virus,

equine herpesvirus 1/4, botulism, rotavirus, plus or minus autogenous *Salmonella typhimurium* and *Clostridium perfringens* in the last 6 weeks of pregnancy, were enrolled. All foals were determined healthy and had adequate postnursing IgG. None received hyperimmunized plasma. Blood was drawn monthly and submitted to Kansas State University rabies laboratory to quantify antirabies neutralizing antibody via the rapid fluorescent focus inhibition test. Foals were allocated with a generalized, randomized block design, based on rabies titer at 1 month of age. Foals received a commercial combination EEE, WEE, WNV, tetanus, rabies vaccine beginning at either 4 or 6 months of age. Foals received a booster 30 days after their initial injection. Rabies antibody concentrations were analyzed to establish the rate of decline prior to and after vaccination immune responses. Prior to vaccination, geometric mean rabies titers were 3.5, 1.1, 0.6, and 0.4 IU/ml at 1, 2, 3, and 4 months of age, respectively. Through the first 4 months of life, antibody titer decline was 90%. At 4 months of age, 33/49 (67%) foals had rabies titer ≤ 0.5 IU/ml. The geometric mean postvaccination rabies titers were 0.8 and 1.0 IU/ml in foals that began initial vaccination series at 4 and 6 months of age, respectively. Postvaccination, 20/25 (80%) foals in the 4-months age group and 20/24 (83%) foals in the 6-months age group had rabies titer ≥ 0.5 IU/ml. In our study, the rate of rabies antibody declined over the first 4 months of life, indicating that most foals are expected to have a titer below 0.5 IU/ml prior to 4 months of age. The immune response to vaccination was similar between foals that started their immunization series at 4 months of age compared to those started at 6 months of age. future is unknown.

Keywords: Rabies, vaccination, titers, immunization, immunity

Prospective ultrasonographic evaluation of caudal placenta and cervix in pregnant mares in relationship to foaling outcomes and placental abnormalities

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Clinical diagnostic procedures to evaluate the late pregnant mare are largely limited to physical examination and ultrasonographic evaluation of the fetus as well as the combined thickness of the uterus and placenta (CTUP). The cervix of a pregnant mare is a key physical and immune barrier to contamination of the pregnant uterus from the vagina yet there is limited research that evaluated changes in the cervix, which might be indicative of potential problems during equine pregnancy. Objective was to evaluate changes in CTUP and mean cervical diameter (CX) determined by transrectal ultrasonography (Sonoscape S9 with 9.5-15 MHz linear probe; Seattle, WA), and the relationship of these parameters to subsequent foaling outcomes and placental abnormalities. The study was conducted in Thoroughbred mares in central Kentucky during 2017 (n = 112 mares), 2018 (n =109 mares), and 2019 (n = 139 mares). Mares were examined by 1 of 2 examiners on a monthly basis from 4 months gestational age (GA) until term (total examinations; n = 1810). At term, outcomes were classified as normal or abnormal foal and normal or abnormal placenta (based on observation of a fetal membranes inspection at the farm). Data were analyzed by a random-effects mixed model including mare as the random effect, gestational age as a covariate and foaling outcome, placenta as well as examiner as a fixed-effects (JMP ver 14.0). Correlations were evaluated by a Pearson's coefficient. The CTUP was higher ($p = 0.001$) in mares with abnormal placenta at term but was not related ($p = 0.3$) to foal outcome. The CTUP increased ($p < 0.001$) with GA and was affected ($p < 0.001$) by examiner. The CX increased ($p = 0.05$) in mares with abnormal foaling outcome but was not related ($p = 0.2$) to abnormal placenta at term. Again, CX increased ($p < 0.001$) with GA and varied ($p < 0.001$) with examiner. As noted, CX and CTUP increased with GA and were positively correlated ($r = 0.26$; $p < 0.01$). Our findings suggested that measurement of CX and CTUP in mares are related to foaling outcome and placental abnormalities at term, respectively. Future studies should examine the predictability of foaling outcomes in mares based upon prospective evaluation of these parameters.

Keywords: Mare, pregnancy, placenta, cervix, ultrasonography

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Oocyte collection rate and in vitro embryonic development with low dose deslorelin in mares

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Equine assisted reproductive technologies have become increasingly popular throughout the past 20 years, especially ovum pick up (OPU) and intracytoplasmic sperm injection (ICSI). Successful production of in vitro embryos through OPU/ICSI often relates to the number of oocytes, but limited research has been devoted towards ovarian super stimulation for this purpose. This study aimed to determine if low doses of the gonadotropin releasing hormone agonist, deslorelin, (LDD) would increase number of follicles, increase number of oocytes collected or affect oocyte quality. Mares (n = 11, 5 - 13 years) were assigned for this study. Each mare served as her own control in a cross over design. All estrous cycles were monitored through routine transrectal ultrasonography and all visible follicles recorded. Routine transrectal vaginal aspiration was performed on mares in both groups at ~ 20 hours after ovulation induction treatment. For treated estrous cycles, when at least

1 follicle measured ≥ 15mm in diameter, 50 µg deslorelin in aqueous solution (Precision Pharmacy, Bakersfield, CA) was given intramuscularly every 12 hours until at least 1 follicle reached 32 mm in diameter with uterine edema. Subsequently, the LDD protocol was discontinued, allowing for 24 hours without hormone treatment. After collection, oocytes deemed mature (expanded cumulus) or immature (compact cumulus) were handled accordingly, prior to ICSI with frozen semen from a single ejaculate. Presumptive zygotes were evaluated at 24 hours for evidence of cleavage and again at 4, 6, and 8 days. Comparisons were made utilizing a Student's and Welch's *t*-tests with significance established at $p \leq 0.05$ and reported as the mean ± standard deviation. In total, 41 estrous cycles were included (24 control and 17 treated). On average, 7.41 ± 2.3 injections of LDD were given during treated estrous cycles. Average number of follicles observed or number of follicles > 25 mm in diameter at OPU did not differ between groups ($p = 0.13$ and $p = 0.46$, respectively). Average number of follicles aspirated in the treated group (2.1 ± 1.9) tended to be higher ($p = 0.09$) than control (1.9 ± 1.4); however, the number of oocytes collected in treated cycles (1.2 ± 1.4) compared to control (0.75 ± 1.0) did not differ ($p = 0.28$). A total of 36 oocytes were subjected to ICSI from the treated and 38 from control estrous cycles. The cleavage rate was similar ($p = 0.89$) for treated (56%; 20/36) compared to control (61%; 23/38). Finally, the blastocyst rate for treated was similar ($p = 0.66$) (19%; 7/36) compared to control (24%; 9/38). In conclusion, the utilization of a low dose deslorelin protocol did not affect the number of follicles or oocytes collected within estrous cycles for OPU/ICSI. Additionally, cleavage and blastocyst rates were similar between the 2 groups indicating that this protocol did not affect the quality of oocytes. More research is necessary to identify an ovarian superstimulation protocol effective for OPU/ICSI.

Keywords: Mare, oocytes, follicles, deslorelin

Pregnancy rates and ovarian activity after uterotubal infusion of n-butyl cyanoacrylate via a hysteroscopic approach in mares: a pilot study

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Sterilization of mares requires surgical removal of ovaries or ligation of uterine tubes and is performed for behavioral reasons, treatment of ovarian pathology, and preparation of tease or mount mares. However, it is invasive, costly, and may be associated with adverse outcomes. Objective was to determine the effect of bilateral uterine tube infusion with n-butyl cyanoacrylate on fertility and estrous cycle activity of mares. We hypothesized that bilateral infusion of n-butyl cyanoacrylate in the uterine tubes causes infertility but does not affect mare's estrous cycle activity. Light horse mares (n = 8) ranging in age from 5 - 23 years, were selected for potential fertility using transrectal ultrasonography examination, uterine culture and cytology. A proven fertile Thoroughbred stallion that successfully bred mares in 2015 was used. Mares were sedated, perineum cleansed, and the uterine tubes of treated mares (n = 6) were infused with 0.5 ml n-butyl cyanoacrylate, and control mares (n = 2) were infused with 10 ml saline, using the endoscopic hydrotubation method.[1] Treated mares were hand mated through 2 or 3 breeding seasons (n = 78 estrous cycles), and control mares were hand mated through 1 breeding season (n = 4 estrous cycles). Biweekly blood samples were obtained from mares during the breeding season for progesterone (P_4) concentrations by RIA. Per cycle pregnancy rate, established by transrectal ultrasonography of the uterus and interestrus intervals, were recorded. Stallion's seasonal pregnancy rate (SPR) in 2015 (n = 24 mares) was compared to the SPR of treated (n = 6) and control mares (n = 2) for years 2016 - 2018. Data were evaluated using Fisher's Exact test at $p < 0.05$. Per cycle pregnancy rates of treated (0/78) and control mares (1/4) were different ($p = 0.048$). SPR for the stallion in 2015 prestudy breeding season (20/24 mares pregnant) was different ($p < 0.001$) than the SPR of treated mares, wherein 0/6 became pregnant in each year. Interestrus intervals of 6 treated mares averaged 20 days (range 18 - 22 days), and serum P^4 profiles were consistent with estrous cycle activity. The n-butyl cyanoacrylate may function to physically obstruct sperm, oocyte and embryo movement in the uterotubal junction or uterine tube, disturb the milieu required for sperm capacitation, oocyte fertilization, and embryonic development, or a combination of these. Uterotubal infusion of n-butyl cyanoacrylate via a hysteroscopic approach may serve as an effective management strategy for induced infertility in mares of at least 3 years duration and may cause permanent sterilization.

Keywords: Mare, infertility, n-butyl cyanoacrylate, uterine tube, progesterone, estrous cycle

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Daily sperm output, spermatogenic efficiency, and sexual behavior of donkey jacks mounting jennies in estrus

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Assessment of daily sperm output (DSO) and spermatogenic efficiency are critical components of breeding soundness evaluation

to diagnose reproductive diseases and estimate the number of females that may be bred in a season. However, there is no consensus on how to determine these parameters in stallions or donkeys. Part of the discrepancies is due to estimation of testicular volume (TV) by several ways. Additionally, donkeys are thought to be less consistent breeders when required to collect daily. We hypothesized that a donkey's DSO varies with the equation utilized for calculation of TV and his behavior parameters are influenced by increased numbers of collection days. Aim was to assess the sexual behavior of jacks mounting jennies in estrus and determine the DSO and spermatogenic efficiency. Eight sexually rested jacks had semen collected once a day for 10 consecutive days using jennies in good standing estrus for mounting. Sexual behavior and semen parameters were assessed during each collection. Testicular measurements of height, width, and length were taken immediately before the first semen collection, and these measurements were used to calculate the TV using a nonellipsoid equation (TV1 [cm^3]: 33.57 × height [in cm] – 56.57) and an ellipsoid equation (TV2 [cm^3]: 4/3π length/2 x height/2 x width/2) used for stallions. After that, the TVs were used to predict the DSO. The average total sperm number (TSN) obtained on days 8 -10 was deemed the actual DSO. Differences in the predicted versus the actual DSO were used to calculate the spermatogenic efficiency. In addition, the actual DSO was also used to calculate the number of inseminating doses a jack could produce for both on- and off-site breeding. Data were analyzed with the Shapiro-Wilk test and then with ANOVA followed by Tukey's test or Kruskal-Wallis. Sexual rest did not affect ($p < 0.05$) sperm motility. Jack's sexual behavior did not vary across collection days ($p < 0.05$). Sperm concentration and TSN reduced ($p < 0.05$) over time. The actual DSO was 9.1 ± 4.1 x 10^9, and the predicted DSO varied from 4.7 to 18 x 10^9. Spermatogenic efficiency ranged from 50 to 150% based on the jack and the equation used to calculate TV. The predicted-DSO obtained with TV1 demonstrated a strong and positive correlation with the actual-DSO ($r = 0.76$, $p < 0.05$); however, there was no significant correlation between predicted-DSO and TV2 ($p > 0.05$). The number of inseminating doses ranged from 15 to 47 at 300 - 500 x 10^6 progressively motile sperm (PMS) /dose for on-site breeding. In contrast, the number of breeding doses with cooled-shipped semen (1 x 10^9 PMS/dose) varied from 4 to 14 doses across donkeys. In conclusion, sexual behavior was not affected by daily semen collections. Sexual rest did not affect sperm motility. The predicted DSO varied with the equation used to determine TV. Clinically normal donkeys have high spermatogenic efficiency that confirms previous histology reports. A distinct approach to calculate DSO in donkeys is described. Further studies are warranted to apply the findings of the present study in reproductively abnormal donkeys.

