Dr. Jerry Rains Memorial Abstracts

Antimüllerian hormone concentrations and antral follicles in hormonally treated mares

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Antimüllerian hormone (AMH) concentrations are correlated with antral follicle count (AFC) in the mare. Because of this relationship, potential lies in the ability of AMH to act as a prognostic tool for assessing a mare's overall fertility or, more specifically, predicting a mare's potential as an oocyte donor for the production of in vitro-derived embryos. It is unknown if AMH concentrations remain correlated when mares are undergoing hormone therapy protocols aimed at increasing AFC to promote oocyte aspiration efficiency. Our aim was to determine if AFC is correlated to AMH concentrations in mares undergoing cycle manipulation with only follicle ablation (controls) or undergoing hormone therapy targeted to increase AFC with either progesterone and estradiol (P&E) or with a proprietary drug that supports follicular growth. We hypothesized that AMH concentrations are positively correlated with AFC in mares undergoing cycle manipulation. Data from 2 previous experiments were analyzed. In the first experiment, 10 mares underwent a crossover design in which mares did (P&E group) or did not (control group) receive a dose of a slow-release formulation of P&E (1.5 g and 50 mg, respectively) on day 0. On day 0, all mares underwent transvaginal follicle ablation. On days 0, 3, 6, and 10, AFC was determined via transrectal ultrasonography and serum was stored for AMH enzyme-linked immunosorbent assay (ELISA). In the second experiment, a group of 6 mares (proprietary drug group) underwent transvaginal follicle ablation (day -10) followed by prostaglandin (intramuscular dinoprost tromethamine [5 mg], day -4) to lyse existing luteal tissue and act as a means of estrous cycle synchronization. Proprietary drug was given on days 0 through 4. On day 0 through 4 and day 7, AFC was determined via transrectal ultrasonography and serum samples were stored for AMH ELISA. Data were analyzed for normality via Shapiro-Wilk and correlation was determined by Kendall rank correlation coefficient. Day 0 AMH and AFC data were removed prior to analysis, as this was the first day in which estrous cycle manipulation (i.e. drug treatment or ablation) occurred in most mares. Significance was set at $p < 0.05$ for all analyses and performed in R version 4.3.1. There was no correlation ($r = 0.17$; $p = 0.19$) between AMH and AFC in the control group ($n = 10$), a moderately positive correlation ($r = 0.51$; $p = 0.00011$) in the P&E group ($n = 10$), and a weak positive correlation ($r = 0.36$; $p = 0.0062$) in the proprietary drug group ($n = 6$). This study demonstrated that there was a significant positive correlation between AMH concentrations and AFC in mares undergoing 2 treatments that support follicular growth. Future studies are needed to determine if AMH concentrations can be a tool used to help assess oocyte aspiration potential and fertility in the mare.

Keywords: Mare, antimüllerian hormone, antral follicle count

L-carnitine and pyruvate increased longevity of ram sperm

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Cooled and cryopreserved ram sperm have poor longevity; additionally, ewe's cervix is convoluted and complex, necessitating insemination via invasive laparoscopic techniques. We hypothesized that sperm longevity improves by avoiding cooling during storage as demonstrated in other species. This would enable utilization of less invasive artificial insemination techniques and, increase the use of sex-sorted sperm. Our aim was to determine if supplementation with L-carnitine and pyruvate increases the lifespan of ambient temperature liquid stored ram sperm. Following semen collection (12 ejaculates from 4 rams via artificial vagina), ejaculates were immediately extended to 4 ml total volume with Biggers, Whitter, and Whittingham (BWW) solution prior to sperm isolation using a 90% Percoll density gradient. Sperm pellets were resuspended into 4 treatment groups: 1. BWW; 2. BWW + pyruvate (5 mM); 3. BWW + L-carnitine (100 mM); and 4. BWW + pyruvate (5 mM) + L-carnitine (100 mM). Media were osmotically balanced to 315 mOsm, and 1 mM of D-penicillamine was added to reduce cell to cell agglutination during storage. Total and progressive motility were assessed via computer assisted sperm analysis at 7 time points between 0 and 168 hours. Sperm were assessed for morphology by a single blinded assessor, whereas another single assessor assigned subjective agglutination scores. Total and progressive motility decreased across all samples to 120 hours. Pyruvate, L-carnitine and combination treatments had greater ($p < 0.05$) total motility ($13.11\% \leq 12.30$, $15.41\% \leq 2.22$ and $12.931\% \leq 1.97$) compared to control ($6.37\% \leq 2.19$). Additionally, there was improvement ($p < 0.05$) in progressive motility at 168 hours in the pyruvate, L-carnitine, and combination treatments ($11.27\% \leq 1.89$, $13.64\% \leq 1.96$, $11.28\% \leq 1.68$) compared to control ($5.5\% \leq 1.85$). Furthermore, at 168 hours, the L-carnitine supplemented samples had greater ($p < 0.005$)

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

total and progressive motility than other treatments. Cell to cell sperm agglutination occurred from 24 hours of storage, despite the addition of D-penicillamine. In conclusion, despite relatively low motility rates, L-carnitine and pyruvate significantly enhanced the longevity of ambient temperature-stored ram sperm after 7 days. Further research is required to explore optimized additive concentrations, methods to minimize agglutination and manage contamination.

Keywords: Ram, sperm, storage, ambient temperature, pyruvate, L-carnitine

Amniotic membrane transcriptome analysis identifies genes and mechanisms associated with inflammatory response during ascending placentitis

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Ascending placentitis (AP) in mares is a leading cause of late-term pregnancy loss, premature birth, and delivery of weak foals. Although AP causes substantial economic losses to equine industry, its pathophysiology is not fully understood. Recently, it has been elucidated that AP is associated with alteration of amniotic fluid proteomics. However, the molecular mechanisms underlying these changes remain unexplored. We hypothesized that AP is associated with critical transcriptional alterations in amniotic membrane. Aim was to characterize the amniotic membrane transcriptome in mares with AP (n = 6) compared to gestationally matched negative controls (n = 6). At 280 days of pregnancy, mares assigned to the placentitis group underwent an intracervical inoculation of *Streptococcus equi* ssp. *zooepidemicus* (1×10^6 CFU) using a semiflexible artificial insemination pipette, to induce placentitis. After 8 days, mares had an increase ($p < 0.01$) in combined thickness of uterus and placenta. Mares in the control group did not receive any treatment. Eight days after inoculation, mares were euthanized. Samples of the amniotic membrane were collected and stored in RNAlater (#AM7021; Invitrogen) until RNA isolation. Paired samples were also fixed in 10% formaldehyde for 24 hours and then transferred to 100% methanol for further histopathological determination of the inflammation score. Following RNA extraction via commercial kits, next-generation RNA sequencing of the samples was performed (NovaSeq 6000; 150bp, paired-end). Following quality assessment, trimming, and gene-level count matrix construction via an HISAT2/StringTie2 workflow, raw gene counts were imported to R 4.1.2. Data were filtered for zero-counts, normalized with the trimmed mean of M-values method (TMM), and then converted into log2-counts-per-million values (log2CPM). Weighted gene coexpression network analysis was used for expression network and module construction. According to signed Kendall correlation coefficients, the 32,004 genes were constructed into coexpression modules; each module was set to have a minimum of 30 genes, and an intermodular correlation above 0.25 was the threshold to merge the modules. Each module was assigned to have unique color identification. Module-trait Kendall's Tau correlation coefficients were considered significant at $|r| > 0.4$ and $p < 0.05$. PANTHER classification system v18.0 was utilized for overrepresentation analysis to functionally annotate genes based on gene ontology (FDR < 0.05). One module ('brown') was highly positively correlated ($r = 0.81$, $p < 0.001$) with the inflammation score trait. This module enriched for toll-like receptor (TLRs) signaling that includes a series of genes (*TLR2*, *TLR8*, *LY96*) described as upregulated in the chorioallantois and endometrium of mares with placentitis; this mechanism has been determined as a of the critical regulator eliciting inflammation during equine placentitis. TLRs are crucial in recognizing pathogenic bacterial infections, triggering inflammatory cascade; however, activation of the inflammatory response at the fetomaternal interface level must be suppressed to maintain pregnancy, as its early activation can lead to preterm delivery. Interestingly, intra-amniotic treatments using TLR antagonists contributed to downregulating the placental inflammatory response in primates. Therefore, future research leveraging these TLR mechanisms and associated gene products may result in a potential treatment for placentitis in mares, delaying infection-associated premature birth or abortions.

Keywords: Mare, placentitis, transcriptome, inflammation, toll-like receptor signaling

Investigating canine infertility through evaluation of full thickness uterine biopsies

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Uterine biopsies have been used routinely in some species (predominantly in mares) to evaluate endometrial health. Evaluation of endometrial tissue has the potential to assist with breeding soundness in dogs. However, there is limited information regarding histopathological lesions of the endometrium of dogs with infertility compared to fertile dogs. Objective of this study was to compare histopathological differences of uterine biopsies between fertile and subfertile female dogs. We hypothesized that subfertile dogs have a higher incidence and severity of endometrial inflammation, periglandular fibrosis, and endometrial cysts compared to fertile dogs. Full thickness uterine biopsies were collected at hysterotomy site during cesarean surgery from fertile dogs (control; n = 103) or dogs with known history of infertility (experimental; n = 263). Samples were processed for histopathology. Paraffin embedded samples were sectioned, placed on a glass slide, and stained with hematoxylin and eosin. Slides were randomized, blinded, and evaluated independently by 2 experienced examiners. Samples were assessed for the presence and severity of periglandular fibrosis (number of glands affected and layers), cysts (number), and inflammation. Inflammation was further categorized by cell type (neutrophilic, eosinophilic, lymphoplasmacytic, and

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

histiocytic) and severity (number of inflammatory cells per 400 x field). Chi-square tests were used to compare the categorical variables between controls and experimental samples. Wilcoxon Rank Sum test was used to compare age between groups. Statistical significance was set at $p < 0.05$. Dogs in the control group were younger ($p = 0.022$) than the experimental group (median 3.5 versus 4.0 years). Cellular inflammation was observed more often ($p = 0.004$) in experimental samples (76%) compared to controls (61%). Percentage of samples having neutrophilic inflammation between control and experimental groups was not different ($p = 0.714$; 45.6 versus 43.5%,). In those with inflammation, chronic inflammation was observed more often ($p < 0.001$) in experimental group than control group (70 versus 43%). In addition, there was no statistical difference between groups regarding periglandular fibrosis and cyst formation. Contrary to our hypothesis, endometrial cysts, periglandular fibrosis, and neutrophilic inflammation were observed at similar frequency in both groups. Therefore, these endometrial changes may not be necessarily indicative of fertility issues in dogs. However, subfertile dogs had a higher incidence of chronic inflammation than controls. In mares, periglandular fibrosis is associated with a reduced ability to carry pregnancy to term. Based on our results, this may not be the case in dogs and it potentially relates to differences in placentation between species. In conclusion, histopathology is an important tool to evaluate potential fertility in dogs. However, findings should be interpreted carefully and considered species-specific.

Keywords: Dog, uterine biopsy, infertility, endometrium

Comparison of commercial semen extenders for long-term storage of stallion semen at various temperatures

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Use of short-term cooled-stored semen is routine in the equine breeding industry. In most instances stallion semen is stored at 5 to 8°C for 24 to 48 hours prior to insemination. Goal was to evaluate the effect on sperm progressive motility of prolonged storage (7 to 15 days) of stallion semen diluted in each of 3 commercial extenders and maintained at 5° or 17°C. Our hypothesis was that progressive sperm motility is maintained at a higher percentage in the Beyond® extender (Minitube) after prolonged storage at 5° or 17°C than either INRA96 (IMV Technologies) or BotuSemen Gold (Botupharma USA) extenders. Semen was collected from 10 light horse stallions and evaluated for volume (graduated cylinder), sperm concentration (Nucleocounter® 200, ChemoMetic) and progressive motility percentage (SpermVision®, Minitube). Two aliquots of semen from each ejaculate were prepared for each of the 3 extenders by diluting raw semen to a concentration of 25 x 10^6 progressively motile sperm per ml and stored in a Whirl-Pak® bag. One aliquot of extended semen (x 3 extenders) from each ejaculate was stored at 5°C within a Styrofoam™ box inside a refrigerator. The second aliquot of each extended sample for each stallion was stored in a commercial thermoelectric box (Minitube) at 17°C. A small (0.5 ml) volume of extended semen was removed each day from each of the 6 aliquots, warmed to 37°C for 10 minutes and evaluated for progressive motility by computer assisted semen analysis. Samples stored at 5 and 17°C were evaluated daily for 15 and 7 days, respectively. Data were evaluated by one-way analysis of variance and Tukey HSD. For aliquots stored at 5°C, there was no difference ($p > 0.05$) in progressive sperm motility among extenders during the first 10 days of cooled storage. From days 11 to 15, the progressive motility of sperm diluted in the Beyond® extender was greater ($p < 0.05$) than other 2 extenders. For aliquots stored at 17°C, there was no difference ($p > 0.05$) in progressive sperm motility among 3 extenders during the first 5 days of storage. On days 6 and 7 of storage, progressive motility of sperm diluted in the Beyond® extender was greater ($p < 0.05$) than that of sperm diluted in INRA96 or BotuSemen Gold extenders. In summary, there was no difference in progressive motility among 3 extenders during short-term storage at 5 or 17°C. In contrast, the progressive motility of stallion sperm was higher in the Beyond® extender during long-term liquid (nonfrozen) storage (≥ 11 days at 5°C or ≥ 6 days at 17°C). Fertility trials evaluating storage time and temperatures and microbial culture studies are indicated before long-term storage of liquid stallion semen can be recommended.

Keywords: Stallion, semen, extender, progressive motility, storage

Gallium nitrate: a potential alternative to traditional antimicrobial treatment of equine endometritis

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Bacterial endometritis commonly contributes to infertility in mares. As rates of antimicrobial resistance rise in equine uterine pathogens, there is a need to identify alternative antimicrobial compounds such as Gallium. Bacteria confuse gallium and iron due to their similar chemical properties, causing disruption of iron-dependent processes and subsequently bacterial cell death. Our goal was to assess in vitro effects of gallium on common bacterial pathogens associated with equine endometritis. We hypothesized that gallium nitrate, a highly soluble crystalline form of gallium, inhibits common equine uterine pathogens growth resulting in a concentration-dependent reduction of bacteria. Twelve unique bacterial isolates collected via uterine lavage from 10 mares were used to determine the minimum inhibitory concentration (MIC) of gallium nitrate for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (n = 4 isolates each). Identification of each clinical isolate was confirmed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Isolated colonies were then added to sterile water until a 0.5 McFarland turbidity standard (1.5 x 10^8 CFU/ml) was achieved using a premade standard and a nephelometer. Each isolate was further diluted in a cation-balanced iron- depleted Muller-Hinton (ID-CAMH) broth to a final concentration of 10^5 CFU/ml. Equal molar amounts of gallium nitrate (0.20 g) and tri-sodium citrate (0.23 g) were combined and added to deionized water to create a 100 ml stock solution of gallium nitrate at 2 mg/ml (2,000 µl/ml). The pH of the gallium nitrate solution was measured by a benchtop pH meter (Aquasearcher) and adjusted to 7.2 using 0.1M sodium bicarbonate. In a 96-well

plate, 50 µl of sterile water was added to each well to facilitate 2-fold serial dilutions of gallium nitrate, starting at a concentration of 2,000 µl/ml. In replicates of 3, 50 µl of each bacterial suspension was added to each of their respective wells for a total volume of 100 µl per well. Plates were then sealed and incubated for 24 hours at 37°C. Growth was visually assessed, and turbidity was quantified using optical density at 600 nm. Results were then processed through a statistical analysis system to determine the mean MIC of gallium nitrate for each pathogen. Mean MIC for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* was 1,437.5 ± 864.8 µg/ml, 7.2 ± 5.6 µg/ml, and 2,000 ± 0.0 µg/ml (mean ± SD), respectively. There was inhibition ($p < 0.001$) for each isolate with *Pseudomonas aeruginosa*. There was also wide variability among individual *Escherichia coli* isolates as demonstrated by a large standard deviation that is thought to be attributed to the variability of the pathogen itself. These data suggested that gallium inhibited the growth of common equine uterine pathogens and maybe an alternative to traditional antimicrobial treatment. Additionally, these results allow for future studies testing the safety and efficiency of the solution as an antimicrobial compound for treatment of equine endometritis.

Keywords: Gallium, antimicrobial resistance, endometritis, isolate, pathogen

Prognostic indicators for survival in goats treated for toxic mastitis

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Severe clinical mastitis, also referred to as toxic or gangrenous mastitis, is a relatively uncommon but life-threatening disease of lactating does. Currently, scientific reports of prognostic indicators for survival of goats with toxic mastitis are unavailable. Our goal was to evaluate records of goats presented to a veterinary teaching hospital for toxic mastitis in order to identify prognostic indicators for survival to discharge. We hypothesized that the physical examination findings of heart rate, respiratory rate, and rectal temperature upon presentation to the hospital differ between survival and nonsurvival groups. Additionally, we hypothesized that medical management factors such as length of hospitalization, treatment with intramammary antibiotics, and treatment with dexamethasone are positively associated with survival. A retrospective study was conducted to evaluate medical records from 2014 to 2024 of goats treated for toxic mastitis. Adult does with severe clinical mastitis and signs of systemic compromise (e.g. abnormal body temperature, dehydration, anorexia, and others) were included in the study. A total of 26 medical records met the inclusion criteria, and the association between independent variables and outcome (discharge from the hospital versus nondischarge) was assessed by Chi Square and Fisher's exact tests. Of the 26 goats included, 76.9% (20/26) survived to discharge and 23.1% (6/26) died or were euthanized. Initial physical examination findings of heart rate, respiratory rate, and rectal temperature were not associated ($p > 0.05$) with the outcome. In contrast, hospitalization length ≥ 10 days ($p = 0.006$), treatment with intramammary antibiotics ($p = 0.01$), and treatment with dexamethasone ($p = 0.04$) were independently associated with survival to discharge. Treatment of goats with toxic mastitis at this institution carried a fair to good prognosis to hospital discharge. Inclusion of intramammary antibiotics and steroids in the treatment regimen may improve survival in some cases of toxic mastitis.

Keywords: Goat, toxic, mastitis, survival, intramammary, dexamethasone

Effect of inactivated parapoxvirus ovis intramuscular injection on endometrial gene expression in chronic infectious endometritis in mares

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Chronic infectious endometritis (CIE) is a major cause of equine subfertility.[1] Persistence of CIE is partially related to impaired uterine immune response and clearance.[2] Inactivated *parapoxvirus ovis* (Zylexis®) is an immunomodulator used in the treatment of respiratory disease in horses[3]; nevertheless, it has not been used for endometritis in the mare. Preliminary data indicated that pregnancy rates increased in postpartum mares after Zylexis® treatment.[4] We studied the effect of Zylexis® treatment on endometrial gene expression in mares with CIE. We hypothesized that Zylexis® affects endometrial genes associated with innate immunity (tumor necrosis factor [TNF]- α, interleukin [IL]-1β, IL-6, IL-8, IL-10, IL-1 receptor antagonist [ra], interferon [IFN]-γ) and mediators of uterine contractility (cytosolic phospholipase A2 [cPLA2], secretory PLA2 [sPLA2], and prostaglandin-endoperoxide synthase-2 [PTGS2]) in mares with CIE. Eight cycling mares (12-15 years) diagnosed with CIE due to *Escherichia coli* infection and positive cytology (1-5 PMNs/hpf) that were refractory to treatment, were enrolled in this experiment. Endometrial biopsies were collected at 0, 4, 8, 24, and 48 hours, starting on day 3 of estrus when mares had a ≥ 35 mm follicle and endometrial edema. After 4 hours, all mares were treated with 2 ml of Zylexis® intramuscularly. Relative gene expression of TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-1ra, IFN-γ, cPLA2, sPLA2, and PTGS2 in the endometrial tissue were measured using RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase was selected as a housekeeping gene. Results were expressed as fold changes calculated using delta-delta (ΔΔ) CT method. Data were tested for normality using Shapiro-Wilk test, and fold changes for all genes were analyzed using repeated measures ANOVA or Friedman test, followed by LSD or Wilcoxon signed-rank test, respectively. Significance was set at ($p < 0.05$). Expression of IFN-γ and PTGS2 differed ($p < 0.05$) among time points. Compared to 4 hours: expression of IFN-γ increased 129-fold at 8 hours, and decreased to 20-fold by 24 hours; and expression of PTGS2 decreased 2-fold at 24 hours. Compared to 4 hours: TNF-α and cPLA2 tended to decrease ($p = 0.07$) 2- and 1.7-fold at 8 hours, respectively. There was no effect ($p > 0.05$) of treatment on IL-1β, IL-1ra, IL-6, IL-8, IL-10, and sPLA2. In this study, IFN-γ and TNF-α (innate immune

response genes and proinflammatory cytokines[5]) and (cPLA2 and PTGS2 (mediators of uterine contractility and key enzymes in prostaglandin production[6]) were affected by Zylexis® treatment. Upregulation of IFN-γ as a proinflammatory cytokine after Zylexis® treatment may have a therapeutic role in the impaired immune response in the mare endometrium. Following the spike of IFN-γ at 8 hours, the declining phase of the upregulated IFN- γ coupled with decreased PTGS2 expression at 24 hours may indicate the end of inflammatory phase and the initiation of endometritis resolution. Further studies are needed to determine the effectiveness of Zylexis® in treatment of CIE.

Keywords: Mare, inactivated *parapoxvirus ovis*, chronic infectious endometritis, gene expression

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Leiomyoma of tunica albuginea affected breeding performance in a Quarter Horse stallion

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A 9-year, Quarter Horse stallion, was presented in October 2023 for potential oligospermia and a history of reluctance to mount mares during live-cover. Oligospermia was noted in a prior semen evaluation. Owners also noted that stallion was unwilling to mount and breed mares during last breeding season. At presentation, stallion was hesitant to mount a phantom. Semen was collected twice with a Missouri model artificial vagina, 1 hour apart, utilizing a hormone-treated, ovariectomized tease mare. During both collections, stallion displayed reluctance to mount the phantom with no signs of obvious lameness that would explain this behavior. First ejaculate had 16.08 x 10^9 sperm with a total and progressive motility of 64 and 49%, respectively, whereas second ejaculate had 1.18 x 10^9 sperm with a total and progressive motility of 57 and 31%. Approximately 55% of sperm were normal in both ejaculates. We assumed that the second ejaculate was only partial (low sperm number) because stallion had discomfort during collection. Therefore, daily sperm output was calculated based on testicular measurements. Daily sperm output was estimated to be 6.8 x 10^9 sperm per day. Ultrasonographic examination of the accessory sex glands revealed a 1 cm cyst in caudal left ampulla. Testicular ultrasonography revealed a mass (1.65 x 1.53 cm) associated with right epididymis head and related dilation of right pampiniform plexus vasculature. Mass was firm on palpation and stallion elicited severe pain when pressed. Hemicastration (right testis) was performed and the mass was submitted for histopathology, which revealed that the mass arose from tunica albuginea. There were no substantial microscopic abnormalities of epididymis, ductus deferens, pampiniform plexus, and spermatic cord fascia or testis. Mass was identified as benign mesenchymal neoplasm, most likely, of smooth muscle origin. Pathologists classified the mass as a leiomyoma arising from tunica albuginea, and excision of the mass was complete. Stallion fully recovered from surgery and was discharged the following day with antibiotics and nonsteroidal antiinflammatories. Most commonly reported tumors associated with equine testis are seminomas, interstitial cell tumors, teratomas, Sertoli cell tumors, and teratocarcinomas; leiomyomas are uncommon. Testicular leiomyomas have been described in stallions.1-3 Two of these were associated with tunica albuginea, and the third was associated with testicular parenchyma. This is a unique case description of a paratesticular neoplasm that caused discomfort and affected this stallion's breeding performance.