Keywords: Sexual behavior, daily sperm output, spermatogenic efficiency

MIXED ANIMAL SESSION

Infertility, pregnancy, and concurrent pyometra in a dog

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A 3-year old Greater Swiss Mountain dog was presented for breeding management. The patient had a history of 2 previous failed pregnancy attempts, 1 with ovulation timing and vaginal insemination, and the other with ovulation timing and transcervical insemination. Regardless of failure to become pregnant, the owner wanted to breed once more due to high sentimental value. The patient was placed on enrofloxacin and carprofen at LH surge until 6 days postovulation. The patient had a negative vaginal culture at last breeding. Based on ovulation timing, 2 transcervical inseminations were performed with fresh collected semen. At 25 days postovulation, the patient was confirmed pregnant on abdominal ultrasonography. Only 1 fetus appeared to be viable, 1 fetus appeared to be in the early stages of resorption with no heartbeat, and 5 other resorptive sites were identified. The patient was placed on amoxicillin. Taking into account the patient's history of infertility, a concern for hypoluteoidism prompted us to assess the patient's serum progesterone concentrations and were elevated (31.42 ng/ml). Due to owner's concern, progesterone concentrations were determined 3 days later. Progesterone concentration decreased to 14.38 ng/ml. Due to this unexpected degree of decline in 3 days, we continued to monitor progesterone concentrations throughout pregnancy. Progesterone concentrations remained adequate for the rest of pregnancy. At 48 days postovulation, ultrasonography revealed 1 viable fetus and a fluid-filled uterus. The patient was asymptomatic for a pyometra, and the owner elected to maintain the pregnancy. The antibiotics were switched to amoxicillin/clavulanate potassium; patient was continually monitored via rectal temperature assessment and ultrasonographic examinations, and for behavioral changes. At 63 days postovulation, an elective cesarean section was performed. The fetus was viable and resuscitated quickly. Substantial amount of purulent material was present in the uterus (> 1 liter). The serosal surface of the uterus did not have any defects and appeared normal. Pathological diagnosis, based on a tissues sample from the right uterine horn, was 'focal extensive eosinophilic endometritis and multifocal moderate lymphoplasmacytic endometritis/myometritis with multifocal mild cysts endometrial hyperplasia.' Infertility in this bitch was accompanied by multiple lesions and this case demonstrated that most 'missed breeding' may be due to an underlying cause, even in a young, otherwise healthy bitch.

Keywords: Pyometra, infertility, cystic endometrial hyperplasia, pregnancy

Factors affecting survival and future foaling rates in Thoroughbred mares with hydrops

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Prognosis for life and future fertility in broodmares following hydrops is reportedly good, and the role of inheritance in the development of hydrops has been suggested but lacks large-scale evidence. Aims of this case series were to evaluate the prognosis for survival and fertility in mares following hydrops diagnosis and to attempt to identify if inheritance is a predisposing factor. Thirty mares presented with hydrops were included. Data collected included history (pregnancy and sire of the foal), clinical findings at presentation and throughout hospitalization (complications, treatments provided, and survival to discharge) and future foaling rates. Ninety percent (90%) of mares survived (hydrallantois: 94.7%; hydramnios: 75%) and 95.2% of mares successfully had a future foal, of which 75% foaled the first year following hydrops. No reoccurrence of hydrops was identified, despite being bred back to the same stallion. Transcervical gradual fluid drainage was associated with improved ($p = 0.05$) survival. Complications associated with poor survival and decreased future foaling rates included hypovolemic shock ($p < 0.005$ and $p = 0.010$, respectively), hemorrhage ($p < 0.005$ and $p = 0.025$, respectively), peritonitis ($p < 0.005$ and $p = 0.01$, respectively) and abdominal wall rupture ($p = 0.01$ and $p = 0.005$, respectively). Laminitis was associated with poor survival ($p < 0.05$). One mare suffered a uterine tear and was euthanized. These results suggest that prognosis for survival, future breeding and fertility following a diagnosis of hydrops is good, provided the hydrops is diagnosed and treated appropriately and no damage to the reproductive tract or body wall occurs. These data did not provide evidence for heritability and further investigation is required.

Keywords: Mare, pregnancy, hydramnios, hydrallantois, fluid drainage

Comparison of 2D and 3D ultrasonography for gestational aging in dogs

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Gestational aging in the bitch by two-dimensional (2D) ultrasonographic measurements of fetal and extra-fetal structures has been well studied. In early pregnancy, the inner chorionic cavity (ICC) diameter was the most reliable parameter, but its accuracy for parturition day prediction within 1 day error decreased from 81% in week 4 to 67.7% in week 5 of pregnancy.1 We hypothesized that three-dimensional (3D) volume ultrasonography, which has previously been studied only in a limited number of bitches, improves gestational age determination in dogs. The aim of our study was to compare embryonic vesicle measurements by 3D to 2D ultrasonography for gestational aging in early to mid-pregnant bitches. Thirty-two pregnancy examinations were performed in 25 bitches of several breeds by abdominal ultrasound between 21 and 34 days after the first mating. ICC diameter and length were measured by 2D, and ICC volume by 3D ultrasonography (Voluson® i, GE Healthcare). ICC volume was calculated using the virtual organ computer-aided analysis (VOCAL™) software with 30° rotational angle. Measurements on 2 embryonic vesicles per examinations were averaged for statistical calculations (ICC volume was available from only 1 embryo in 5 examinations). The associations between the dependent variables ICC diameter, length and volume, and the independent variable time, calculated as either 'days from ovulation' (n = 15 dogs, ovulation day based on serum progesterone concentrations) or 'days before parturition' (n = 22 dogs, planned cesarean sections or bitches under progesterone supplementation excluded) were analyzed by linear or exponential regression using IBM® SPSS® Statistics v. 26.0.0.0. Statistical significance was set at $p < 0.05$. Counting from the day of ovulation, regression lines for ICC diameter, ICC length and ICC volume had a good fit ($p < 0.001$) with our data points ($R^2 = 0.707$, $R^2 = 0.728$ and $R^2 = 0.718$, respectively). Regression curves had improved fit ($p < 0.001$) for all ICC measurements when time 'days before parturition' was used; ICC length and ICC volume gave slightly better estimates than ICC diameter ($R^2 = 0.810$, $R^2 = 0.818$ and $R^2 = 0.800$, respectively. In conclusion, regression analysis of conceptus size measured by ICC length and ICC volume were slightly more accurate to estimate conceptus age than ICC diameter. Furthermore, on the basis of ICC volume and ICC length regression curves, it is apparent that the development of canine conceptus follows an exponential growth curve already during the early stages of pregnancy that is not reflected by ICC diameter measurements. ICC length measured by 2D ultrasonography may represent a more feasible target for improving accuracy of canine parturition date prediction than 3D volume calculations, which require specialized, expensive equipment and more time to process data.

Keywords: Dog, 3D ultrasonography, embryo, pregnancy, parturition prediction

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Vaginal vault diverticulum causing functional urinary obstruction in a maiden bitch

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A 14-month-old German Shepherd bitch presented with a bloody vaginal discharge, followed by an inability to void her bladder. Approximately 6 weeks prior, she had a dark colored vaginal discharge interpreted as her first estrus. The vaginal discharge at presentation was initially assumed to be associated with the development of a pyometra. Ultrasonography revealed normal (no fluid) uterus and an overly distended urinary bladder. Work up included comprehensive physical examination, radiographs, contrast study of her bladder and urethra, CBC, chemistry panel, urinalysis, vaginal cytology, vaginoscopy, culture of the vaginal discharge, and ultrasonography by a board-certified radiologist. No cystic calculi were detected in any imaging. Fluid was present caudal to the cervix. Her bladder required multiple episodes of catheterization to manage her functional urinary obstruction, pending surgery. Exploratory laparotomy was performed to assess the structural abnormality. The urinary bladder, uterus and ovaries were normal. No other abnormalities were observed in the abdominal cavity. A large diverticulum containing dark red blood-tinged fluid was the only structural abnormality detected at exploratory. The ventral aspect of the diverticulum was incised and evaluated. The cervix appeared ~ 4 times the size of a normal cervical os at this stage of estrus. The dorsal median fold was pronounced. The remaining vaginal vault was normal with the exception of the diameter. Redundant vaginal tissue was excised, and the vaginal vault was closed with a two-layer continuous absorbable suture. Redundant tissues were submitted for histopathology. Celiotomy incision was closed routinely after the area was explored and lavaged. Bitch recovered unremarkably and was subsequently able to void her urinary bladder voluntarily. Her uterus and ovaries were spared with the anticipated use as a brood bitch. As of this report, she remains clinically normal. She has not yet had a subsequent estrus and her reproductive future is unknown.

Keywords: Vaginal diverticulum, functional urinary obstruction, vaginal distension, excess vaginal fluid

Screening canine sera for smooth brucella strain antibodies via *Brucella abortus* fluorescent polarization assay

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Dogs can serve as hosts for all 4 of the most common zoonotic strains of *brucella*: the smooth strains (i.e. *Brucella abortus* [*B. abortus*], *B. melitensis*, *B. suis*, and the rough strains [i.e. *B. canis*]). However, due to differences in cell wall morphologies between the smooth and rough strains, the only validated serologic test currently available for brucellosis screening in dogs is limited to the identification of the rough strains (i.e. *B. canis*) alone. Recently, the USDA added the fluorescent polarization assay (FPA) as an approved test for the confirmation of brucellosis infection in cattle, bison, and pigs, due to the demonstration of sufficient cross reactivity between *B. abortus*, *B. melitensis*, and *B. suis*. Thus, the goal of our study was to utilize the FPA test to identify antibodies to smooth brucella strains in canine sera, and to compare the results of the FPA test to the commonly utilized *B. abortus* card agglutination (BCA) test. Sera from 95 clinically healthy dogs, including 45 hog hunting dogs, were screened for circulating antibodies utilizing a combination of canine *brucella* slide agglutination test (CBSA), *Brucella canis* agar gel immunodiffusion II test (AGID), and BCA and FPA tests. Suggested test interpretation results yielded a 0% (0/95) smooth *brucella* strain seropositivity rate, with 38/95 (40%) BCA positive results, and 0/95 (0%) FPA positive results. Rough brucella strain serology yielded an inconclusive result (0 - 2% rough strain seropositivity rate) in 2% (2/95) of dogs. Additionally, a retrospective portion of the study was performed to identify sera containing circulating antibodies to any of the smooth strains of *brucella* via *Brucella abortus* FPA by testing banked canine serum samples that had been submitted to Cornell's veterinary diagnostic laboratory between 2018 - 2019 and previously screened by CBSA and AGID for *B. canis* with a positive or inconclusive test interpretation result. Of the 769 serum samples tested, 30/769 (4%) yielded a positive FPA test result, 13/769 (1.7%) yielded an inconclusive result, 725/769 (94.2%) were negative, and 1/769 (0.1%) sample excluded due to insufficient sample remaining to perform the diagnostic test. Of the 30 FPA suspect positive canine serum samples, 97% (29/30) also tested positive on CBSA. Additionally, the signalment of FPA suspect positive dogs was much more likely ($p < 0.0001$) to be spayed or neutered compared to intact, and mixed breed compared to purebred.

Keywords: Brucellosis, canine, abortion, smooth strain, fluorescence polarization test

Fetal loss at time of elective cesarean section in a dog

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A 3-year-old Greater Swiss Mountain dog was presented exhibiting prolonged signs of stage 1 labor. Abdominal radiographs obtained 6 days prior revealed 8 fetuses appearing viable with heartbeats >180 bpm on ultrasonographic examination. Owner elected for cesarean section. Surgical site was prepared while the patient was given supplemental oxygen. An anesthetic protocol of alfaxalone for induction and isoflurane for maintenance were utilized. Time from anesthetic induction to removal of the first puppy from the uterus was ~ 8 minutes. The first, fourth, and seventh puppy delivered resuscitated in < 2 minutes. Five remaining puppies did not respond to any resuscitation efforts including accordion technique, GV26 acupuncture point, and epinephrine. When GV26 acupuncture points were performed substantial bleeding was noted from the site. On physical examination the 5 puppies were also judged to have distended abdomens. Paracentesis was performed and abdomen of each puppy was full of what was suspected to be frank blood. Blood was sent for CBC/coagulations panels. Radiographic images of the neonates were obtained and 4 of 5 puppies had fluid in their lungs. Three of the 5 struggling puppies had faint heartbeats. After 40 minutes of resuscitation efforts with no improvement the owner elected to discontinue the efforts. Blood had markedly decreased platelets with very minor platelet clumping observed. Many of the platelets were enlarged, suggesting accelerated platelet production of the bone marrow. A neutropenia was present. Prothrombin time and APTT were markedly increased, fibrinogen was markedly decreased, and D-Dimer was severely elevated. Three fetuses that resuscitated quickly had no abnormalities on physical examination and there were no concerns going forward. Multiple consults and discussions about this case did not provide answers as to why the fetal loss occurred. P2Y12 is a platelet disorder present in greater Swiss Mountain dogs. The sire was negative, and the dam was negative or a carrier. It is unclear if a natural whelping would have yielded better results. The purpose of this case is to present an occurrence of multiple unexplained fetal losses and bring awareness to a possible phenomenon. Despite much criticism from the greater Swiss Mountain dog breed club for isoflurane use during cesarean sections due to a supposed breed sensitivity, we have not changed our anesthetic protocol. Multiple cesareans have been performed on greater Swiss Mountain dogs since this case with a 100% fetal resuscitation rate.

Keywords: Fetal loss, cesarean section, clotting disorder

Sperm acrosome associated 3 protein expression in the feline ovary decreases with age

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Sperm acrosome associated 3 (SPACA3) is a lysozyme-like protein previously identified in 5 to 7.5-month-old cat ovarian follicles.[1] The objective of this research was to compare SPACA3 expression in young (2 months [n = 3]) and adult (>12 months [n = 3]) queens in different follicular stages. We hypothesized that SPACA3 expression does not differ by age. Routine immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections, followed by image analysis using FIJI software. Heat-induced epitope retrieval with sodium citrate (pH 6.1) was used. AntiSPACA3 polyclonal antibody (#HPA023633, Atlas Antibodies) was applied at 1:200 dilution and immunostaining specificity was verified by replacing the primary antibody with negative control rabbit serum on adjacent sections. Sections were then reacted with one-step horseradish peroxidase-conjugated polymer antirabbit IgG (IH-8064-custom-OrSU, ImmunoBioScience), followed by a NovaRED peroxidase substrate (#SK-4800, Vector Labs). Representative images of each follicle stage from each ovary were digitally captured using QCapturePro image capture software by a single observer at 200 x magnification. Cellular expression of SPACA3 was then quantified in primordial (0°), primary (1°), secondary (2°), and tertiary (3°) follicles using FIJI software with RGB stack and manual thresholding to isolate areas of staining. The oocyte nucleus, granulosa cells, and theca cells were outlined using the freehand selection tool and mean gray value was measured. Results (mean ± SEM) were compared between young and adult queens using a Student's *t*-test and significance was defined as $p < 0.05$. There was greater SPACA3 expression in young compared to adult queens (Figure) in the oocyte nucleus of 0° ($p < 0.001$), 1° ($p = 0.006$), 2° follicles ($p < 0.001$), in granulosa cells of 1° ($p = 0.016$), 2° ($p < 0.001$), 3° follicles ($p = 0.004$), in theca cells of 2° ($p = 0.006$), and in 3° follicles ($p = 0.043$). This is the first study to evaluate differences in SPACA3 expression by age in any species. More research is needed on the mechanisms that regulate ovarian SPACA3 expression and its role in female fertility.

Keywords: Cat, granulosa cell, oocyte nucleus, ovary, theca cell

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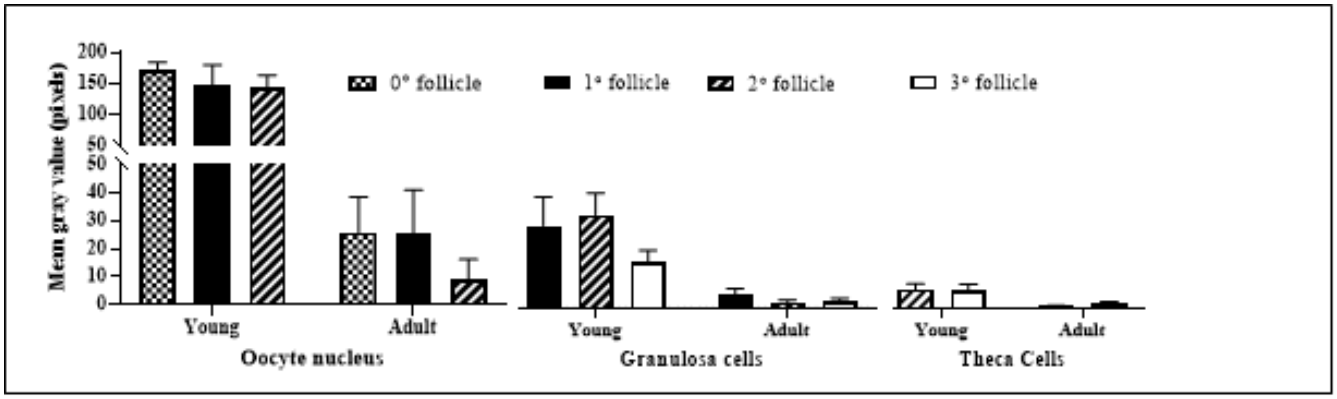

Using reflection to optimize student engagement during theriogenology practical classes: the benefits of mentoring and peer support in teaching

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Veterinary training is a resource consuming process. Practical sessions throughout the curriculum are considered vital aspects of professional training and consume large amounts of time, space and human resources and are not without risks to human and animal health.[1] It is therefore, important that the educational experience is optimized during practical sessions. Mentee dialogue with mentors, as part of institution peer teaching support, is aimed to optimize the practical sessions in order to develop professional expertise (knowledge and skills) of veterinary students learning theriogenology. We used the theory of experiential learning and reflective observation as a means of enhancing learning during the practical session.[2] We attempted to do this by discussing abstract concepts associated with the authentic learning tasks covered in each practical session. Anonymous end-of-course student feed-back revealed that the process encouraged in depth and alternative critical thinking and discussion in the groups that was a fun way for them to embed the knowledge and develop the skills being taught. The use of 'abstract reflection' appears to be a really useful and efficient

way of enhancing the value of laboratory practical teaching and learning resources within the veterinary theriogenology curriculum. Interestingly, a few of the reflective questions were formulated so as to not have answers that were known by the academic staff. The dissonance was relished by some of the cohort, those excited by investigation and research, but appeared to induce chagrin in a minority that were uncomfortable with the unknown. This was despite the whole group being made aware that this was a safe and fun environment that developed during the session. An important aspect of the peer review process has been the continued discussion between mentors and mentees in relation to enhancing teaching in general, and practical sessions in particular. The vibrancy associated with collegiate interactions between academic staff members and educational designers results in a more enthusiastic and beneficial teaching and learning environment.

Keywords: Abstract reflection, experiential learning, peer support

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Agreement level among 4 techniques for analysis of stallion sperm morphology

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Analysis of sperm morphology is important for assessment of sperm quality and prognostication of fertility potential of stallions. Previous studies have compared various techniques for morphologic analysis of stallion sperm using correlation analysis or mean comparisons. We hypothesized that such approaches might lead to erroneous interpretations when comparing different methods of sperm morphologic analysis. The present study sought to determine the agreement level among four techniques for analysis of stallion sperm morphology: eosin/nigrosin staining (EN); diff-quick staining (DQ); wet mount using phase-contrast microscopy (PH); or wet mount using differential interference contrast microscopy (DIC – gold standard). Ejaculates from 12 sexually active stallions (n = 36) were collected and analyzed using each technique. A total of 100 sperm were observed under 1,000 x magnification and classified as described by the Society for Theriogenology (normal [N], abnormal head [AH], abnormal acrosome [AA], proximal droplet [PD], distal droplet [DD], abnormal midpiece [AMP], bent midpiece [BMP], and coiled tail [CT]). Based on the percent normal sperm observed by DIC microscopy, sperm morphology was categorized in each of 3 categories (n = 12/category) as high (H): > 57% normal sperm, average (A): 23 - 56% normal sperm, or low (L): < 23% normal sperm. Within each morphology category (H, A, L), the agreement level (bias, 95% lower and upper limits of agreement) was determined (Table). The results from this study indicate that the use of EN or DQ leads to an overestimation (negative bias value) of normal sperm, as well as an underestimation (positive bias value) of morphological defects that are known to impact stallion fertility, such as AH or AMP (values in bold). Most discrepancies among methods were observed in categories A and L. These results may affect the interpretation of sperm morphology evaluation and, thus, the estimation of stallion potential fertility.