Keywords: Equine, leiomyoma, tunica albuginea, stallion

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Double type 3 jejunal atresia in a neonatal pup

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A 1-day-old female Dachshund pup was presented with weight loss and decreased suckle response. Pup weighed 208 g at birth, which decreased to 199 g the next day. At presentation, pup was depressed and had a subnormal body temperature (35.6˚C) with no evidence of meconium on the rectal thermometer. Pup also appeared to have mild abdominal distension but did not act painful during abdominal palpation. A urine sample was collected by perineal stimulation. The urine parameters were all within normal limits for a neonatal dog (USG: 1.017 [range 1.006-1.017]; trace protein; trace glucose).[1] Abdominal ultrasonography revealed some gas-filled bowel with no peristalsis. Initial diagnosis was hypothermia with secondary ileus. Pup was given prewarmed lactated Ringer's solution subcutaneously (5 ml), glucose (karo syrup) on the oral mucosa, and placed in a humidified incubator (35.5˚C, 50% humidity). Within 4 hours, pup's rectal temperature had increased to 36.7˚C and milk (1 ml) provided by the breeder was given via an orogastric tube. However, within the next hour, pup became agonal, and the decision was made to euthanize. At necropsy, there was small amount of milk in the stomach. Serosa of the duodenum and proximal jejunum was dark red, and the lumen was distended along its length to a blind end. After a 1-cm gap, jejunum resumed for 6-cm and then there was a second blind end with a 1-cm gap. Distal to the second blind end, the intestinal tract continued to a patent anus. Diameter of the jejunum distal to the first blind end was about half that of the diameter proximal to the blind end. On histological examination, the jejunum proximal to the first blind end

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

had transmural congestion and serosal lymphatics were dilated. All other organs were grossly and histologically normal. Congenital intestinal atresia can vary from complete blockage by a membrane (Type 1) to sections joined by an imperforate cord (Type 2) to blind-end atresia in which a segment of intestine is missing (Type 3).[2] Pup in the current report had a double type 3 jejunal atresia. Type 3 intestinal stenosis has been reported in 1 foal, 9 calves, 2 lambs, 3 piglets, 1 pup, and 3 kittens.[2] There are several hypothetical causes of congenital intestinal atresia, but mesenteric ischemia has been experimentally shown to cause intestinal atresia in fetal dogs.[3] Mortality can occur in neonatal pups for multiple reasons.[1] Performing necropsies allows veterinarians to make definitive diagnoses in cases of congenital defects.

Keywords: Canine, congenital defect, neonatal mortality

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Transient Addisonian-like crisis in a late pregnant dog

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A 3-year, pregnant Golden Retriever dog, was presented 52 days after ovulation for nausea, hyporexia, regurgitation, polyuria, and polydipsia. Combined blood count had abnormalities consistent with late-term pregnancy; blood chemistry revealed mild metabolic acidosis, hypocalcemia, hyponatremia, hyperkalemia, and hyperphosphatemia; and there were traces of ketones in urine without glucose. Reproductive ultrasonography revealed several viable fetuses. Dog was treated with fluids, calcium, and antinausea drugs. Two days later, dog was hospitalized because of hyporexia, vomiting, abdominal distention, and acute onset of bleeding from the rectum. A nasogastric tube was placed, and dog received fluids, antiemetics, antinausea, antibiotics, and calcium. Despite treatment, dog deteriorated, became tachypneic, and tachycardic, had progressive regurgitation, generalized weakness, and was transferred to an O2 cage. Combined blood count was compatible with sepsis/infection and blood chemistry revealed metabolic acidosis, hyponatremia, hyperkalemia, a low Na: K ratio (24:1), hypochloremia, and hypocalcemia, raising the suspicion of an Addisonian-like crisis. Baseline serum cortisol concentrations on the next day were 7.89 ug/dl (reference range 1.00- 7.13 ug/dl). Aiming to improve fetal maturation, dexamethasone was started on day 2 of hospitalization and cesarean surgery was performed 2 days later (57 days of pregnancy) because of dog's continued deterioration despite aggressive medical therapies. Evidence of a moderate to a marked increase in allantoic fluid and hiatal hernia were noted at surgery. All 11 pups were delivered alive and had Apgar scores of 8-9/10 at discharge on the same day. Dog remained hospitalized and progressively improved after aggressive medical management. An ACTH stimulation test was performed as a differential diagnosis for pregnancy-associated Addisonian-crisis and the result was within the reference range. Dog was discharged 4 days after surgery and continued to be treated with prednisone for 45 days and recovered well in the following days. She had a follow-up ACTH stimulation test after discontinuation of prednisone that was within the reference range. Pups progressively faded and died a few days after birth. One pup survived and is doing well at the time of this report. Necropsy with histopathology on 6 pups that died on various days after birth had lung immaturity with mild edema; conclusion was respiratory failure due to pulmonary immaturity. Based on the clinical and laboratory findings and improvement after intensive management of the dog, we hypothesized that the dam had a transient Addisonian-like crisis. Similar findings were described[1,2] despite cortisol concentrations were within the normal range. Corticosteroid treatment to improve fetal maturity appeared successful as reported.[3] However, according to necropsy and histopathology reports, pups' lungs stopped maturing after birth, suggesting that glucocorticoids were insufficient to have a persistent influence on lung maturation to prevent respiratory distress syndrome after delivery.[4] Literature is limited, and based on our knowledge, only a few case reports described similar findings.[1,2] This case highlighted that an Addisonian-like crisis can be a differential for late pregnant dogs presenting with nausea, vomiting, and hyporexia in addition to conditions such as pregnancy toxemia and hypocalcemia.

Keywords: Pregnancy, canine, hyponatremia, hypochloremia, glucocorticoids

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Mastitis-induced laminitis suspected in a maiden Fell pony mare

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Mastitis is described as inflammation and infection of mammary tissue and is less commonly observed in mares. This is due to the smaller size and hidden nature of mammary gland in mares compared to other domestic species.[1] Mares afflicted with this disease are typically lactating mares that have recently been weaned. Mares present systemically ill with clinical signs such as fever, anorexia, swollen mammary gland, and abnormal mammary secretions.[1] Sequelae to mastitis include fibrosis of the mammary gland, transmission of infectious agent to foal, foal malnutrition, and abortion. We describe a case of suspected mastitis-induced laminitis in a 6-year, Fell Pony maiden mare that was diagnosed with unilateral mastitis and laminitis. Mare was presented for bilateral forelimb lameness. Mare had been treated for mastitis for 2 weeks with nonsteroidal antiinflammatories and an antibiotic. At presentation, mare was lame and reluctant to bear weight on her forelimbs and swelling was observed in the left mammary gland. Complete blood count and blood chemistry were normal. Mammary gland was swollen, painful to the touch, and purulent discharge was milked from the left teat and submitted for aerobic culture and cytology. Culture results revealed growth of methicillin-resistant *Staphylococcus aureus*. Metabolic testing was performed due to mare's breed predisposition to metabolic disorders; results were within normal limits. Ultrasonography of the udder revealed an abnormal area deep to the skin; due to the small size and location, drainage was not performed. Radiographs of all feet were obtained and rotation of front coffin bones was confirmed. Ice therapy, broad-spectrum antibiotics, nonsteroidal antiinflammatories, pentoxifylline, and probiotics were implemented as part of mare's treatment plan, along with frequent milking of the affected udder and cold hosing. A venogram was performed of the front feet that revealed bilateral compromise to the circumflex artery with dorsal flip into the lamellar wedge area and the distal corium was shut down during initial weight bearing views. Mare was placed in Nanric ultimate cuff shoes with ACS padding on the front feet and was scheduled with our veterinary podiatrist for re-evaluation in 30 days. Our veterinary podiatrist and surgeon worked closely to manage the mare and keep her comfortable; treatments over the course of several months included therapeutic shoeing, a hoof wall resection, and bilateral deep digital flexor tenotomies. Three months after the initial insult, udder was swollen, painful, and hot and an abscess was observed on ultrasonography. Abscess was lanced, drained, and flushed over several days and antibiotic therapy was reinstated. Mare has had intermittent swelling of the left mammary gland, with and without drainage. Courses of various antibiotics have been utilized unsuccessfully to resolve the underlying infection. Ultimately, a unilateral mastectomy was performed and tissue was submitted for histopathology. Diagnosis was moderate, multifocal, chronic, nodular, pyogranulomatous mastitis with severe fibrosis. Mare recovered well from the surgery and minimal swelling was present at the last recheck.

Keywords: Mastitis, laminitis, methicillin-resistant *Staphylococcus aureus*

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Management of persistent overgrowth on routine contagious equine metritis cultures in a Warmblood mare

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A 4-year, maiden Warmblood mare, was presented for routine *Taylorella equigenitalis (T. equigenitalis)*, organism responsible for contagious equine metritis (CEM) cultures after imported to United States from Netherlands. As per United States Department of Agriculture (USDA) requirements, cultures have to be obtained a total of 3 times, 3 days apart, prior to beginning 5 consecutive days of clitoral washing with chlorhexidine and packing with nitrofurazone. Sites to be cultured include clitoral fossa, clitoral sinuses, and endometrium in nonpregnant mares. Initial culture samples were obtained from the clitoral fossa and sinuses and submitted to the University of Kentucky Veterinary Diagnostic Laboratory. All cultures were unable to be assessed for growth of *T. equigenitalis* due to overgrowth of other bacteria. After discussion of results and options with the state veterinarian, it was decided to repeat cultures after rinsing clitoris with distilled white vinegar. These cultures again were overgrown with bacteria and were not useful for CEM evaluation. A routine aerobic clitoral culture sample was then submitted to the laboratory at Hagyard Equine Medical Institute to identify and determine the sensitivity of organism(s) contributing to the overgrowth to guide a targeted treatment plan, with state veterinarian's approval. Culture revealed heavy growth of *Pseudomonas aeruginosa (P. aeruginosa)*, sensitive to polymyxin B. Clitoris was then treated topically for 5 consecutive days with cleansing with dilute betadine scrub and application of polymyxin B ointment compounded at the Hagyard Pharmacy. As per USDA requirements, 21 days elapsed between the final topical treatment and restarting culture procurement. Once again, these clitoral cultures had overgrowth of bacteria. After further discussion with state veterinarian and client, a more robust diagnostic approach was pursued. Samples were obtained from endometrium, vagina, and clitoris for routine culture and sensitivity. Endometrial culture was obtained via low-volume sterile lavage 1 day after instillation of N-acetylcysteine. Endometrial sample yielded scant beta *Streptococcus* species, the vaginal sample grew scant *Staphylococcus aureus* and scant *P. aeruginosa*, and clitoral sample grew moderate *P. aeruginosa*. Mare was then started on an oral probiotic to promote systemic microflora homeostasis. Local treatment was initiated as follows for 5 consecutive days: sterile uterine lavage and infusion of 2 grams of ampicillin, application of MediHoney® to the vaginal vault, and application of compounded polymyxin B ointment to the clitoris. Systemic treatment with ciprofloxacin and probenecid was initiated concurrently for a total of 14 days. All antimicrobial selection was based on sensitivity results. As per USDA guidelines, CEM cultures can be procured 21 days after the final topical treatment or 7 days after cessation of systemic antimicrobial treatment. First CEM culture sample obtained after this treatment period was again overgrown. After further discussion with state veterinarian, another culture was performed the following day after a

more vigorous application of distilled white vinegar and sterile water to clitoris. All subsequent cultures were negative for overgrowth and for *T. equigenitalis* and after 5 days of clitoral washing and packing, the mare was released from quarantine.

Keywords: Mare, contagious equine metritis, culture, overgrowth

Transcervical approach for treatment of pyometra in a snow leopard (*Panthera uncia)*

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Pyometra is a serious condition characterized by accumulation of purulent material in the uterus, secondary to bacterial infection. Although pyometra has been described in big cats, medical management has not been widely reported. An 8-year, nulliparous snow leopard, underwent exogenous hormonal stimulation and laparoscopic oviductal artificial insemination (AI). On day 60 postAI, moderate amount of purulent serosanguineous vaginal discharge was observed, and clavamox and enrofloxacin were initiated. Oral misoprostol treatment (200 µg SID) was given on day 65 postAI. Snow leopard underwent immobilization for a comprehensive reproductive health examination on day 68 postAI. Transabdominal ultrasonography identified small pockets of hypoechoic fluid in the uterine body and right uterine horn. Transvaginal endoscopic examination with a 43 cm rigid 9.5-13.5Fr hysteroscope revealed mild purulent discharge within the vaginal lumen, making visualization difficult. A 5-Fr cannula was passed through the cervix, and purulent discharge was released from the uterus. Ten ml of sterile saline was flushed into the uterus and then aspirated to recover as much fluid as possible; this was repeated 2 more times. Aspirated volume initially appeared as a light pink, purulent fluid that transitioned to a mild cloudy consistency by the third series. Intramuscular dinoprost (3.4 mg) was given and transcervical cannula remained in place for 28 minutes postinjection. Peak back flow occurred 17-21 minutes postinjection. After completing the examination, a 200-µg misoprostol tablet was dissolved in 1 ml sterile saline and given intravaginally. Subcutaneous aglepristone was given during the examination (16.8 mg). However, retrospective serum progesterone concentrations (0.4 ng/ml) revealed that the snow leopard was not in luteal phase at examination. A second dose of dinoprost (3.4 mg) was voluntarily given the next day, and a partial dose (due to patient compliance) was given the following day. Misoprostol, enrofloxacin, and clavamox were continued 3 days after dinoprost treatment. Two-weeks after the initial evaluation, snow leopard was reexamined and was in estrus. Uterus and uterine horns (from body to tip) were examined via ultrasonography and no hypoechoic pockets of fluid were noted. A small amount of serosanguineous-purulent discharge was present in her vulvar area. During vaginal endoscopy, vestibule appeared to be the source with a minimal amount of discharge, whereas vaginal canal revealed normal pink mucosa with a clear sheen. Due to narrowing of the vaginal lumen (reported in domestic cats during estrus), the scope could not be advanced to cervix level. Vestibule sample was cultured and treated per the sensitivity results with an additional 10 days of marbofloxacin, after which, the patient recovered uneventfully. Due to the disadvantages associated with ovariohysterectomy in large cats, medical management should be considered when treating pyometra. Outcome of this procedure provided evidence that combination of antibiotics, prostaglandins, and transcervical therapy can be used to manage the reproductive health of female snow leopards in a zoo-based setting. Furthermore, it also increases the chance of identifying animals with pathology linked to genetics.