Keywords: Stallion sperm, sperm morphology, stained smear, wet mount, agreement

Category	Comparison	% N	% AH	% AA	% PD	% DD	% AMP	% BMP	% CT
High	DIC vs. EN	**-4 (-15, 7)**	**11 (7, 16)**	4 (-5, 15)	-2 (-10, 7)	-1 (-17, 15)	**4 (-3, 11)**	4 (-6, 13)	0 (-4, 3)
	DIC vs. DQ	**-5 (-14, 5)**	**12 (3, 20)**	4 (-5, 14)	4 (-12, 4)	3 (-11, 18)	**5 (-3, 12)**	3 (-8, 13)	0 (-3, 3)
	DIC vs. PH	**0 (-7, 8)**	**4 (-19, 10)**	4 (-5, 12)	2 (-10, 13)	6 (-10, 22)	**-1 (-13, 11)**	3 (-6, 11)	0 (-2, 3)
Average	DIC vs. EN	**-11 (-29, -6)**	**12 (6, 18)**	3 (-3, 8)	6 (-17, 28)	-1 (-11, 9)	**8 (-9, 24)**	4 (-7, 15)	0 (-2, 2)
	DIC vs. DQ	**-7 (-23, 9)**	**4 (-4, 12)**	3 (-4, 10)	3 (-13, 18)	0 (-14, 14)	**3 (-15, 21)**	5 (-6, 16)	0 (-3, 3)
	DIC vs. PH	**-1 (-15, 13)**	**3 (-22, 27)**	1 (-10, 12)	1 (-15, 16)	0 (-10, 10)	**-4 (-20, 13)**	1 (-8, 11)	0 (-2, 3)
Low	DIC vs. EN	**-11 (-25, -5)**	**20 (17, 24)**	5 (-7, 17)	6 (-22, 34)	-2 (-11, 9)	**7 (-7, 20)**	7 (-12, 25)	0 (-8, 9)
	DIC vs. DQ	**-9 (-20, 3)**	**19 (14, 24)**	3 (-3, 10)	13 (-12, 40)	0 (-20, 21)	**2 (-14, 17)**	7 (-9, 23)	-1 (-8, 6)
	DIC vs. PH	**-2 (-10, 7)**	**6 (-26, 38)**	1 (-16, 17)	5 (-22, 33)	-2 (-17, 12)	**-3 (-23, 17)**	5 (-14, 24)	-1 (-8, 10)

PRODUCTION ANIMAL SESSION

Preliminary report on bovine prenatal sex determination using PCR in maternal peripheral blood

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Fetal sex identification at pregnancy diagnosis may prove beneficial to beef producers as they could make herd management decisions in an organized manner well in advance. Realtime ultrasonography is an ideal tool to obtain immediate information about the pregnancy status and fetal sex; however, the method is time-consuming, needs expertise, and lacks accuracy beyond 90 days of pregnancy. Alternatively, sex-chromosome-specific genes of the fetal DNA circulating in maternal peripheral blood can be targeted using polymerase chain reaction (PCR), but the technique needs further validation before its commercialization. Therefore, the objective of the present study was to identify an appropriate PCR methodology to target sex-chromosome-specific genes to detect fetal DNA in maternal plasma for determining fetal sex in pregnant cows. We hypothesized that both bovine amelogenin (bAML) and Y-chromosome specific genes are targets via PCR to predict fetal sex in cattle. In this initial experiment, we enrolled dairy cows (n = 5; 4 - 5 months pregnant) carrying male fetuses (confirmed by ultrasonography) from the Rayner Dairy Research and Teaching Centre at the University of Saskatchewan, Saskatoon, Canada. We collected 18 ml blood from each cow in K2 EDTA vials to harvest plasma. Fresh plasma and frozen aliquots were processed for DNA extraction using DNeasy Blood and Tissue, MagMAX cfDNA isolation, KAPA express extract, Nucleomag cfDNA isolation, QIAamp DNA Blood Midi, and QIAamp DSP Virus Kits. In addition, blood cells from bulls (n = 5) and nonpregnant heifers (n = 5) were processed for DNA isolation and subjected to PCR to validate primers and identify the PCR conditions. Isolated DNA from the plasma of pregnant cows was used as a template for bAML and Y-specific gene PCR to predict fetal sex. No statistical comparisons were carried out due to the small sample size. The experiment results indicated that when frozen plasma samples were processed for DNA isolation (DNeasy Blood and Tissue Kit, MagMAX cfDNA isolation Kit), PCR failed to predict fetal sex. However, PCR on DNA isolated from fresh maternal plasma using the QIAamp DSP Virus, DNeasy Blood and Tissue, Nucleomag cfDNA isolation, and MagMAX cfDNA isolation Kits correctly predicted the presence of male fetii in 3/5, 2/5, 2/5, and 2/5 cows, respectively. However, PCR on the DNA obtained from both QIAamp DNA Midi and KAPA express Kits failed to predict male fetus in all 5 cows. In conclusion, the DNA isolation methods compared so far had variation in their ability to isolate fetal DNA from the maternal plasma of pregnant cows. We are pursuing spiking experiments with fetal DNA from aborted fetuses to further validate DNA extraction methodology for cell-free DNA from maternal plasma.

Keywords: Bovine amelogenin, fetal DNA, Y-chromosome specific gene, fetal sex, pregnant cattle

Melatonin and l-arginine mitigate heat stress-induced reductions in quality of frozen-thawed ram sperm

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Adding melatonin or l-arginine to semen extender enhanced postthaw sperm quality and protected against cryopreservation-induced oxidative stress.[1,2] The objective was to determine the effects of melatonin or l-arginine on quality of frozen-thawed sperm from rams subjected to heat stress (HS). We hypothesized that addition of melatonin or l-arginine mitigates the effects of heat stress on frozen-thawed ram sperm. Ten Dorset rams with good semen quality were group-housed indoors (~ 18˚C), randomly allocated into 2 equal groups and subjected to either whole-scrotum insulation for 96 hours or placed in a warm room (28˚C, 30 - 34% relative humidity) for 8 hours per day for 4 consecutive days. Semen was collected weekly for 1 - 5 weeks after HS, extended (Steridyl CSS One Step®) and divided into 5 aliquots: no additives (control) or 0.5 or 1 mM of either melatonin or l-arginine. For cryopreservation, semen was refrigerated for 2 - 3 hours, cooled to 5˚C, then loaded into 0.5 ml straws that were placed in straw racks and held horizontally in a styrofoam box, 5 cm above liquid N_2 for 10 minutes and then plunged into liquid N_2. Straws were subsequently thawed at 37˚C for 35 seconds and immediately evaluated for postthaw motility using CASA (Sperm Vision®),

morphology using eosin-nigrosin, and acrosome integrity using FITC-PSA. Data were analyzed using repeated measures, with a post-hoc Bonferroni test. For total and progressive motility, there were effects of group ($p = 0.023$ and $p = 0.0008$, respectively); for total abnormalities, there were effects of group ($p = 0.001$ and a group*week interaction ($p = 0.003$); and for acrosome integrity, there were effects of group ($p = 0.046$) and week ($p = 0.0001$). On all days, all end points were significantly improved for all treatments compared to control. All 4 treatments improved motility, whereas improvements in total abnormalities and acrosomal integrity were dose-dependent (greatest improvement with 1 mM). Total and progressive motility were improved by ~ 5 - 10 percentage points, whereas total abnormalities and intact acrosomes were improved by ~ 7 and 12 percentage points, respectively. Bowed midpiece, ruffled acrosome and distal midpiece reflex were highest in the control group. In summary, exogenous melatonin or l-arginine in semen extender mitigated HS-induced reductions in quality of frozen-thawed ram sperm by improving motility and acrosome integrity and reducing total abnormalities.

Keywords: Ram, sperm, melatonin, l-arginine, Heat stress

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An example of incorrect storage of bull semen samples on spermiogram assessment

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Sperm morphology assessment and interpretation is an integral part of bull breeding soundness evaluation. Historically the spermiogram has been classified into primary, secondary, and tertiary; major or minor; compensable or uncompensable abnormalities; and reporting individual sperm defects. Abnormalities that might occur after semen collection (tertiary abnormalities) are often discussed. However, these abnormalities are generally not well defined nor explained. We report tertiary abnormalities that were detected because of incorrect samples submitted for morphological assessment. Samples from a cohort of bulls were collected for morphological assessment as part of breeding soundness examination. The samples were examined grossly, crush side motility was assessed by diluting the sample in isotonic phosphate buffered saline (PBS). An ejaculate aliquot was placed in 10% buffered formol saline (BFS) for morphological assessment via differential interference contrast microscopy at 1000 x magnification. In this case, some of the PBS diluted samples were inadvertently sent for morphological assessment in the first instance. The results appeared aberrant, with a large proportion of loose and detached heads, and abnormal tails. The correctly stored samples were located and subsequently assessed. PBS and BFS samples ($n = 13$) had substantial differences in spermiograms between the storage methods. The BFS samples had 12/13 spermiograms with ≥ 68% morphologically normal sperm. By comparison, 1/13 of the PBS samples had ≥ 68%, with 5/13 having fewer than 20% normal sperm. There were 2 samples in the BFS cohort that had ≥ 19% loose or detached heads, compared to 12 in the PBS cohort that had ≥ 35%, 7 of which had ≥ 55% loose or detached heads. Most of the abnormalities detected in the PBS samples were a combination of loose and detached heads. Interestingly, the tails, particularly the detached tails, were noticeably devoid of the plasma membrane for some or most of the principal piece and other parts of the tail. These were typically not documented in the interpretation of the spermiogram, as most were recorded as detached heads. Particular tertiary abnormalities are not often described in the literature. The inadvertent error of assessing the incorrect samples has given an opportunity to report abnormalities that are most likely due to incorrect storage of samples for morphological assessment of an ejaculate. It is clear from these observations that appropriate collection and storage of samples for morphological assessment is carried out when assessing spermiograms. Incorrect sample preparation and storage should be considered as a reason for an abnormal spermiogram, especially when a large proportion of detached heads, with or without tail plasma membrane abnormalities, are detected in a semen sample.