Keywords: Pyometra treatment, transcervical endoscope, snow leopard

Mycobacterium avium subsp. *hominissuis* induced abortion in a mare

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Equine bacterial abortion can be caused by opportunistic organisms ascending from vagina, hematogenous invasion or unknown as in cases of nocardioform placentitis. The result is placental infection, and potentially fetal death. This is the first report of abortion due to placental and fetal infection with *Mycobacterium avium* subsp. *hominissuis* (MAH) in Australia. Although rare, abortion caused by nontuberculous *Mycobacterium* spp. has been reported in Japan (MAH), USA (*M. avium* complex and *M. avium* Runyon group IV), Canada (*M. avium* complex), and Australia (*M. avium* subsp. *terrae*).[1] Furthermore, a role for *Mycobacterium* spp. in cases of nocardioform placentitis has been suggested.[2] A multiparous, 7-year, Thoroughbred mare, was exported for live-cover from Australia to Hokkaido Island, Japan and returned on day 108 of pregnancy. Mare developed premature mammary gland development on day 134 with unremarkable changes to fetoplacental unit (via transrectal and transabdominal ultrasonography). Treatment with intramuscular altrenogest (0.3 mg/kg, once every 7 days) and oral pentoxifylline (8.5 mg/kg, twice daily) was initiated and fetoplacental health was regularly monitored via transrectal and transabdominal ultrasonography. Treatment was discontinued when the clinical signs subsided. On day 229 of pregnancy, mild placental edema at the cervical star was noted. Given the mare's history, treatment was started with intramuscular altrenogest (0.3 mg/kg, once every 7 days), oral pentoxifylline (8.5 mg/kg twice daily), oral phenylbutazone (2 grams, once daily), and oral sulfadimidine/trimethoprim (25 mg sulfadimidine/5 mg trimethoprim/kg, twice daily) that normalized all parameters after 5 days of therapy. Pentoxyfylline, phenylbutazone, and sulfadimidine/trimethoprim were discontinued but altrenogest treatment

continued. On day 243, mare aborted an emaciated fetus with fetal membranes. Grossly, cervical star region of the chorioallantois was edematous with well circumscribed area of caseous exudate. Umbilical cord (58 cm) was thickened and there was prominent perivascular edema of the amnion at the point of umbilical cord attachment. Pinpoint white foci were noted in fetal liver and white nodules within the caudal lung lobes. Final diagnosis based on histopathologic examination was necrotizing granulomatous hepatitis, splenitis and fetal pneumonia associated with a mild multifocal granulomatous amnionitis, funisitis, and placentitis with intralesional modified Ziehl-Neelsen positive bacilli; MAH was identified using 16S rRNA PCR and genome sequencing. Endometrial biopsy collected 6 months after abortion indicated a severe chronic active lymphoplasmacytic neutrophilic, granulomatous endometritis with moderately extensive early fibrosis. A Ziehl-Neelsen and Fite stain failed to reveal any acid-fast bacteria within the endometrium. Low volume lavage prior to breeding revealed gram negative bacteria and neutrophils on cytology and heavy growth of *Klebsiella oxytoca* and *Proteus mirabilis* was isolated from the fluid. Extended culture for Mycobacterium species remained negative. Based on sensitivity testing, the mare was treated with ceftiofur and Tris-EDTA. Mare subsequently conceived and carried to term. Origin of MAH in this case, is uncertain, but the mare could have acquired the organism in Japan during her residence for live-cover, where MAH has previously been described as a cause of equine abortion. The bacteria could have remained dormant until unknown favourable conditions allowed MAH to become opportunistic within the fetal membranes, with subsequent inflammation and fetal death.

Keywords: Equine, abortion, placentitis, fetus, *Mycoplasma avium* subsp. *hominissuis*

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Stranguria due to cervical sarcoma in a Nubian doe

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Female reproductive tract tumors in animals may include vaginal leiomyosarcoma, vaginal fibrosarcoma, uterine lymphosarcoma, adenocarcinoma, and leiomyosarcoma. Literature on reproductive tract tumors in goats is limited, with sporadic reports of uterine and cervical leiomyomas. Approximately 10-50% of genital tumors originate from smooth muscle, of which only 10% are considered malignant. A 7-year, 55 kg maiden Nubian doe, was referred for evaluation of a vaginal mass and dysuria. Before presentation, owner noticed that the doe had a mucoid, slightly hemorrhagic vulvar discharge, and weight loss. At presentation, the goat's vitals were within normal limits. However, she had stranguria and abdominal tenderness. Abdominal palpation indicated a mass in the caudal abdomen that was confirmed via transrectal and transabdominal ultrasonography. On transabdominal ultrasonography, a 10 cm mass extending from cervix to uterus, compressing urinary bladder neck, was observed. Vaginal endoscopy revealed a multinodular, firm and pink-blueish mass extending from caudal vagina to cervix. Cervix could not be visualized. Vagina was hyperemic and a milky mucoid discharge was present. Computed tomography (CT) was performed to determine the lesion's extent and whether surgery was an option. CT scan revealed a multilobular, heterogeneously enhancing mass with punctate foci of mineralization in the intrapelvic vagina and uterine body, measuring 16.3 (length) x 11.4 (width) x 11.0 cm (height). Large vessels branching from the uterine arteries were observed entering mass center from its cranial aspect. There was no evidence of metastasis in CT. However, there was evidence of parasitic cysts or abscessation in the lungs. Considering the complex vascularization of the mass, size and location, risks associated with anesthesia, poor to grave prognosis and the invasiveness of surgery, owner opted for humane euthanasia. Histopathology of the cervical mass revealed cervical sarcoma, cystic endometrial hyperplasia, and endometritis. Presumptive diagnosis was cervical leiomyosarcoma based on tumor location and tissue of origin. Leiomyosarcomas are rare malignant neoplasia that develop in smooth muscle of the reproductive tract. They usually exhibit a low-grade malignancy growth pattern, slow invasion, and rarely metastasize.[1] Diagnosing genital tract tumors in goats is important in USA, where many people own goats as companion animals. Although tumors of the female reproductive tract in small ruminants are rare, they can have substantial health implications. This case demonstrated the complex nature of these tumors and highlighted the challenges in diagnosis and treatment. This case also underlined the importance of using vaginoscopy in the diagnosis of cases that present with abnormal vaginal discharge. It allowed direct visual examination of the vaginal and cervix and facilitated the detection of abnormalities that might otherwise be missed.

Keywords: Goat, leiomyosarcoma, neoplasia, stranguria, vagina, cervix,

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Competitive Poster Abstracts

Sperm chromatin structure assay use in diagnosis of canine infertility

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A 2-year, maiden Pembroke Welsh Corgi dog, was examined prior to planned artificial insemination. Physical examination was unremarkable. Serial progesterone measurements were used to estimate the day of LH surge. Dog was bred on days 4 and 6 postLH surge. Transabdominal ultrasonography (30 days postLH surge) revealed 6 anembryonic vesicles. Owner elected to medically induce abortion with aglepristone injections, while empirically treating with enrofloxacin (8 mg/kg, once daily). Follow up ultrasonography (by referring veterinarian) confirmed successful evacuation. At next estrus, dog was presented for repeated breeding management; same stud dog was used. Thirty days later, dog was presented with PU/PD. Transabdominal ultrasonography revealed 3 anembryonic vesicles, 1 reabsorption, and 2 fetuses had visible heart beats. Complete blood count, chemistry, and urinalysis unremarkable. Cesarean surgery (planned) was performed; submitted uterine biopsy tissue was normal. Singleton pup died 3 days postdelivery from an unknown cause. Due to the high number of anembryonic vesicles, a genetic cause for the infertility was suspected. An Embark panel was previously submitted for the dog that was clear, except for a coefficient of inbreeding (COI) of 42%, with the breed average being 22%. This was considered a possible contribution to the early embryonic loss, and ultimately the dog was retired from breeding. Stud dog (proven 11-year, Pembroke Welsh Corgi) had a COI of 14%. A fresh semen sample was flash frozen and submitted to SCSA Diagnostics, Brookings SD (sperm chromatin structure assay [SCSA] measures DNA fragmentation index [DFI], percentage of sperm containing measurable DNA damage along with a high DNA stainability [HDS], and percentage of sperm with immature, abnormal tertiary chromatin structure). The DFI for this dog was 26.42 and 27.80% (mean 27.1) with an HDS of 5.20 and 5.50% (mean 5.4). Although the threshold for the DFI of fertile versus infertile dogs has not yet been determined, the mean value is 5.9% compared to subfertile dogs (13.2%).[1] With a higher DFI, there is an increased risk of lethal early embryonic defects. The high DFI in this dog likely contributed to the reproductive failure observed. This case demonstrated the use of SCSA to aid in diagnosing male canine infertility.

Keywords: Pembroke Welsh Corgi, sperm chromatin structure assay, DNA fragmentation

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Vulvar hemorrhage in a nulliparous aged Arabian mare

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A 24-year, nulliparous Arabian mare (493.2 kg [1,085 lb]), was presented to Rhinebeck equine facility for evaluation of acute onset of hemorrhagic vulvar discharge. Mare was bright, alert, and responsive with vital parameters within normal limits. Perineal blood staining was evident and the mare frequently passed small volumes of serosanguinous discharge from the vulva, independent of urination. Point of care blood work was within normal limits. Transrectal palpation revealed no detectable abnormalities within the urinary bladder or the uterus; transrectal ultrasonography confirmed a moderately full urinary bladder and a diestrous uterus, with a corpus luteum on left ovary, no uterine edema, and multiple small follicles on both ovaries. There was a mild echogenic fluid (3-5 cm) collection within the uterine lumen and an irregular spheroid structure (~ 5 x 8 cm) was identified in the mid-uterine body. Vaginal speculum examination revealed frank blood in the vagina that appeared to originate from cranial to the cervix. Uterus was distended with 2 liters of sterile lactated Ringer's solution. Repeat transrectal ultrasonography confirmed that the mass was pendulous and appeared to communicate with the endometrium (cranial to uterine body) via a short stalk. At this time the differential diagnoses included hematoma, abscess, or neoplasia. Hysteroscopic examination was performed with a 1.5-meter endoscope, confirming the presence of a hemorrhagic and heterogeneous mass within the cranial uterine body close to the base of the right uterine horn and in direct communication with the endometrium via a vascular stalk. Sampling of the mass via endoscope biopsy channel was unsuccessful due to its markedly friable nature. Cloprostenol sodium [Estrumate, Merck Animal Health] was given to induce luteolysis and facilitate cervical relaxation in anticipation of transcervical mass removal. Next day, mare was sedated (intravenous detomidine [0.01 mg/kg] and intravenous butorphanol [0.01 mg/kg]), the perineum was cleaned, and a sterile sleeved hand was passed into the mare's vagina. Gentle digital manipulations were used to dilate the cervix; however, complete dilation was unsuccessful. A caudal epidural was performed with 2% lidocaine (3 mg/kg) and detomidine (0.02 mg/kg) and a 1.5-meter endoscope was again passed transcervically into the uterus. After insufflating the uterus, a disposable electrosurgical snare [SnareMaster 20 mm, Olympus Medical Systems Corporations] was passed through the scope's biopsy channel and tightened around the base of the stalk; electrocautery was used to ligate and provide hemostasis. Having freed the mass from its endometrial attachment, gentle transrectal manipulations were used to break off portions of the mass to allow transcervical removal, followed by copious uterine lavage with sterile lactated Ringer's solution. Mass fragments were submitted for histopathology that revealed adenocarcinoma. Although a specific diagnosis was not possible, this report is valuable to practitioners due to its

thorough approach to management of an intrauterine mass, and most importantly, identification of the condition based on clinical signs. Removal method adopted possibly failed to achieve complete margins; however, it provided adequate resolution of mare's clinical signs.

Keywords: Mare, vulvar hemorrhage, uterine mass, electrocautery, adenocarcinoma.

Incidental masses during a routine transvaginal reproductive examination in a grizzly bear (*Ursus arctos horribilis)*

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An 18.5-year, wild-caught multiparous brown bear, underwent routine reproductive examination prior to shipment. At last year's examination, a multinodular (cauliflower-like) soft tissue mass (2.5 x 1.9 x 0.6 cm) was observed on the right side of the ventral vulvar mucosa and an incisional biopsy was submitted for histopathology. Results revealed benign collagenous expansile lesion suggestive of a polyp. The goal of this year's procedure was to perform a reproductive examination to characterize and document reproductive pathology among Ursidae. Vulva measured 76 x 32 x 61 mm (length x width x protrusion). Vulvar growth appeared unchanged on the ventral side and measured 2.4 x 1.7 cm. A cotton swab moistened in PBS was passed through a short (~ 10 cm) speculum to sample vaginal epithelial cells. Swabs were rolled on glass slides, fixed and stained to differentiate cell types using a Papanicolaou stain kit. Cytology analysis revealed 4.75% parabasal cells, 43% intermediate cells, 24.75% superficial cells, and 27.5% anuclear superficial cells. Aerobic and anaerobic cultures were obtained from perivulvar region, and caudal and deep vagina. All 3 areas cultured *Klebsiella* sp and *Pseudomonas aeruginosa* with no anaerobic growth. Perivulvar region also grew *Aeromonas punctata* and *Enterococcus faecalis*, whereas caudal and deep vaginal cultures grew *Proteus hauseri* and *Staphylococcus schleiferi*. Vaginal endoscopy with a 43 cm rigid 9.5-13.5Fr transcervical insemination (TCI) endoscope (Karl Storz, Tuttlingen, Germany) with manual insufflation was performed. Scope was advanced through the vestibule until the vestibulovaginal junction demarcation. Normal light pink mucosa was noted in the vestibule with minimal horizontal striation. A growth protruding from the ventral right vaginal wall was noted adjacent to the urethral opening. It was estimated to be 3-5 mm and was similar in appearance to the vulvar mass. No samples of the mass were taken. Vaginal mucosa appeared slightly darker pink with horizontal folds. Scope was advanced through the vaginal canal until cervix was visualized. Cervix appeared as a normal dorsal out pocketing with pink mucosa at the cranial end of the vaginal canal, and no excessive mucus or abnormalities were noted. Distance from the vulva to vestibulovaginal junction was 17 cm and from the vulva to cervical os was 24 cm. Bear recovered uneventfully and with no clinical issues related to the masses at this time. Although brown bears are not bred for genetic conservation in zoos, they offer valuable insights for comparison with threatened species like polar bears, sloth bears, and Andean bears. Reproductive examination to collect baseline data on reproductive anatomy and pathology may help assess factors influencing reproductive success or failure. Crucial for wildlife conservation, these examinations offer insights into overall health, reproductive trends, and potential threats faced by bear populations. This knowledge aids in developing targeted conservation strategies and contributes to maintaining genetic diversity and sustainable management of bear species, even if not bred in captivity.

Keywords: Transcervical endoscope, reproductive exams, brown bears, vaginal mass

Bilateral cryptorchidism in a Miniature Horse and a Mustang

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Cryptorchidism is the most common disorder of sex development in males. In horses, the prevalence ranges from 2 to 12%, with unilateral cryptorchidism more common (85.7-95.2%) than bilateral (4.8-14.3%).[1,2] This condition is easily diagnosed in unilateral cryptorchid stallions and horses with a good history. However, it becomes challenging in presumed geldings presented with stallion-like behavior. This report describes 2 cases of bilateral cryptorchid stallions, a Miniature Horse and a Mustang, purchased as geldings but later exhibited stallion-like behavior, and were referred for localization of testes and cryptorchidectomy. Endocrine evaluation was performed by the referring veterinarian. Miniature Horse' serum concentrations of estrone sulfate and testosterone were 25.94 ng/ml (geldings < 0.1 ng/ml; cryptorchid 35-60 ng/ml; intact stallions 140-200 ng/ml) and < 10 pg/ml (geldings < 50 pg/ml; cryptorchid 100-500 pg/ml; intact stallions 800-2000 pg/ml) respectively. Mustang, testosterone concentrations were 987.6 pg/ml (after hCG stimulation). In the miniature horse, transcutaneous inguinal ultrasonography revealed a 3 cm soft tissue structure, consistent with testis and located in the right abdomen dorsal to the vaginal ring. Left testis was not identified and transrectal ultrasonography was not performed due to horse's size. In the Mustang, both transrectal and transabdominal ultrasonography revealed 2 soft tissue structures (3 cm), consistent with testes, located in the abdomen dorsal to the respective vaginal rings. Before surgery, a blood sample was collected from horses for antimüllerian hormone (AMH). Serum AMH concentrations were 12.2 ng/ml in the Miniature Horse and 10 ng/ml in the Mustang (geldings < 0.15 ng/ml; cryptorchid and intact stallions > 0.15 ng/ml). Laparoscopic cryptorchidectomy was performed. Miniature Horse underwent general anesthesia and a laparoscopically assisted technique in dorsal recumbency in Trendelenburg position. The Mustang's surgery was performed standing using a flank laparoscopic approach, and testes were removed via morcellator.[3] Testes were successfully visualized above the vaginal rings and removed. Histopathology had the following: no evidence of spermatogenesis in seminiferous tubules with enlarged lumen, and vacuolization (consistent with testicular

degeneration due to abnormal thermoregulation). These cases highlighted the importance of endocrine testing to confirm cryptorchidism in presumed geldings exhibiting stallion-like behavior. Testosterone estimation may not be reliable (sensitivity 85% and specificity 91%) due to diurnal fluctuations, seasonal effects, and age-related variations, making stimulation by hCG or GnRH necessary to improve diagnostic accuracy. Estrone sulfate sensitivity is 88%, and specificity is 84%, and it is not accurate in horses < 3 years of age. AMH sensitivity and specificity have not been determined; however, it is considered the best biomarker in cryptorchid horses because only 1 blood sample is required, and the serum concentration is not affected by hourly or seasonal variation. Furthermore, these cases suggested that bilateral cryptorchidism is likely underdiagnosed in horses sold as geldings and not exhibiting stallion-like behavior, placing them at risk of developing retained testicular tumors later in life.

Keywords: Stallion, gelding, cryptorchid, antimüllerian hormone, laparoscopy

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Vaginal polyp prolapse in a queen

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A 9-year, domestic shorthair queen, was presented for evaluation of tissue acutely protruding from the vulva and lethargy. Her last estrous cycle was 3-4 months prior, and she was reportedly primiparous. On presentation, the queen was systemically stable. Intake diagnostics included PCV/TS (42% and 7.0 g/dl, respectively), blood glucose (304 mg/dl), and blood lactate (2.9 mmol/l). These results supported the clinical findings of mild dehydration, in-hospital stress and local urogenital tissue hypoxia. Physical examination revealed a spherical, turgid protrusion, of hyperemic tissue measuring ~ 6 x 4 cm appearing from the vulva. Interestingly, the tissue appeared to have a lumen, and a small circular region of ischemia was identified at the caudal-most aspect of the protruding tissue. Quick assessment via ultrasonography noted no free fluid within the prolapsed structure and an intact urinary bladder in situ. Comprehensive bloodwork and abdominal imaging were performed. Complete blood count revealed mild thrombocytopenia (deemed artifact), lymphopenia, and monocytosis (stress leukogram). Chemistry panel revealed mild hypoalbuminemia (2.67 g/dl), elevated creatinine kinase, and mild electrolyte derangements and no evidence of urinary obstruction (e.g. hyperkalemia or azotemia). Diagnostic imaging included transabdominal ultrasonography and abdominal radiographs. Right uterine horn was minimally dilated with anechoic fluid, beginning at the bifurcation. A cystic structure (~ 1 cm in diameter) associated with the cervix was identified near the pelvic inlet, dorsal to the urethra. Urinary bladder and urethra were noted to be moderately distended, and echogenically stippled urine was appreciated within the bladder lumen. Due to the inability to reduce the prolapsed and necrotic tissue, the queen underwent ovariohysterectomy and vaginal tissue resection. Uterus, ovaries, and prolapsed vaginal tissue were submitted for histological evaluation. Maturing oocytes and stroma were identified in the ovaries that were otherwise unremarkable. Cystic endometrial hyperplasia was identified in uterine horns. Vaginal tissue was hyperplastic in nature, and an edematous fibrovascular polyp was identified. There were no complications postoperatively and the patient was able to urinate and defecate normally after the procedure. Fibrovascular vaginal polyps are uncommon, benign neoplastic masses that are more often reported in middle-aged to older dogs, with limited case reports published in the queen.[1,2] Often incidental findings, these can go undetected or be managed conservatively. Prolapsed hyperplastic vaginal tissue is more common, and a sequela to estrogen dominance during estrus or prepartum. In this case, the presence of follicular tissue may have contributed to the hyperplastic vaginal tissue identified, resulting in prolapse of the polyp. Another consideration is that the polyp reached a certain threshold in size, leading to discomfort and increased abdominal press. Surgical excision in an attempt to preserve normal anatomy and fertility could be considered in the event that these are identified at an early stage, prior to prolapse and tissue necrosis. Thus, though less common, a vaginal polyp is an important differential to consider when presented with prolapsed tissue from the vulva in a dog or cat.

Keywords: Prolapse, polyp, hyperplasia

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Use of reproductive tract extracellular vesicles to capacitate bull sperm in vitro

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Sperm cryopreservation induces plasma membrane damage and premature acrosome exocytosis resulting in reduced sperm longevity, viability, and fertilizing potential (in vivo and in vitro [IVF]). Extracellular vesicles (EVs) have a physiological role in sperm function through posttesticular modification of sperm, yet that role is not completely understood. Objective of this study was to determine if EVs from the reproductive tract can affect sperm physiology and fertility. We collected EVs from oviducts and accessory sex glands and from in vitro grown 3-D organoid cultures that were stimulated with steroid hormones to mimic in vivo estrus conditions. We hypothesized that EVs collected from seminal plasma (SP) and in vivo-derived diestrous oviductal cells (DO) would delay sperm capacitation, whereas EVs collected from in vivo-derived estrous oviductal cells (EO), and in vitro oviductal organoids (OO) would enhance capacitation. Frozen- thawed sperm from 6 bulls with proven fertility with IVF were treated with: 1. no EVs (control); 2. SP; 3. DO; 4. EO; 5. OO; or 6. dilauroylphosphatidylcholine (PC12; + control) after thawing. Samples were incubated at 38°C and assessed at 0.5, 1, 2, and 4 hours posttreatment for motility using microscopy/computer-assisted sperm analysis system (CASA), viability (PI), acrosome integrity (FITC-PNA), and tyrosine phosphorylation (pY20 antibody) using flow cytometry. Sperm fertilization potential was determined using IVF. Data were analyzed using ANOVA. Addition of OO and EO resulted in higher percentages of live-acrosome reacted sperm at 4 hours (8 ± 1 and 6 ± 1, respectively), than sperm treated with SP (2 ± 1; $p < 0.05$). Additionally, a higher percentage of sperm exhibited capacitation changes at 4 hours (assessed by tyrosine phosphorylation) when treated with OO or EO (67% ± 3 and 55% ± 3, respectively) than control sperm (51% ± 3, $p < 0.05$). Sperm treated with OO tended to fertilize more oocytes in vitro (76%) than control sperm (67%; $p = 0.13$). This study indicated that oviductal EVs enhanced sperm capacitation, and SP EVs improved frozen-thawed sperm longevity. These preliminary findings have positive implications to improve fertility rates in commercial bovine assisted reproductive technology and artificial insemination breeding programs.