Keywords: Bull, spermiogram, tertiary morphological abnormalities, detached heads

Obstructive urolithiasis in a dromedary camel

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A 9-year-old, castrated, male dromedary camel was presented with an inability to urinate for 1.5 days. The camel had a history of severe malnutrition and had been castrated prior to onset of puberty. The camel was maintained in a petting zoo and had received an excessive amount of grain prior to his presentation. The animal was bright, alert and responsive with moist and pink mucous membranes, but had mild icteric sclera and was posturing to urinate. There were perineal urethral pulsations accompanied by tail flagging. Severe enlargement of the bladder was diagnosed via transrectal palpation and ultrasonography. The bladder was ~ 12 inches in diameter with a thickened wall and mucus debris within

the fluid. On abdominal ultrasonography, there was no free abdominal fluid observed. Ultrasonography of the penis and urethra revealed a hyperechoic structure proximal to the glans penis, and the urethra was intact. The penis could not be extended for examination due to nonseparation of the penis from the prepuce as a result of early castration. The urine pH was 6.0 and specific gravity was 1.028. On microscopic examination of the urine sample urate crystals were observed. Serum chemistry had an elevated BUN (52.3 mg/dl; normal range 11 - 30), creatinine (7.6 mg/dl; normal range 1-2.3), AST (79 U/L) and serum iron (22 µg/dl). The diagnosis was obstructive urolithiasis. A tube cystotomy surgery was done in a 'cushed' position using the following intravenous anesthetic protocol: detomidine (0.03 mg/kg), torbugesic (0.06 mg/kg) and ketamine (2.5 mg/kg). The bladder was accessed with a blind stick using a scalpel blade. A Foley catheter with a stylet was inserted through the incision and the bladder was flushed with saline solution. The tube was sutured in place to allow for urine expression. Antibiotic therapy with ceftiofur crystalline free acid (6.6 mg/kg subcutaneous, daily) and sulfadimethoxine (55 mg/kg initial dose, 27.5 mg/kg subsequent doses, intravenously, daily) and an antiinflammatory (flunixin meglumine 1.1 mg/kg, intravenously, once every 12 hours) was started. Acepromazine (20 mg intravenously) to cause urinary tract relaxation and fluid therapy (5 liter bolus, once every 12 hours) were done. There was a continuous and steady urine dripping from the Foley catheter after the surgery and during the next 3 days, so the treatment plan was continued. However, on the fourth day after surgery, the animal was seen posturing to urinate and the bladder was lavaged with 5 liters of saline solution, during which bloody drops were noted in the prepuce. Urine had a pH of 7 and struvite crystals were seen. Hence, a total of 120 ml of Walpol's solution was placed in the bladder. Then, 30 minutes later, another 5 liters lavage was done. The next day, the Foley catheter had become occluded due to fibrin deposition. The animal was posturing more frequently, although the bladder was small on ultrasound. Animal was sedated in accordance with the previous surgery and an epidural was given with 3 ml of lidocaine. A perineal urethrostomy and penile amputation was performed while the animal was in a cushed position. The urethra was then spatulated at the level of the skin incision and a catheter was placed. The camel continued to urinate successfully, and treatment was discontinued. The animal was sent home on allopurinol to be given every other day to aid in reduction of uric acid and was instructed to receive a low grain diet. Early castration in camelids can predispose to a narrowed urethra that coupled with a high grain diet may lead to urolithiasis.

Keywords: Camelid, urogenital system, bladder, urethra

Evaluation of ovarian response to PG600 in alpacas

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The hormonal preparation PG600, a combination of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), is commonly used for swine estrus synchronization. However, it is often used off-label in sheep and goats to replace the eCG formulation not available in the US. In alpacas, eCG has been used alone or in combination with FSH to induce superovulation, with variable results. The aim of this preliminary trial was to investigate the effect of PG600 on follicular dynamics in alpacas. We hypothesized that a single treatment of PG600 (800 IU eCG and 400 IU hCG in 5 ml) 2 days after ovulation results in ovarian follicular superstimulation in alpacas. Adult multiparous alpacas (n = 9) were used in the experiment. Ovarian follicular activity was monitored by transrectal ultrasonography. Ovulation was induced with GnRH (100 µg) given intramuscularly when a dominant follicle reached at least 8 mm in diameter and uterine tone and edema were present. All females received 5 ml PG600 intramuscularly 2 days after induction of ovulation. Ovarian follicular response was assessed by transrectal ultrasonography on day 7 after PG600 treatment and the follicles were recorded and counted by 2 clinicians. Ovulation was induced with GnRH (100 µg) given intramuscularly. The mean number of follicles between 7 and 12 mm in diameter present in the ovaries after the treatment was 14.9 ± 13.8 (mean ± SD). There was a large variation among females in the number of follicles, which ranged from 1 to 38. The ovulation rate (number of corpora lutea) following induction was very low 1.1 ± 1.6 (mean ± SD). The maximum ovulation rate (5) was observed in a female that had 6 follicles after stimulation. All females presented anovulatory hemorrhagic follicles 2 days after induction of ovulation (10.0 ± 11.5, mean ± SD). In conclusion, PG600 induced ovarian follicular stimulation (> 2 mature follicles) in 7 out 9 alpacas. However, the follicular response had large individual variability. The ovulation rate after ovarian superstimulation with PG600 was poor. However, this poor ovulatory response has been also observed with eCG and FSH. This preliminary trial suggests that PG600 may not be appropriate for ovarian superstimulation in multiple ovulation embryo transfer programs in alpacas but could be used for oocyte recovery within other advanced reproductive techniques.

Keywords: Ovulation, superovulation, camelids, anovulatory follicles

Morphometric characteristics, testicular histology, and semen parameters in mature hybrid bucks born from white-tailed deer dams sired by a mule deer buck

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Despite white-tailed deer (WTD) and mule deer (MD) sharing similar number of chromosomes (n = 70), the 2 species have distinct features making the crossing of species unsuccessful in wild or captive conditions. Across mammals, hybrids are regarded as infertile, yet anecdotal reports suggest that WTD-MD hybrids are fertile. However, there have been limited reports of hybrid animals producing fully formed sperm, though none on hybrids of WTD-MD. We aimed to describe the somatic morphometric features, testicular histology and semen features of captive hybrids of WTD-MD. Four 1.5-year-old bucks were enrolled in this study, 2 hybrids WTD-MD, 1 WTD, and 1 MD from a captive farm. The hybrid animals were born from different WTD, but were sired by the same MD. Morphometric profile included thorax circumference, crown-rump length, metacarpus and metatarsus diameter, tail length, tail color, location of the metatarsal gland, antler configuration, antler inside spread, ear length, metatarsal tuft color, scrotal circumference and length of the penis. Semen collected via electroejaculation was evaluated for the presence of sperm and the concentration of alkaline phosphatase (ALP). Testicular biopsies were collected from both testes using a split needle biopsy tool. The scrotal circumference was 22 cm for the WTD, 19 cm for the MD, and both hybrids measured 12 and 14 cm, respectively. The penis length was 28.6 for the WTD, 27.4 for the MD and 12 and 14 cm for the hybrids. It has been suggested that testosterone regulates penis growth after puberty; perhaps these animals had lower testosterone production hence the shorter penis. Semen collection yielded ~1 ml of yellow and viscous fluid. No sperm were visualized under the phase-contrast microscope. Histologic evaluation revealed the presence of hypoplastic seminiferous tubules in both animals populated with spermatogonia in the basal compartment and normal Sertoli cells. One animal had primary spermatocytes in the adluminal compartment and scattered spermatids could be seen in a few seminiferous tubules. The basal membrane of the seminiferous tubules was surrounded by dense, irregular connective tissue. The Leydig cells were present in the interstitium and appeared morphologically normal; this explains why the hybrids were able to produce intermediate sized antlers that hardened at the peak of rut. Concentrations of ALP in hybrid 2 was 1620 U/l. The color of the tail of the hybrids was brown on the dorsal surface, but white on the ventral part and resembled 1 of the WTD. The metatarsal gland was in the proximal segment of the metatarsus in the hybrids and WTD, whereas it was below in the MD. Only the MD presented dichotomous antlers. The metatarsal tuft color was brown in the hybrids and MD, but white in the WTD. In conclusion, hybrid 1 male was unable to complete spermatocytogenesis, and the second could not complete spermiogenesis, making then unable to fully form sperm. The high concentrations of ALP confirmed ejaculation in these 2 animals; however, they were deemed infertile.

Keywords: Azoospermia, infertility, mule deer, white-tailed deer, hybrids

Association of metabolic status with uterine diseases and reproductive outcomes in lactating Holstein dairy cows

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Selection of high yielding dairy cows has predisposed them to develop metabolic and uterine diseases. While the association of hyperketonemia (HRK) with metritis and decreased reproductive performance is known, little data exists examining the association of HRK and concurrent hypoglycemia with metritis and reproductive outcomes. Our hypothesis was that cows with HRK have a higher incidence of puerperal metritis and poorer reproductive outcomes than cows without HRK and those effects are more profound in cows with hypoglycemia. The objectives of this study were to analyze the association of metabolic status with metritis incidence and reproductive outcomes in lactating dairy cows. Cows (n = 2651) had blood samples collected between 3 - 9 days postpartum (DPP) and whole blood beta-hydroxybutyrate (BHBA) and whole blood glucose was measured using a hand-held cow-side device validated in dairy cows (Precision Xtra, Abbott, Mississauga, ON, Canada). Hyperketonemia was defined as BHBA ≥ 1.2 mmol/liter and hypoglycemia was defined as glucose ≤ 2.2 mmol/liter. Cows were then categorized into the following 4 groups: first those having no metabolic abnormality (Norm, n = 1996), those having HRK only (BHBA, n = 260), those having hypoglycemia only (HG, n = 181), and those having both HRK and hypoglycemia (BHBA + HG, n = 214). Incidence of puerperal metritis (defined as watery, fetid discharge present at time of blood collection), and the reproductive outcomes first insemination pregnancy per AI (P/AI), pregnancy loss, average days open (DOPN), and proportion of cows pregnant at 150 DIM (P150) were compared for the 4 metabolic statuses enrolled in the study. The cow-level prevalence of hyperketonemia was 17.9% (474/2651), and the cow-level prevalence of hypoglycemia was 14.9% (395/2651). The cow-level prevalence for each metabolic category was as follows: Norm, 75.3% (1996/2651); BHBA, 9.8% (260/2651); HG, 6.8% (181/2651); and BHBA + HG, 8.1% (214/2651). Statistical analysis was performed using ANOVA and logistic regression with JMP Pro 13 (SAS Institute Inc. Cary, NC, US). Parity (Parity 1 (P1) versus Parity ≥ 2 (P2)), season, and farm were retained in the model. P2 prevalence of puerperal metritis was significantly less for cows in the HG group compared to cows in the BHBA and BHBA+HG groups

(HG, 1.9%; BHBA, 16.8%; BHBA+HG, 9.4%). P2 prevalence of puerperal metritis was significantly less in the Norm group compared to cows in the BHBA group but similar to cows in HG group (Norm, 6.7%; BHBA, 16.8%; HG, 1.9%). For primiparous cows there was no difference in incidence of puerperal metritis amongst metabolic categories. There were no differences between metabolic groups for P/AI, pregnancy loss, average DOPN, or P150. In conclusion, hyperketonemia of multiparous cows was associated with increased puerperal metritis; however, hypoglycemia alone was associated with decreased puerperal metritis compared to cows with elevated BHBA with or without concurrent hypoglycemia.