Keywords: Sperm, cryopreservation, extracellular vesicles, organoids

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

Equine Abstracts

Novel management of chronic pyometra in the mare

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A 24-year, Standardbred mare, with an unknown breeding history was presented on farm with mild colic signs and purulent vulvar discharge. Transrectal palpation and ultrasonography performed by the referring veterinarian revealed an enlarged uterus filled with fluid, and the mare was referred for further management. On presentation, the mare was bright and alert with normal vital parameters and point of care blood work within reference ranges. Purulent discharge was evident on the mare's perineum, tail and hindlegs. Transrectal palpation and ultrasonography revealed a distended uterus with > 15 cm echogenic fluid. Intrauterine catheter was passed through the cervix into the uterus; the resulting effluent was thick and diluted as needed with lactated Ringer's solution to facilitate drainage. Approximately 50 liters of thick off-white effluent was obtained. Mare was treated daily with uterine lavage followed by intrauterine infusion with ceftiofur sodium (CeftiFlex, Aspen Veterinary Resources, Loveland, CO) and intracervical topical steroids (Animax, Dechra, Overland Park, KS) to reduce adhesion formation. Purulent effluent became more hemorrhagic as treatments continued; hysteroscopy was performed and the endometrium was diffusely inflamed, ulcerated, and hyperemic. Mare was discharged on oral sulfadiazine/trimethoprim (Equisul, Aurora Pharmaceutical, Northfield, MN) and flunixin meglumine (Covetrus, Portland, ME) and was scheduled to return in 3 - 4 weeks for further treatment after allowing the endometrium to heal. Due to noncompliance, 11 months passed before the mare was presented for reevaluation. Mare's tail was clean and no vulvar discharge was appreciated. Transrectal palpation and ultrasonography confirmed a distended uterus with > 10 cm of moderately echogenic fluid. Manual cervical examination confirmed the presence of transcervical adhesions that had effectively sealed the cervix closed. Careful manual dissection through adhesions and scar tissue was performed until a finger could pass through external os. Once again, uterine evacuation, culture, lavage, and antimicrobial infusion were performed serially for 5 treatments. In order to maintain cervical patency and prevent ongoing adhesion formation, an intracervical stent was made with a short, 14 mm internal diameter silicone tracheostomy tube (Bivona, Gary, IN) placed through the cervix, with the cuff inflated at the internal os and the tracheostomy tube flange at the external os. Mare was examined at gradually increasing intervals to ensure that the cuff remained inflated and the stent in place. After 3 months, the stent was expelled. Manual examination confirmed healing around the stent, and ultrasonography confirmed the absence of intrauterine fluid. Without an inflammatory nidus passing through the cervix, it has remained patent for 10 months thus far. Chronic pyometra in the mare is an uncommon but potentially significant occurrence, typically secondary to outflow (i.e. cervical) obstruction. This condition may be seen following dystocia without appropriate after care. Several options have been described for the management and treatment of pyometra, including bilateral ovariectomy to induce an anestrous state in which the cervix may be moderately relaxed so as to allow drainage, cervical wedge resection[1] or a cervicectomy using a fetotome.[2] In this case, the mare's financial value, owner compliance, and cervical anatomy made her a poor candidate for surgical intervention.

Keywords: Mare, pyometra, cervix, adhesions

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Incubation of frozen/thawed stallion sperm in a lactate-modified Whitten's medium induced molecular changes associated with the acquisition of fertilizing capacity

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Molecular events associated with the acquisition of fertilizing competence (capacitation) include protein tyrosine phosphorylation (PTY), acrosomal exocytosis (AE), and relocation of sperm- oocyte interaction proteins. Fresh stallion sperm undergo spontaneous AE while retaining viability (AE/Viable),[1,2] when incubated under presumed capacitating conditions (presence of calcium, bicarbonate, and albumin) in a medium with lactate as the only energy substrate (lactate-modified Whitten's medium [Lac-MW]). Following incubation in Lac-MW, a time-dependent change in the occurrence of PTY has been reported in fresh stallion sperm.[2] In addition, the percentage of AE/Viable sperm in fresh and frozen/thawed semen has been reported to be similar.[2] Whether the occurrence of AE/viable sperm in frozen/thawed stallion sperm is temporally related to changes such as PTY or relocation of the sperm-oolemma fusion protein IZUMO1 is unknown. We processed frozen/thawed semen from 5 fertile stallions by density gradient centrifugation (40% silica particle solution), diluted to 30 x 10^6 sperm/ml in Lac-MW, and incubated at 38.2°C in 5% CO2 for up to 6 hours. At 2, 4, and 6 hours of incubation, aliquots were analyzed for AE/Viable sperm via flow cytometry, whereas PTY and IZUMO1 expression were

examined by immunofluorescence. Data were analyzed using the mixed linear model. Statistical significance was set at $p < 0.05$. The mean percent AE/Viable sperm was lower ($p < 0.05$) at 2 hours than at 4 or 6 hours (17, 31, and 44%, respectively). During incubation in Lac-MW, the immunofluorescence signal locations generally changed from a caudal position on the sperm to acrosome for both PTY and IZUMO1 and were specifically characterized as follows: PTY transitioned from the equator, midpiece and principal piece (Location I [L1]) to the acrosome, equator, and midpiece (Location II [LII]); and IZUMO1 transitioned from the equator (Location A [LA]) to acrosome region (Location B [LB]). At 2 hours, the mean percent of sperm displaying PTY-LI was higher ($p < 0.05$) than LII (43 versus 12%), whereas by 4 and 6 hours, the percent of sperm displaying LII was higher ($p < 0.05$) than LI (28 and 22%, respectively). At 2 hours, the mean percent of sperm displaying IZUMO1-LA was higher ($p < 0.05$) than for LB (32 versus 11%). By 4 and 6 hours of incubation, the mean percentages of IZUMO1-LB were 34 and 41%, respectively. These values were higher ($p < 0.05$) than those observed at 2 hours. Overall, the sequence of molecular events in frozen/thawed stallion sperm incubated in Lac-MW was characterized by the movement of PTY from the principal piece to the acrosome, followed by an increase in AE/Viable sperm, and then by IZUMO1 relocation to the acrosome. These molecular changes resembled to similar sequence of events described for capacitation in other species.[3] Current studies are focused on determining whether incubation in Lac-MW could be used to prepare frozen/thawed stallion sperm for conventional in vitro fertilization of in vitro-matured equine oocytes.

Keywords: Stallion sperm, frozen/thawed semen, acrosomal exocytosis, tyrosine phosphorylation, IZUMO1

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Blastocyst development of in vitro-matured equine oocytes fertilized by intracytoplasmic sperm injection using nonsorted or sex-sorted frozen/thawed stallion sperm

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Intracytoplasmic sperm injection (ICSI) using frozen/thawed (F/T) stallion sperm is a common procedure in equine breeding industry. Historically, sex-sorted (SS) F/T stallion sperm has yielded lower cleavage (< 30%)[1] and blastocyst rates (< 5%)[2] after ICSI compared to nonsorted (NS) F/T sperm. A new technology for sperm sex-sorting (Genesis III; Cytonome®) has been validated by a commercial company (ST Genetics), in which bovine sperm quality and in vivo fertility (62 versus 64%, respectively) are comparable to NS-F/T sperm.[3] Our objective was to determine the efficiency of ICSI on in vitro-matured equine oocytes fertilized using SS-F/T stallion sperm processed through Genesis III technology. We hypothesized that in vitro-matured equine oocytes fertilized by ICSI with SS-F/T sperm have similar cleavage and blastocyst rates to NS-F/T sperm. Immature oocytes were collected via transvaginal oocyte aspiration from 8 mares during 65 sessions. A total of 324 in vitro-matured oocytes were fertilized by Piezo-driven ICSI using NS- F/T (n = 160 oocytes) or SS-F/T (n = 164 oocytes) from a fertile stallion. After ICSI, injected oocytes in both groups were cultured for 5 days, cleavage rate was assessed (i.e. > 8-16 blastomeres/embryo), and only cleaved embryos were further cultured. On days 7 to 10 postICSI, blastocyst development was assessed. Cleavage and blastocyst rates were analyzed by Fisher's Exact Test, whereas postthaw sperm quality data were analyzed by One-way ANOVA with LS Means-Tukey's adjustment test. Significance was set at $p < 0.05$. Overall, higher ($p < 0.05$) cleavage (NS-F/T: 113/160 [71%] versus SS-F/T: 79/164 [48%]) and blastocyst rates (NS-F/T: 42/160 [26%] versus SS-F/T: 23/164 [15%]) were observed in NS-F/T than in SS-F/T sperm. Blastocyst rate per cleaved oocytes (NS-F/T: 42/113 [37%] versus SS-F/T: 23/79 [32%]) was not different ($p > 0.05$). Percentage of blastocysts developing on days 7 (33 versus 26%), 8 (19 versus 35%), 9 (38 versus 22%), or 10 (10 versus 17%) was similar ($p > 0.05$). Number of embryos/session was numerically, but not statistically higher ($p > 0.05$) between NS-F/T (1.4) versus SS-F/T (0.68) sperm. Considering these results, in a separate experiment, NS-F/T and SS- F/T sperm from 3 fertile stallions were analyzed for postthaw sperm motility, viability, and DNA damage. Postthaw sperm motility was higher ($p < 0.05$) in NS-F/T than in SS-F/T sperm (41 versus 12%), whereas sperm viability was similar ($p > 0.05$) between groups (54 versus 57%). Interestingly, percentage of sperm with damaged DNA was considerably higher ($p < 0.05$) in SS- F/T than in NS-F/T sperm (80 versus 5%). Compared to earlier reports,[1,2] although an improvement in blastocyst development after ICSI with SS-F/T sperm was observed, efficiency of the Genesis. III technology for in vitro equine embryo production was lower than NS-F/T sperm. Lower cleavage rates observed may be associated with higher percentage of DNA damage in SS-F/T sperm, Current studies are focused on determining the effect of SS-F/T on initial steps of embryonic development postICSI.

Keywords: Equine embryo, sex-sorted sperm, cleavage, blastocyst, sperm DNA

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Effect of lactobacillus application on mare clitoral microbiome

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Mare's lower reproductive tract (LRT) harbors a community of commensal organisms. Dysbiosis of LRT may have a role in clinical diseases, including endometritis, metritis, and placentitis. Metagenomic/metagenetic analysis of bacterial DNA can identify organisms that are not readily cultured and thus may go undetected. Recent metagenetic studies have indicated that uterus, placenta and vagina of the mare accommodate microorganisms in varying diversity, abundance and richness. Clitoral microbiome has yet to be described in the mare. We tested the following hypotheses: 1. clitoris of estrous mares harbors a unique resident microbiome; 2. topical lactobacillus (LB)-containing probiotic alters clitoral microbiome; and 3. mare conception rates after clitoral LB application will not differ significantly from industry standards. Mares (n = 12) with normal breeding soundness evaluation were utilized in the same breeding season. Sterile swabs of the clitoral fossa were collected (0) prior to daily topical LB (1 gram) for 4 days. Pharmaceutical grade LB was a silicone-based gel containing *Lactobacillus pentosus*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* in equal parts totaling 10^9 -10^{10} CFU per gram. A second (12 hours) and third swab (48 hours) of the clitoral fossa was collected 12 and 48 hours, respectively, following final LB application. Swabs were frozen and stored at –80°C until DNA extraction. On the next estrus, mares were bred by artificial insemination with 1 x 10^9 motile sperm from a single fertile stallion. Conception rates were calculated based on reproductive ultrasonography 14-16 days after ovulation. Genomic DNA was extracted from frozen swab using Qiagen DNeasy PowerSoil extraction kits, screened by gel electrophoresis followed by PCR amplification of the 16S rRNA gene prior to multiplex sequencing of the V4 region using an Illumina Miseq platform. The top 3 most abundant phylum resident in the equine clitoral fossa (0) were Firmicutes, Bacteroidota, and Fusobacteriota; contradictory to equine uterine and vaginal microbiome, with Proteobacteria in the top 3 phylum. Abundance was evaluated via Friedman test with pairwise Dunn's post hoc comparisons. Statistical significance was set at $p < 0.05$. Compared to time 0, Desulfobacterota decreased at 12 and 48 hours, whereas Synergistota only at 48 hours, and Fusobacteriota increased only at 12 hours ($p < 0.05$). At the genus level, *Lactobacillus* sp. was increased in the clitoral fossa at 12 hours but returned ($p < 0.05$) to 0 levels by 48 hours *Corynebacterium* sp. increased at 12 and 48 hours compared to 0, whereas *Actinobacillus sp.* increased ($p < 0.05$) in a time-dependent manner. Furthermore, *Mobiluncus* sp. and *Christensenellacea_R-7_group* decreased ($p < 0.05$) at 12 and 48 hours compared to 0. LB had an effect ($p<0.05$) on beta but not alpha diversity at both 12 and 48 hours. Finally, LB treated mareshad an 80% pregnancy rate. Our results indicated that mare clitoral fossa microbiome is unique to the vagina and uterus measured by metagenetics, LB application dynamically alters the resident clitoral microbiome, but does not interfere with fertility of normal mares. Future investigations are needed to understand the role of the LRT microbiome and probiotics in equine breeding.