Keywords: Hyperketonemia, hypoglycemia, dairy cows, uterine disease

Effect of GnRH at artificial insemination for dairy cows detected in estrus by an activity monitoring system or by conventional estrus detection

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Artificial insemination (AI) after detected estrus constitutes a substantial proportion of AI's that occur in the US. Moreover, AI after detected estrus may be increasing with the use of activity monitors. We hypothesized that GnRH treatment at AI increases both ovulation and circulating progesterone concentrations, thereby improving pregnancies per AI (P/AI); additionally, we hypothesized that this effect is higher in farms using activity monitors. The objectives of the study were to determine if GnRH treatment at AI increases P/AI for lactating dairy cows detected in estrus on farms using activity monitors (AM) or not (NAM). Holstein cows were blocked by parity and randomly assigned to receive an injection of GnRH at AI (G-AI) or to receive no injection of GnRH (NG-AI) at AI on a farm using AM for estrus detection and on a group of 4 farms using NAM. On the farm with AM, 409 cows were enrolled (G-AI, n = 207; NG-AI, n = 202) and for the farms using NAM, 398 cows were enrolled (G-AI, n = 197; NG-AI, n = 201). Ovarian structures and plasma progesterone concentrations were assessed in a subset of cows (G-AI, n = 52; NG-AI, n = 55) detected in estrus by conventional methods at the time of AI and 7 days later. Data were categorized by milk production quartiles, genomic daughter pregnancy rate (High: > the median, Low: < the median), activity level (AL) for the farm using AM (High: AL > the median AL versus Low: AL < the median AL) and DIM (> 150 DIM versus < 150 DIM). Statistical analyses were performed using logistic regression and a Student's *t*-test. There were no differences in ovulation rate (G-AI = 83.2 ± 6.1%; NG-AI = 77.9 ± 5.5%) between G-AI and NG-AI. There were no differences in plasma progesterone concentrations at day of estrus detection (day 0) (G-AI = 0.16 ± 0.11 ng/ml; NG-AI = 0.09 ± 0.10 ng/ml) nor at day 7 after enrollment between G-AI and NG-AI (G-AI = 2.17 ± 0.15 ng/ml; NG-AI = 2.04 ± 0.15 ng/ml). Data for all farms were analyzed together for P/AI; no difference for P/AI at first pregnancy diagnosis (G-AI = 38.7 ± 3.9%; NG-AI = 40.9 ± 3.9%) or second pregnancy diagnosis (G-AI = 35.1 ± 4.1%; NG-AI = 35.7 ± 4.2%) was identified. No difference in P/AI between G-AI and NG-AI when farms were analyzed separately based on estrus detection method (AM separate from NAM) at first pregnancy diagnosis (AM: G-AI = 39.1 ± 5.0%; NG-AI = 38.6 ± 5.1%; NAM: G-AI = 38.3 ± 5.2%; NG-AI = 43.3 ± 5.2%) or second pregnancy diagnosis (AM: G-AI = 36.3 ± 5.1%; NG-AI = 33.8 ± 5.2%; NAM: G-AI = 33.8 ± 5.3%; NG-AI = 37.8 ± 5.2%) was identified. There was no interaction between treatment and method of estrus detection. For the farm using AM, there was a significant interaction between treatment and AL with the injection of GnRH having a greater impact on cows with high AL. In conclusion, GnRH treatment did not enhance P/AI. Additional studies are warranted to understand the interaction between treating cows with GnRH at AI and AL in herds using activity monitoring systems.

Keywords: Dairy cows, estrus detection, activity monitors, GnRH

POSTER SESSION

Uterus unicornis in a maiden mare

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Uterus unicornis is a congenital abnormality that has been reported in several domestic species, but occurs uncommonly in mares. A previous report described a successful foaling in a Warmblood mare with this condition, but the foal was small and dysmature.[1] Two additional cases reported in American paint mares have been described.[2,3] The case reported herein corresponded with a maiden Standardbred mare that was imported from Europe. Breeding management was attempted over 1 estrous cycle but the mare did not become pregnant. She was referred for a breeding soundness evaluation, as an abnormality was suspected on transrectal palpation of her reproductive tract. Palpation and transrectal ultrasonography of the reproductive tract revealed the absence of a normal left uterine horn. Both ovaries were normal in shape and size and ovary was active. The left ureter appeared normal and was traced to the left kidney. Urine was visualized moving through the left ureter. The right uterine horn appeared normal and no uterine edema or fluid was observed. Cervix was toned and had a competent canal. Hysteroscopic examination was performed and a normal uterine body, right uterine horn, and right oviductal papilla were observed. There was no evidence of a left uterine horn presence. Based on these findings, a diagnosis of uterus unicornis or segmental aplasia of the Müllerian ducts was made. This abnormality has been observed in other species. In cattle, it is associated with a white coat phenotype in breeds such as Shorthorn and Belgian blue.[4] This condition is associated with failure of the development of the Mullerian ducts and appears to be genetically inherited. Breeding this mare to carry to term was discouraged and assisted reproductive techniques such as embryo transfer and ovum pick up were recommended. The client decided not to utilize this mare for breeding. This is the first report of uterus unicornis in a Standardbred mare. Although rare, this condition can cause infertility in mares and may accompany other genetic or congenital abnormalities. It is important to pursue genetic testing to ensure the mare does not have karyotypic abnormalities. Further diagnostic procedures to assess genitourinary function are also important as renal agenesis and ureteral abnormalities can occur ipsilateral to the affected uterine horn.[3]

Keywords: Uterus unicornis, congenital abnormality, mare infertility

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A congenital abnormality in a mare: nonpatent uterus

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Failure of normal uterine development was diagnosed in in a 4-year-old Thoroughbred mare. Congenital abnormalities of mares have been described; segmental aplasia in other species, such as cattle, is often associated with genetic abnormalities. This mare had no history of uterine infusions, breeding, or vaginal examination prior to initial evaluation performed at 3 years of age (in winter) while in training. Transrectal palpation and speculum examination revealed no abnormalities. Mare was presented again 2 months later for a prebreeding evaluation. Mare had normal overall appearance, body condition score 6/9, normal mammae and external genitalia with a Caslick's in place. Transrectal palpation and ultrasonography revealed a mildly flaccid uterus, normal shape and size to the ovaries with multiple 20 mm follicles. Scant free fluid was observed in the uterine horns with swirling heterogeneous echogenic material and with no endometrial edema. Cervix was short and flaccid. Vaginoscopy revealed a small hypoplastic cervix

and external os and digital evaluation revealed a patent but short canal with no communication into uterine lumen. Hysteroscopy was performed and the cranial cervical canal failed to connect to uterine lumen. Fine spiderweb trabeculae of tissue traversed the area cranial to the cervix, consistent with subepithelial tissue that was distended with air. However, no uterine lumen and there was no evidence of normal endometrium. Chromosomal analysis of the mare revealed normal female karyotype. These findings are consistent with a congenital abnormality and canalization failure of a normal uterine lumen. In some cases, uterine exposure to caustic substances can result in this condition; however, this mare had also had normal endometrium. As diagnosed in this case, congenital malformation of the uterus (that prevented normal ability to carry a pregnancy) can be missed with routine evaluation.

Keywords: Mare, segmental aplasia, uterine and cervical hypoplasia, congenital abnormality

Ovine male pseudohermaphrodite with testes adjacent to mammary gland in a sheep

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Male pseudohermaphrodite sheep have been reported with variations in genotype and phenotype.[1] A Katahdin sheep was born as a triplet with 1 male and 1 female siblings. At birth, the sheep had what was considered normal ewe lamb phenotype. At 1.5 years of age the phenotype of the sheep described included a long-haired mane and heavy muscling. The lamb siblings developed normal ewe and ram phenotypes. The sheep's vulva had a prominent ventral bulge with increased hair and a prominent clitoris, consistent in appearance with a glans penis. Tip of the glans penis had a very short urethral process. The animal demonstrated behavior consistent with a male including protective 'ramming' and mounting of its pasture mates. Transabdominal ultrasonography could not confirm structures consistent with testicular parenchyma. Serum testosterone concentrations were 5.0 pg/ml. Based on the unwanted aggressive behavior, an exploratory laparoscopy was performed in an attempt to identify any testicular tissue, and if present, remove it. Exploratory laparoscopy revealed bilateral tubular structures consistent with the vas deferens originating from the inguinal canal and reaching the dorsal aspect of the urinary bladder. External palpation identified 2 ovoid structures (~ 5 x 3 cm) located between mammary tissue and body wall. Skin consistent with scrotal skin (wrinkled and slightly red) was observed in bilateral regions 2 cm in diameter caudal to the mammary gland. These 2 structures were removed via a 3 cm incision made lateral to the mammary tissue. Histopathology revealed testicular tissue with abortive spermatic tubules, lined by Sertoli cells without germinal cells, and bilateral suppurative epididymitis. No female gonadal tissue was identified by light microscopy. Karyotype revealed a mixed population of genetically female 54, XX (80%), and male XY (20%) lymphocytes. This may indicate blood chimerism or true somatic mosaicism and DNA analysis from an ear punch is pending. A case of an unusual location of extra-abdominal but undescended testes in a male pseudohermaphrodite Katahdin sheep is described.

Keywords: Male pseudohermaphrodite, sheep, bilateral epididymitis, triplets

Reference

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Effect of hyaluronic acid on fresh-cooled extended equine semen: sperm motility

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Hyaluronic acid (HA) is a glycosaminoglycan, has a role in in vitro sperm-oocyte binding and exerts antioxidant properties.[1,2] Conflicting results regarding the benefit of addition of HA to freezing extender on postthaw motility exist.[2] This study determined whether addition of sodium hyaluronate (Hytryl®, 10 mg/ml, KineticVet, Lexington, KY) at varying concentrations to 2 milk-based extenders affected motility parameters of fresh-cooled equine semen stored at 5°C for up to 72 hours. We hypothesized that the addition of Hytryl®, 10 mg/ml to fresh-cooled equine semen increases total and/or progressive motility, benefitting the longevity and quality of equine fresh-cooled semen. Ejaculates from 8 stallions were extended in either INRA (IMV Technologies, IMV Technologies, L'Aigle, France) or CST (Animal Reproduction Systems, Chino, CA) with no or differing concentrations of Hytryl® at 0, 100, and 1,000 µg/ml. The samples were stored in a passive cooling device (EquiSaver, IntegriTemp, Omaha, NE) and cooled to 5°C for up to 72 hours and aliquots were incubated at 37°C for 5 - 10 minutes prior to motility evaluations with a computer assisted sperm analysis (CASA; SpermVision®, Minitube, Verona, WI). Sperm motility of each treatment group was compared at time 0, 24, 48, and 72 hours postcollection. Means of total and progressive motility parameters were subjected to the mixed procedure of SAS® for statistical analysis. Means were compared using Tukey's range test at a significance level of alpha = 0.05. There were no significant differences in total or progressive motility

among treatments at any time point. In conclusion, addition of hyaluronic acid to 2 milk-based extenders did not affect motility parameters of fresh-cooled equine semen. Additional work is necessary to determine whether there is any benefit to stallion fertility with HA in semen extenders.