Comparison of commercial vitrification kits for vitrifying equine embryos

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Several commercial vitrification kits have been used for vitrifying equine embryos. However, the optimum types and concentrations of intra- and extra-cellular cryoprotectants for equine embryos have not been investigated. Our aim was to compare the pregnancy rate of embryos vitrified using 2 commercial human vitrification kits (Kitazato; www.kitazato.co.jp or VitKit- Freeze; www.irvinesci.com). Each kit contained an equilibration solution (ES) and vitrification solution (VS). The Kitazato ES comprised 7.5% dimethyl sulfoxide (DMSO) and 7.5% ethylene glycol (EG) in medium with HEPES, gentamycin, and 0.06 mg/ml hydroxypropyl cellulose (HPC). The VitKit-Freeze ES varied only in that it contained 20% dextran serum supplement (DSS) instead of HPC. The VS of both kits contained 15% DMSO and 15% EG in their respective base media, but with the addition of 0.5M trelahose or sucrose for the Kitazato and VitKit-Freeze, respectively. Our hypothesis was trelahose and HPC versus sucrose and DSS in the VS improve embryo survival after vitrification. Sixty-eight, unpunctured, embryos ≤ 500 mm were vitrified using Kitazato (n = 35) or VitKit-Freeze (n = 33). Embryos were placed in Kitazato or VitKit-Freeze ES for 15 minutes at room temperature (RT), then in Kitazato or VitKit-Freeeze VS for £ 90 seconds at RT, loaded onto a Cryolock and plunged into LN2. Before transfer to a day 6 recipient mare, vitrified embryos were warmed in thawing solution (1 mol/l sucrose) at 42°C for 1 minute, then warming solution (0.5 mol/l sucrose) for 4 minutes at RT, before transfer to a holding medium (Vigro Hold) for 4 minutes. Conceptus development in pregnant recipients was checked until day 25 of pregnancy, or day 34 in 2 recipients per vitrification method and size group (G1: ≤ 300 µm; G2: > 300-400 µm; G3: > 400-500 µm). Percentages of pregnant mares at day 25 overall, and per size group, were compared between vitrification kits using a z-test. Differences in mean embryo size between G1-3 for each vitrification kit were assessed using t-test. Significance was set at $p < 0.05$. No difference existed ($p = 0.14$) in the overall pregnancy rate between the Kitazato or VitKit- Freeze vitrification kits (28/35 [80%] versus 20/33 [60.6%], respectively). The mean (± SEM) diameter of embryos in G1, G2, and G3 did not vary between kits (G1: 230.8 ± 9.2 versus 253.0± 15.0 mm [$p = 0.21$]; G2: 349.1 ± 7.6 versus 344.5 ± 9.4 mm [$p = 0.71$]; G3: 444.2 ± 8.6 versus 451.9 ± 8.7 mm [$p = 0.53$]; Kitazato versus VitKit-Freeze, respectively). Pregnancy rates for G1 and G2 were not different between

kits (G1:10/12 [83.3%] versus 8/10 [80%], p = 0.72; G2: 9/11 [81.8%] versus 9/10 [90%], p = 0.93; Kitazato versus VitKit-Freeze, respectively). However, for G3, the pregnancy rate was higher (p = 0.03) with Kitazato versus VitKit-Freeze (9/12 [75%] versus 3/13 [23.1%]). Three recipients lost their pregnancies before day 20, all 3 embryos (225, 350, and 460 mm) were vitrified with the Kitazato kit. In conclusion, Kitazato improved pregnancy rate over VitKit-Freeze for embryos > 400 - 500 µm. Trelahose and HPC have been reported to support a higher glass transition temperature and membrane stability than sucrose, and these properties may have aided the vitrification of larger embryos. Higher numbers of Kitazato vitrified embryos are required to ensure pregnancy loss is not a risk factor of this methodology.

Effect of novel water-dispersive ubiquinol supplementation in extender on postthaw stallion sperm quality

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Supplementation of various preparations of ubiquinone oxidized, ubiquinol reduced coenzyme Q10 (CoQ19) in semen extenders has an antioxidant effect, resulting in sperm cryoprotection. CoQ10 supplementation can also improve mitochondrial function in sperm resulting in improved postthaw sperm viability and motility. However, the effects of extenders supplemented with CoQ10 on postthaw semen quality have been variable. Extender supplementation with powdered CoQ10 did not improve postthaw stallion sperm quality.[1] CoQ10 is not water soluble and may precipitate at the bottom of the supplemented extender resulting in decreased CoQ10 bioavailability in semen extenders. Thus, our aim was to test the hypothesis that postthaw CoQ10 supplementation, using a novel water-dispersive ubiquinol commercial preparation (ShiroQE®), improves postthaw sperm quality and longevity. Dispersion was composed of 13% ubiquinol, 13.4% modified starch, 6.5% sodium ascorbate, 3% dextrin, 3.5% Arabic gum, and 60.6% water. A total of 15 replicates (straws) from 3 stallions frozen in E-Z Freezing 'LE'® (EZ) were evaluated for sperm progressive motility (PM), total motility (TM) and motion kinetics (VCL, STR, LIN, ALH) using computer-assisted sperm analysis (CASA). After thawing, the semen was diluted with 4 treatments: EZ, INRA96® (I), INRA96® plus 300 micromolar ubiquinol (I300), and INRA96® plus 150 micromolar ubiquinol (I150). The CASA evaluations were performed at 10, 60, and 120 minutes postthawing. Data were analyzed using 2-way ANOVA under repeated measures. The means were compared using Tukey's test and presented as mean ± SEM. Effect of time and treatment interaction was not significant for any CASA parameters. Effects of time and treatment were significant for PM, TM and VCL but not for STR, LIN and ALH. The mean sperm PM, TM, and VCL did not differ among various treatments at 10 minutes and 60 minutes postthawing. At 120 minutes postthawing, the mean sperm PM, TM and VCL were significantly higher for I (21.80 ± 2.62, 28.31 ± 2.66 and 96.60 ± 3.82, respectively) than for I300 (8.405 ± 1.86, 14.71 ± 2.38, 74.03 ± 4.61, respectively). At 120 minutes postthawing, the mean sperm PM, TM, and VCL did not significantly differ among EZ versus I, EZ versus I300, EZ versus I150, I versus I150, and I300 versus I150. Sperm TM, PM, and VCL at 60 and 120 minutes were nonsignificantly higher for I than for EZ extender. This confirmed that diluting semen, postthaw, in INRA96 did not negatively affect sperm quality and longevity. This can be used for insemination under field conditions. In conclusion, adding water-dispersive ubiquinol preparation did not improve postthaw quality of stallion sperm. Viscosity of the dispersion can negatively affect sperm quality. However, a study[2] suggested that CoQ10 supplementation of semen extenders enhanced postthaw sperm.

Keywords: Coenzyme Q10, stallion, semen, postthaw

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Relationship between sire size and breeding dose in dogs

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Minimum dose for artificial insemination (AI) in dogs has been widely disputed. Multiple recent studies proposed that the accepted canine breeding dose is 150-200 x 10^9 total sperm. However, pregnancies have been reported via surgical insemination with as little as 20 x 10^6 total fresh sperm. We hypothesized that the minimum total progressive morphologically normal sperm (TPMNS) necessary to achieve pregnancy depends on sire size. Aim of this retrospective study was to determine the minimum sperm dose likely to establish a pregnancy by either vaginal insemination or transcervical insemination, and to establish recommendations for insemination based on sire size. Medical records were evaluated from sires presented for semen collection and AI at 2 veterinary clinics specialized in animal reproduction between 2021-2024. Information gathered included age, breed, weight, semen evaluation (volume, color, concentration, and morphology), pregnancy rate, and litter size. Sires were categorized into 2 groups: 1. PREG: pregnant female and 2. NP: nonpregnant female. Within each group, sires were classified as: Large (> 56 lb), medium (35-55 lb), small (10-34 lb), and toy (< 10 lb). Sperm dose was determined by calculating total volume, total sperm (TS), total progressive motile sperm (TPMS), and TPMNS. For dogs that were bred multiple times during estrus, total semen dose was calculated by combining individual ejaculates. Semen volume, TS, TPMS, and TPMNS were compared by sire size and pregnancy status using a one-way ANOVA, and significance was set at $p < 0.05$. Twenty-one breeds were included, and sires had a mean age of 3.9 years (range: 1-9 years). For the PREG group (n = 46), mean volume, TS, TPMS, and TPMNS were 5.9 ml, 2.41, 2.01, and 1.74 x 10^9, respectively, compared to NP group (n = 16) of 7.6 ml, 1.06 x 10^9, 914, and 758 x 10^6. Semen volume was significantly less in the toy group (n = 6) than medium (n = 22, p = 0.03) or large (n = 20, p = 0.01), but no difference was observed in volume between PREG and NP groups. Although no difference was observed in TS by sire size, TS was different (p = 0.01) in NP group than PREG group. Similarly, no difference was observed in TPMS or TPMNS by sire size; however, TPMS and TPMNS was different (p = 0.02) in NP group than PREG group. Pregnancy rate was significantly worse when dogs were bred with < 200 x 10^6 TPMNS or > 5 x 10^9 TPMNS, with 1/7 dogs conceiving when bred with < 200 x 10^6 TPMNS, 19/22 when bred with 200-1200 x 10^6 TPMNS, 26/29 when bred with 1.21 to 4.9 x 10^6 TPMNS, and 0/4 when bred with > 5 x 10^9 TPMNS. In conclusion, there appeared to be minimal and maximal recommended TMPNS doses for dogs, and even toy breeds benefitted from a minimum breeding dose of at least 200 x 10^6 TMPNS to achieve pregnancy.

Keywords: Dogs, sperm, artificial insemination, pregnancy rate

Dag-like sperm defect in a dog suspected of reproductive infection

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A 7-year, 50 kg Rhodesian ridgeback dog, was evaluated for chronic poor semen quality and prostatitis. Dog was not used for breeding nor his semen was used for inseminations. Two years prior, 50% of sperm had coiled tails. Four months prior, the dog had 80% immotile sperm with 95% coiled tails, and semen culture identified *Escherichia coli*, *Proteus*, and an *Enterococcus* species. Therapy with enrofloxacin and later doxycycline did not improve spermiogram and dog was referred. On initial examination, dog appeared clinically healthy. Two scrotal testes were present, with normal orientation, symmetry, and turgidity on palpation and with normal appearance of all scrotal content on ultrasonographic examination. Epididymides and spermatic cords were symmetrical and normal on palpation. Total scrotal width (TSW) by caliper was 43 mm. Prostate was nonpainful on transrectal palpation and measured 35-40 mm in width, with left lobe slightly larger (mild mixed echogenicity on ultrasonographic examination). Two semen collections had 416 and 700 x 10^6 total sperm, respectively, with asthenozoospermia (< 10% total motility) and teratozoospermia (3-11% normal morphology). Cytological evaluation identified rare neutrophils and apparent sperm accumulation plugs with possible bacteria present. Initial New York State Veterinary Diagnostic Laboratory (NYSVDL) semen culture confirmed *Proteus mirabilis* and *Enterococcus faecalis*, and identified mycoplasma. In-house serum *Brucella canis* rapid slide agglutination test was negative. Basal serum thyroid and testosterone concentrations were normal. Therapy, based upon susceptibility testing, was completed with oral ciprofloxacin (10 mg/kg, twice daily, for 8 weeks and concomitant oral amoxicillin (10 mg/kg, twice daily, for 2 weeks). After completion of antibiotic course, *Proteus* and *Enterococcus* were cleared, but *Mycoplasma canis* was identified and susceptibility testing was performed through the NYSVDL. Oral azithromycin therapy (~ 12 mg/kg, once a day for 3 days, then every other day for 2 months) was initiated that cleared mycoplasma from the ejaculate. At 9 months after initial presentation, TSW had declined to 35 mm. Multiple ejaculates were evaluated during the course of care described. Due to continued prominent morphological defects, scanning and transmission electron microscopy were performed. Majority of sperm exhibited dag-like defects

in which the flagella were intensely coiled and enclosed within a common plasma membrane. Sperm exhibiting dag- like defects had coiling of all components of the flagellum including midpiece, principal, and end pieces. Transverse sections of the flagella displayed alterations, disarrangements, and/or absence of outer dense fibers and microtubules. Other histochemical evaluations indicated that sperm from the dog had normal chromatin integrity; however, only 32% had intact acrosome. There was no improvement during 9-month period and owner later elected castration. Testes were not provided for further examination.