Keywords: Stallions, sperm, motility, fertility, hyaluronic acid

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Methods to prepare platelet-rich plasma

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Platelet-rich plasma (PRP) is a biological by-product commonly used in clinical practice to treat orthopedic and dermatologic conditions. Recently, use of PRP has become popular in management of a mare's reproduction to mitigate postbreeding induced endometritis and improve fertility. Currently, there are no standardized methods to prepare PRP for intrauterine use in mares. The aim of this study was to compare 3 methods of PRP preparation. Eighteen clinically healthy mares had blood collected via venipuncture in a blood transfusion bag (method 1), blood tubes (method 2), and a syringe (method 3). In method 1, blood was collected in a 150 ml blood transfusion bag containing 21 ml of citrate-phosphate-dextrose solution with adenine as an anticoagulant (CPD-A). After collection, blood was divided into 50 ml conical tubes and centrifuged at 400 x g for 10 minutes. Resulting plasma was split into 15 ml conical tubes and subjected to centrifugation at 1000 x g for 20 minutes. After second centrifugation, the 2.5 ml in the bottom of each tube was considered PRP, and the remaining plasma as platelet-poor-plasma (PPP). Method 2 involved centrifugation of blood collected in 4.5 ml vacutainer tubes containing 3.2% sodium citrate at 120 x g for 10 minutes. The top third layer of the plasma was deemed as PPP, while the remaining portion was considered PRP. In method 3, blood was collected in a 60 ml syringe containing 7 ml of CPD-A; after collection, each syringe was wrapped in aluminum foil and placed in an upright position for 4 hours. The top 10 ml of plasma was considered PPP, and the remaining plasma (including sedimented blood cells) was deemed PRP. After processing by 3 methods, PRP and PPP were extracted and assessed for red and white blood cell counts, platelet counts, and viability. In a subset of mares (n = 6), samples of PRP were also evaluated at 6 and 24 hours postcooling at 5°C. Method 1 resulted in the highest, and method 3 in the lowest, platelet concentrations; the latter had higher ($p < 0.05$) WBC than others. Platelet viability was similar among treatments. The recovery factor (i.e. the ratio of the PRP volume to the whole blood volume) of plasma recovered as PRP was different ($p < 0.0001$) among methods; method 1, 10.5%; method 2, 33.1%; method 3, 27.2%). Cooling for 24 hours did not affect ($p > 0.05$). platelet counts. However, platelet viability was reduced ($p < 0.05$) after cooling in PRP produced by method 3, and agglutination increased over time among methods. In conclusion, the 3 methods resulted in satisfactory PRP yield without compromising platelet viability. Method 1 (i.e. involving double centrifugation) resulted in the greatest platelet concentrations whereas method 3 (sedimentation) resulted in the lowest platelet concentration and tended to be more contaminated with leukocytes. Cooling affected platelet viability in PRP obtained by method 3 and increased platelet agglutination over time among methods. Clinical efficacy of PRP with these methods of cooling remains to be determined.
Funding: Cesarean section, hypercoagulable, pulmonary embolism, venous thromboembolism

Keywords: Platelet concentrates, horse, endometritis, tissue regeneration

Fetal bones in the uterus of a Thoroughbred mare

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Fetal mummification has been reported occasionally in domestic species. It is more common in polytocous species, but has been observed in the horse, most commonly in twin pregnancies. Mummification is typically a sterile process in which fetal death occurs, the conceptus dehydrates, and is retained within the uterus. This describes a case in a 7-year-old Thoroughbred mare diagnosed pregnant with 1 conceptus (evaluations normal with transrectal ultrasonography at 15, 17, 29, and 42 days postovulation). Transrectal palpation confirmed pregnancy at 5 months of pregnancy. At 7 months of pregnancy, ultrasonography revealed that the mare was not pregnant. Two uterine lavages were performed and 6 grams Timentin was infused into the uterus after lavage. Two months later, uterine cytology and aerobic culture performed prior to breeding season revealed severe inflammation and moderate growth of *Escherichia coli* and *Enterobacter aerogenes*. Mare's uterus was lavaged for 4 days and infused with 2 grams Amikacin, and a Caslick's was placed. Transrectal ultrasonography of the uterus performed 1 month later revealed multiple small (2 cm) hyperechoic linear structures in the uterine lumen at the base of the uterine horns extending

into the right uterine horn. Uterine lavage was performed to aid in the removal of these structures with no success. A repeat uterine culture and cytology revealed moderate inflammation and a light growth of *Escherichia coli*. Hysteroscopy revealed several bony fragments within the right uterine horn and were extracted. There were in total 7 fetal bones, ranging from 1.5 to 2 cm consistent with 2 scapula, 2 pelvic bones, and 3 long bones. Mare was given a dose of broad-spectrum systemic antibiotics, the uterus was lavaged, and acetylcysteine was infused. Uterine lavage was continued for 3 more days. Two weeks later, a culture and cytology were performed and were negative. Mare was bred over 2 estrous cycles (~ 30 and 55 days after the procedure) and became pregnant with twins after the second estrus. One embryonic vesicle was successfully reduced and the mare was confirmed in foal with 1 fetus at 49 days of pregnancy. Due to the low prevalence of fetal mummification in the horse, the underlying cause of this phenomenon has been difficult to discern. When twins are present, placental insufficiency is typically the cause of fetal demise of 1 fetus followed by fetal fluid resorption. In singleton pregnancies, there has been no established cause for fetal mummification and why they are retained within the uterus. This case demonstrated the future fertility of mares after fetal mummification treatment.

Keywords: Mare, fetal mummy, uterine foreign body, endometritis

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Intra-uterine injection of amnion-derived acellular bioscaffold product in mares: systemic and intra-uterine effects over 21 days

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Amnion-derived acellular bioscaffold product (ADABP) has been used as an antiinflammatory agent to promote healing in human and veterinary medicine. Proteins and cytokines present in ADABP are reported to decrease fibroblast formation and fibrosis.[1] Thus, ADABP may be beneficial in the treatment of uterine adhesions, uterine cyst ablations and remodeling of scar tissue. The safety of uterine injection of ADABP is unknown. We studied the systemic and uterine effects after uterine injection. Twelve clinically healthy light-breed mares (mean age 11.5 years; range 5 - 22) were the subjects. Rectal temperature and behavior were recorded for the duration of the study. On day 0, all mares underwent a hysteroscopic examination, control mares (n = 3) received 3 ml injection of sterile saline in the base of 1 uterine horn, and AniCell mares (n = 9) received 3 ml of ADABP (EquusCell StemWrap D™, AniCell Biotech, Arizona) in the base of 1 uterine horn. Blood (for serum amyloid A [SAA], fibrinogen [FIB], and white blood cell count [WBC]), endometrial cytology and aerobic cultures were obtained prior to hysteroscopy. Four days (day 4) after injection, mares were evaluated via transrectal ultrasonography and blood was obtained. Twenty-one days (day 21) after injection, endometrial cytology, aerobic culture, and hysteroscopy were performed. Continuous data were analyzed to determine the main effects of group, day and their interaction using the SAS MIXED procedure with a repeated statement. Categorical data were analyzed using the SAS LOGISTIC procedure. No mares experienced an elevation in rectal temperature during the 21 days after injection. There were no differences in bloodwork for markers of inflammation (SAA, FIB, WBC) from day 0 to day 4 either in the control or AniCell group. Similarly, there were no differences in uterine cytology and culture results between groups or among days within groups. Hysteroscopy following injection demonstrated no gross evidence of detrimental effects in any mare examined. In 1 mare that received a saline injection, a small 1 cm bleb of fibrous tissue was noticed and that remained for 21 days after injection. This study demonstrated that ADABP had no detrimental effect on the systemic health of the mare and it is as safe as hysteroscopy and saline intrauterine injection up to 21 days after injection. Further work is continuing, evaluating histological changes in the mares' endometrium after injection and in clinical cases where injection is performed into uterine tissue, as ADABP may be a useful tool to promote endometrial healing in the mare.

Keywords: Amnion-derived cell product, endometritis, hysteroscopy, uterine injection

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Luteal blood flow and side effects of luteolytic doses of dinoprost tromethamine and cloprostenol sodium in jennies

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Exogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment revolutionized the breeding management of livestock and horses. However, despite 4 decades of its continued use in theriogenology, the optimal luteolytic dose for donkeys has not been determined.

Cloprostenol sodium and dinoprost tromethamine are 2 most widely used and available $PGF_{2\alpha}$, with the former being a natural and the latter a synthetic prostaglandin. Label dose treatment of $PGF_{2\alpha}$ results in colic-like signs in mares, but the impact is unknown in donkeys. The latter species are thought to be more pain-tolerant than horses. This study aimed to objectively assess luteolysis and side effects of jennies receiving standard horse doses of cloprostenol and dinoprost. We hypothesized that the luteolytic doses widely recommended for horses have no side effects in donkeys and both types of $PGF_{2\alpha}$ have equivalent luteolytic properties. Eight jennies (144 ± 22.5 kg; height 95.5 ± 113 cm) were used. Five days after ovulation, jennies were randomly assigned in a cross-over design and received either intramuscular dinoprost (5 mg) or cloprostenol (250 µg). B-Mode and Doppler ultrasonography were performed starting 15 minutes before $PGF_{2\alpha}$, and then repeated at 15 minute intervals until 1 hour after $PGF_{2\alpha}$ and then at 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours. At these times, serum samples were collected for progesterone concentrations by RIA (Beckman Coulter, US). Animals were observed from a distance for side effects (sweating, abdominal discomfort, and diarrhea) at 15 minute intervals starting before and for 1 hour after $PGF_{2\alpha}$. Data normality was assessed with the Shapiro-Wilk's test and comparisons of the CL area and luteal blood flow were performed using PROC MIXED of SAS 9.4. The study was approved by the Ethics Committee on the use of animals – CEUA (UNESP, Brazil) under protocol 0028/2019. Jennies were accounted as random effect whereas time and luteolytic agent were fixed effects. Interactions of fixed effects were also assessed. Significance was considered as $p \leq 0.05$. An increase ($p < 0.05$) in CL blood flow was observed 60 minutes and 45 minutes after treatment with dinoprost and cloprostenol, respectively. There was an increase ($p < 0.05$) in CL blood flow at 4 hours after dinoprost compared to cloprostenol treatment. However, at hours 5, 6, and 7, jennies that received cloprostenol had higher CL vascularity than dinoprost-treated cycles. Blood flow and CL area decreased gradually during the first 24 hours in both groups. Both prostaglandins reduced ($p < 0.005$) serum progesterone concentrations within 30 minutes after treatment with no differences ($p > 0.05$) between groups. Dinoprost resulted in major score of sweating ($p \leq 0.05$) whereas higher ($p \leq 0.05$) abdominal discomfort and diarrhea were detected in cloprostenol. In conclusion, both prostaglandins and doses used were equivalent in inducing luteolysis in donkeys. However, both prostaglandins caused adverse reactions, leading us to believe that horse doses used are inappropriate for small-frame donkeys.

Keywords: Jennies, corpus luteum, Doppler ultrasonography, progesterone, side effects

Sperm-filter enhanced semen parameters and fertility of stallion poor cooled semen

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Cooled-shipped semen is the horse industry's primary approach to breed mares. Whereas most stallion ejaculates tolerate cooling some have inadequate responses to cooling. Despite the development of new extenders in the past 2 decades, some stallions still have poor semen cooling quality. Therefore, there is a critical need to develop tools to process semen for stallions with a poor response to cooling. Sperm-Filter® (SF, Botupharma) is a porous membrane used as an alternative to centrifugation. This technology has yet to be tested in stallions with poor semen cooling. Therefore, this study's objective was to assess semen parameters and fertility of cooled-stored stallion semen processed with SF or conventional centrifugation ([C] 600 x g for 10 minutes) and reextended in 3 commercial extenders. We hypothesized that SF enhances semen parameters and improves the fertility of stallions with poor semen cooling ability. The ejaculates were obtained from 7 stallions known to have poor semen cooling ability (i.e. < 25% total motility (TM) 24 hours postcooling at 5°C). After collection, semen was extended to 50 x 106 sperm/ml with a skim milk-based extender ([SM] BotuSemen, Botupharma) and stored at 5°C for 24 hours. At 24 hours postcooling, samples were split into 7 groups. Control (CT) consisted of cooled semen with no further processing and the remaining 6 groups were submitted to SF or C, then resuspended in either SM, SM containing pentoxifylline ([P] BotuTurbo, Botupharma), or an egg yolk-based extender ([EY] BotuCrio, Botupharma). Total and progressive motility (PM) and percentage of sperm with rapid motility (RAP) were assessed with CASA (IVOS 12, Hamilton Thorne, Beverly, MA). Plasma membrane integrity (PMI), and mitochondrial membrane potential (MMP) were assessed with the combination of Yo-Pro® and MitoStatusRed with flow cytometry (LSR-Fortessa, Becton Dickinson, Mountain View, CA). Five stallions (4 - 8 ejaculates) were used for breeding mares (CT, n = 19; SF-SM-P, n = 9; SF-EY, n = 18 estrous cycles). Data were analyzed with GraphPad Pris 8.0.1. (GraphPad, San Diego, CA). Parametric data were analyzed with ANOVA-RM with Tukey's as post-hoc. Nonparametric data were analyzed by Kruskal-Wallis followed by Wilcoxon–Mann–Whitney. Pregnancy rates were compared by multivariate regression. Significance was set at $p \leq 0.05$. Sperm kinetics (TM, PM, and RAP) increased ($p < 0.05$) in all samples resuspended EY compared to CT, SM, and semen centrifuged and resuspended in SM-P. Semen processed by SF and resuspended in SM-P was similar ($p > 0.05$) to EY groups. SM-P had superior ($p < 0.05$) results in all processed semen by SF compared to CT, whereas centrifuged semen had intermediate values ($p > 0.05$). There were no differences ($p > 0.05$) in PMI between CT and semen processed by SF. However, centrifuged semen had less ($p > 0.05$) PMI than SF processed semen. Additionally, mares inseminated with SF-SM-P (66%) or SF-EY (67%) had higher ($p < 0.05$) pregnancy rates than mares inseminated with CT (13%). In conclusion, sperm parameters of stallions with poor semen cooling ability were enhanced by

the removal of the supernatant and sperm resuspension with YG or SM-P. Additionally, processing semen by SpermFilter enhanced PMI compared to centrifuged semen. Fertility rates of poor cooled semen improved by semen processing by SF and resuspended in SM-P or EY.

Keywords: Stallion, extender, sperm kinetics, bad cooler

Pregnancy rates and subsequent pregnancy losses of in vitro produced embryos from oocytes

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Over the past 5 years there has been an increased utilization of transvaginal aspiration of oocytes and intracytoplasmic sperm injection (ICSI) to produce equine embryos. With this increase in demand has come the increase in the number of commercial ICSI labs and in the number of ICSI-produced embryos being shipped to commercial recipient herds for transfer. There are limited data in the literature describing the pregnancy rates and any subsequent pregnancy losses associated with these shipped ICSI-produced embryos. Data were collected from 2 facilities that performed a total of 572 aspirations over 3 breeding seasons and shipped the oocytes to 5 commercial ICSI labs. Embryos produced were shipped to 2 commercial recipient herds. Pregnancy rates and subsequent losses were calculated for 3 of the ICSI labs; 2 of the labs were not included due to a very small number of embryos transferred from these facilities. In total, 208 fresh embryos were shipped for transfer. Fourteen-day pregnancy rates ranged from 41 to 75%; pregnancy loss rates varied from 12.8 to 60% depending on the TVA Facility/ICSI Lab combination and year. Due to the variability in transfer results both between and within the same facilities, more in-depth research needs to be performed to identify the ideal shipping conditions (media, time in transport, etc.) to maximize pregnancy rates and minimize subsequent pregnancy losses.

TVA Facility/ICSI Lab/Year	Embryos transferred	14-day pregnancies	Pregnancies lost
TVA Facility 1/ICSI Lab A 2018	21	10 (47%)	6 (60%)
TVA Facility 1/ICSI Lab A 2019	12	9 (75%)	2 (22%) - 1 due to twins
TVA Facility 1/ICSI Lab A 2020	53	29 (55%)	8 (27.5%) - 3 due to twins
TVA Facility 1/ICSI Lab B 2019	31	15 (48%)	8 (53%)
TVA Facility 1/ICSI Lab B 2020	24	10 (41%)	5 (50%)
TVA Facility 2/ICSI Lab A 2020	21	14 (66%)	8 (57%) plus 2 late term
TVA Facility 2/ICSI Lab B 2019	13	8 (61%)	2 (25%)
TVA Facility 2/ICSI Lab B 2020	14	8 (57%)	1 (12.8%)
TVA Facility 2/ICSI Lab C 2020	19	13 (68%)	2 (15%)

Keywords: Mare, embryo, ICSI, trans-vaginal aspiration, pregnancy

Incidence rate of reproductive problems in nonpregnant mares

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Broodmares may be affected by a variety of reproductive issues. The goal of this retrospective study was to document reproductive abnormalities encountered in broodmare veterinary practice. Reproductive records of mares managed at Colorado State University were evaluated retrospectively. Reproductive issues were broadly categorized into abnormalities of the ovary, oviduct, uterus, cervix, vagina, perineum, mammary gland, and behavioral concerns. The abnormalities were then assigned to specific subcategories within each broad category. Reproductive records were evaluated for 636 individual mares over a 3-year period (2018 - 2020). A mare was considered positive for an abnormality if the issue was noted at least once during a breeding season. The incidence rate (IR) was calculated as the percentage of mares with a specific abnormality compared to the overall population of mares. Data are presented as the mean ± standard deviation. A total of 862 mare-years were evaluated, as some mares were evaluated over multiple breeding seasons. The average age of the mare population was 11.9 ± 4.8 years and ranged from 3 to 26 years. The most common breeds were American Quarter Horse (383 mares, 60.2% of total), Warmbloods (all breeds combined) (55 mares, 8.6%) and Arabians (26 mares, 4.1%). Most common ovarian issues noted were after 250 µg of cloprostenol treatment were, persistence of luteal tissue (62 cases; 7.2% IR) and hemorrhagic anovulatory follicles

(43 cases; 5.0% IR). Most common oviductal abnormalities were presumptive blocked oviducts (28 cases; 3.2% IR) and parovarian cysts (10 cases; 1.2% IR). Uterine issues comprised the greatest number of abnormalities, including persistent breeding-induced endometritis (PBIE; 189 cases; 21.9% IR), endometrial cysts (137 cases; 15.9% IR), presence of excessive fluid prior to breeding (78 cases; 9.0% IR) and bacterial endometritis (70 cases; 8.1% IR). Most common abnormalities of the caudal reproductive tract were failure of cervical relaxation (36 cases; 4.2% IR), urovagina (6 cases; 0.7% IR), and poor perineal conformation or tone (13 cases; 1.5% IR). Mammary abnormalities were uncommon, with 3 cases of galactorrhea and 2 cases of mastitis. Most common behavioral issues were stallion-like or aggressive behavior (3 cases), recurrent colic or pain (3 cases) that an owner was associating with the reproductive tract and persistent estrus (2 cases). Issues with a higher incidence in mares > 15 years of age included hemorrhagic anovulatory follicles, uterine cysts, persistent breeding-induced endometritis and bacterial endometritis. Mares with a tight cervix or excessive uterine fluid on initial examination had an increased incidence of PBIE (75 and 39.7%, respectively). In conclusion, persistent breeding-induced endometritis was the most common reproductive abnormality and the incidence of reproductive issues increased with advanced age.

Keywords: Equine, mare, reproductive, problems, pathology

Induction of parturition in a late pregnant mare with large colon displacement

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An 11-year-old multiparous pregnant (324 days) Thoroughbred broodmare was presented to Rhinebeck Equine LLP for treatment of right dorsal displacement of the large colon and associated abdominal discomfort. Mare had minimal mammary development. No surgical option was available for the mare; foal survival was the owner's priority. Transrectal and transabdominal ultrasonography examinations revealed a viable fetus in anterior longitudinal presentation with a fetal heart rate of 72 bpm (reference range: 80 - 120 bpm). Mare was treated conservatively with isotonic intravenous fluids (lactated Ringer's 1-2 liters/hour) and intravenous flunixin meglumine (1.1 mg/kg twice daily). Dexamethasone (100 mg once daily) was given intramuscularly at days 325, 326, and 327 to stimulate fetal maturation. Induction of parturition was proposed to allow for delivery of a live foal and possible improvement of colonic displacement postpartum. At 328 days of pregnancy the mare's discomfort persisted and colon displacement was unresolved. Induction of parturition using a low-dose oxytocin protocol was elected. Mare was treated intramuscularly with 5 IU oxytocin; after 25 minutes, a vaginal examination confirmed cervical relaxation. Mare was then treated intravenously with 5 IU oxytocin and had behavioral signs consistent with stage I labor. Following an additional 25 minutes interval, the mare was treated intravenously with 5 IU oxytocin. Seventy minutes after the first oxytocin treatment, stage II labor was initiated with spontaneous rupture of the chorioallantois. Duration of stage II labor was 15 minutes, and the mare delivered a live colt with minimal assistance. Complete and grossly normal fetal membranes were passed within 30 minutes. Foal was given 36 ounces of frozen thawed colostrum via nasogastric intubation, and no gross signs of dysmaturity were noted. The colt received intravenous hyperimmunized plasma and was supplemented with stored mare's milk via esophageal feeding tube for 5 days and remained clinically normal. Mare was started on oral domperidone (1.1 mg/kg, once every 12 hours) to promote mammary development. Colic signs ultimately resolved, and both mare and foal were discharged and remained healthy on farm. Elective induction of parturition in the mare is uncommonly performed due to the marked variation in equine pregnancy length and the relatively late ability of the equine fetal adrenal gland to respond to ACTH. The criteria typically used to assess fetal readiness are length of pregnancy, cervical relaxation, and the presence of colostrum within the mammae. This case highlighted the successful use of oxytocin to induce parturition in a mare despite meeting only 1 of the 3 criteria for fetal readiness. Additionally, dexamethasone was utilized to stimulate precocious fetal maturation prior to induction of parturition.

Keywords: Mare, induction, parturition, oxytocin

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