Keywords: Dogs, infertility, teratozoospermia, asthenozoospermia, sperm morphology

Role of prostaglandin-endoperoxide synthases in parturition and dystocia in dogs

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Prostaglandins have key roles in parturition as they contribute to luteolysis, uterine contractions, and cervical dilation. Prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX 2) is the inducible key enzyme for prostaglandin biosynthesis, although historically, PTGS1 is regarded as the constitutively expressed isoform. In dogs, uteroplacental PTGS2 expression significantly increased at prepartum luteolysis compared to midpregnancy,[1] and uterine PTGS2 expression did not differ between dystocia types.[2] We hypothesized that PTGS2 and PTGS1 are important uterine and placental signals for initiation of parturition in dogs, and their altered expression contributes to primary uterine inertia (PUI). Our aim was to determine and compare gene expression of PTGS1 and PTGS2 in uterus and placenta of dogs at various stages of parturition or dystocia. Uterine biopsies and placental tissues were collected during cesarean surgery in dogs at term pregnancy that are not in labor (TNL, n = 7), in stage 1 labor (TL, n = 6), diagnosed with PUI (n = 6), or with obstructive dystocia having strong contractions (OD, n = 5). Relative gene expression for PTGS1 and PTGS2 was determined by qPCR. Blood was collected before surgery and serum progesterone (P4) concentrations were determined by chemiluminescence. One-way ANOVA, Kruskal-Wallis test, and paired Student's t-test were used for data analysis, and significance was set at $p < 0.05$. Serum progesterone concentrations were significantly higher in TNL (range: 3.4-5.3 ng/ml) than in other groups (0.3-1.3 ng/ml). In uterus, PTGS1 expression was significantly higher in TNL than in OD, and PTGS2 was significantly lower in TNL compared to other groups. Placental gene expression was similar among groups. Comparing gene expression by tissue types, PTGS1 was significantly higher in uterus than in placenta in all parturition groups. Conversely, PTGS2 expression was higher in placenta than in uterus in TNL and TL, but not in PUI and OD. Ratio of uterus/placenta PTGS1 gene expression was significantly higher in TNL and TL compared to OD. Ratio of placenta/uterus PTGS2 expression was significantly higher in TNL than in the PUI and OD groups. In conclusion, these preliminary results using gene expression data indicated that PTGS1 may have an important role in initiating uterine prostaglandin synthesis as labor approaches, with decreasing role during active labor, when PTGS2 becomes more important. In contrast to PTGS2, PTGS1 appeared to be a negligible contributor to placental prostaglandin production in dogs. PTGS1 and PTGS2 expression could not be pinpointed as contributors to PUI.

Keywords: Dogs, parturition, prostaglandins, uterus, placenta

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Chronic cardiac thrombosis in canine neonates

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A 2-year, maiden Cavalier King Charles Spaniel, was presented for initial progesterone timing prior to breeding. The kennel had previous issues with *Mycoplasma canis* causing reproductive failure, and the dog was given oral doxycycline (10 mg/kg, once a day). Physical examination was unremarkable and serum progesterone concentrations were consistent for day 3 postLH surge. Dog was bred artificially on days 4 and 6 postLH surge and doxycycline treatment was discontinued on day 10 postLH surge. Transabdominal ultrasonography was performed on day 37 postLH surge for pregnancy diagnosis and 5 fetuses with heartbeats were identified. Owner elected for planned cesarean surgery on day 61 postLH surge. Transabdominal ultrasonography revealed a hyperechoic cystic structure in the ventricle of 2 fetuses. Both fetuses had normal heart rates and serum progesterone concentrations were 6.48 ng/ml. Following day, cesarean surgery was performed after confirming a drop in serum progesterone concentrations (1.43 ng/ml). Eight live pups were delivered and all had harsh bronchovesicular sounds with no cardiac arrhythmias. Pups were empirically treated with 2.5 mg/kg of ceftiofur, and 6 out of the 8 pups received dextrose orally during resuscitation. Three days after delivery, 2 pups died suddenly and necropsy was performed within 24 hours. Postmortem examination revealed an abnormal cystic mass-like structure in the heart of both neonates and entire organ was submitted for pathology (PennVet Diagnostic Laboratories). Biopsy report described a focally extensive chronic thrombus adhered to the endocardial surface effacing endothelium at the base of the septal aspect of right ventricle composed of neutrophils, macrophages, erythrocytes, and fragmented to granular material. There was no evidence

of bacteria, infectious organisms, or neoplasia. Lesion was noted to be unusual with an unknown etiology. It was hypothesized that an in utero infectious or inflammatory insult spread hematogenously from dam to pup; however, other tissue samples were not submitted. There is limited research on diagnosis, etiology, and management of chronic cardiac thrombi in neonatal dogs, however causation and treatment for neonatal thrombosis has been studied in human medicine. It is reported that 2-4% of human infants diagnosed with thrombosis die as direct result of the pathology;[1] however, this information is not known in canine population. In humans, prenatal fetal thrombosis is rarely identified via ultrasonography; however, when diagnosed the cause was typically multifactorial.[2] Treatment of human neonatal thrombi can be managed with observation, through medication (thrombolytics and anticoagulants) or via a thrombectomy, as the definitive treatment depended on patient's risk of embolization and the underlying cause.[2] This case demonstrated the need for additional research on the etiology of antenatal canine fetal cardiac thrombosis that can aid in management or prevention of pathology. This case also demonstrated the use of ultrasonography to identify potential fetal abnormalities to provide a detailed assessment of fetal viability to breeders.

Keywords: Dogs, neonatal, cardiac thrombosis

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Treatment of male cats with adeno-associated gonadotropin-releasing hormone to achieve nonsurgical sterilization

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Overpopulation of stray cats poses substantial challenges to animal welfare and public health. Although traditional surgical sterilization methods are effective, they are resource- intensive, stressful to animals, and require a veterinarian. A nonsurgical technique would eliminate these barriers, remove physical and psychological stressors associated with surgery, and reduce the number of animals euthanized. We explored the potential of adeno- associated gonadotropin-releasing hormone (AAV-GnRH) treatment as a nonsurgical alternative for cat sterilization. Adeno-associated virus (AAV) is a nonenveloped virus that can be modified to target cells and deliver DNA. Gonadotropin-releasing hormone is produced by the hypothalamus and acts on anterior pituitary gland to produce follicle- stimulation hormone and luteinizing hormone. These hormones act on gonads to stimulate the release of testosterone in male and estrogen in female. Overproduction of GnRH should lead to downregulation of GnRH through constitutive activation of the receptor. We hypothesized that intravenous treatment of AAV containing GnRH to male kittens at 6 weeks induces a reduction in testosterone concentrations and impair sperm characteristics, resulting in infertility. With 4 treated and 4 control (untreated) kittens, the study employed monthly testicular ultrasonography starting at 4 months to calculate total testicular volume (TTV), testicular mass, and percent body weight. Once cats reached 6 months, they underwent monthly electroejaculation (EEJ) to analyze sperm number and characteristics. Preliminary results suggested a decrease in sperm production and quality, elevation of testosterone concentrations, and decreased total testicular volume. Ongoing research will refine and validate the efficacy of this innovative, nonsurgical approach, offering a step towards addressing the challenges posed by feline overpopulation with ethical and practical considerations.

Keywords: Feline contraception, adeno-associated virus, electroejaculation, GnRH

Prostaglandin assisted semen collection and cryopreservation of semen in an aged dog with testicular disease

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An 8-year, intact male golden retriever weighing 27.2 kg, was presented for semen collection and cryopreservation. Dog sired 1 litter in the past and semen was collected successfully once. Prior to visit, a hypoechoic, cyst-like structure of ~ 1 x 1 cm within right testis and multiple smaller cysts within left testis were identified via ultrasonography. Hemicastration was scheduled to remove right testis but was postponed for preservation of genetic material. On presentation, dog was bright, alert and responsive and no abnormalities were noticed on physical examination. First 2 collections, 30 minutes apart, using vaginal swabs of estrous females, were unsuccessful and only partial erection was obtained. Three days later, after several attempts in the presence of an estrous female and vaginal swabs, only a few drops of first fraction of the ejaculate were obtained. Dog's libido was low and did not posture or thrust during semen collection procedures. Four days later, intramuscular dinoprost tromethamine (0.1 mg/kg) was given 20 minutes before semen collection. Dog displayed moderate libido and partial ejaculation was obtained containing 31.2 x 10^6 sperm with 65/40% (total/progressive) motility and 71% morphologically normal sperm. Cryopreservation was not performed due to limited sperm count. Subsequently, semen was collected 4 times (every 4 days) aided by dinoprost treatment and cryopreserved with CaniPlus freeze extender with 20% egg yolk (Minitube, Verona, WI, USA). Results were as follows (mean ± SD): total sperm count of 419.3 ± 131.2 x 10^6 (Nucleocounter, Chemometec, Denmark) with 75.8 ± 3.8% total and 65.5 ± 10.5% progressive motility and 53 ± 6.1% morphologically normal sperm per collection. Predominant defects were coiled and kinked tails and abnormal midpieces. Post thaw motility was 48 ± 19.9% total and 38.3 ± 17.0% progressive. After castration, testes were measured, and

average size was ~ 6.5 x 3.5 x 1.5 cm and cystic structures were not appreciated. On histopathological examination of right testis, a Leydig cell tumor, most likely benign due to the low mitotic index and lack of other tissue involvement, was identified. In left testis, a Sertoli cell tumor was present, most likely benign due its well-demarcated area, mild pleomorphism and low mitotic count. A possible concern was the transmission of genes associated with these types of neoplasias to next generations; however, there is not enough evidence of their heritability and castration is the treatment approach. In this case, the genetic material of the stud dog was successfully collected through medically assisted ejaculation and subsequently cryopreserved.

Keywords: Chemical-assisted ejaculation, dog, prostaglandin, testicular neoplasia

Production Animal Abstracts

Evaluation of bull semen viability before, during, and after scrotal insulation via IZUMO1 expression and sperm-bound antisperm antibodies

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Most common cause of poor semen quality in bulls is testicular degeneration that can be transient. In a study in our laboratory, bulls recovered preinsulation semen parameters 48 days after scrotal insulation. However, there was persistence of antisperm antibodies (ASA), low mitochondrial membrane potential and altered concentrations of antimüllerian hormone and testosterone, in spite of these bulls being classified as satisfactory breeders during routine breeding soundness evaluation.[1] Bovine sperm-bound ASAs impaired acrosome reaction and binding to zona pellucida.[2] Persistence of ASAs, dysfunctional mitochondria and altered hormonal concentrations beyond regaining normospermia reflect a long-term effect of scrotal insulation on testicular and sperm function that is not detected via routine semen evaluation. These undetected changes could be responsible for long-term infertility in bulls recovering from testicular degeneration and with apparently normal microscopic sperm parameters. It is therefore important to understand how long-lasting ASAs affect fertilization to make recommendations for testing and management of bulls after undergoing a scrotal insult. In addition, IZUMO1, a protein involved in sperm-oocyte interaction during fertilization, essential for gamete fusion and for blocking polyspermy, was used to predict bull fertility.[3] We hypothesized that testicular degeneration leads to dysregulation of the IZUMO1 expression, the IZUMO1 expression is associated with semen microscopic parameters over time and IZUMO1 expression is not associated with ASAs. To test this hypothesis, 10 Angus- cross bulls were collected (AUP A2016 01-005-Y3-A0) via an eletroejaculator twice a week for 3 months. An insulated bag was placed around the scrotum for 8 days. Semen sample was evaluated for multiple parameters and semen was cryopreserved using a routine semen freezing protocol. Straws were stored in liquid nitrogen until flow cytometry analysis for evaluation of ASA binding and IZUMO1 expression. Timepoints analyzed were preinsulation (day 7 [during scrotal insulation]) and days 30 and 60 after insulation. These timepoints were selected to represent negative control, normospermic samples (T0), time when changes were observed on sperm exposed to heat within epididymis (T7), time with most pronounced changes in semen quality (T30), and time when 'normospermia' was recovered in the face of persistent ASAs and endocrine disruption (T60).[1,2] Positive correlation was observed between IZUMO1 stained sperm with progressive motility, normal morphology, sperm DNA integrity, and live sperm. Negative correlation was observed between IZUMO1 positive and abnormalities in head shape, midpiece, coiled tails, and failure of the bull to pass the breeding soundness evaluation. IZUMO1 expression was negatively affected in some primary morphological defects, probably related to impairment during spermiogenesis, since the gene is upregulated in testes as observed in a transcriptomic study.[4] These findings supported the hypothesis that IZUMO1 expression is affected by testicular degeneration, potentially compromising sperm-egg fusion and affecting fertility. Moreover, this research opens avenues for further investigations to detect subfertily or infertility in livestock populations.

Keywords: Bulls, infertility, testicular degeneration, sperm, physiology

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Effect of delayed maturation on cloned bovine embryo production

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Cloning by somatic cell nuclear transfer is a powerful technology for producing transgenic animals and for genetic preservation of high value and endangered animals. However, the technology is very time consuming, limiting the number of clones that can be produced in a day, making it less efficient. As in equine oocytes, holding a batch of bovine slaughterhouse oocytes could allow a second opportunity to produce cloned embryos. Our aim was to determine if holding oocytes overnight has detrimental effects on cloning efficiency. We hypothesized that holding bovine oocytes overnight at room temperature before maturation allows a second cloning replicate the following day with similar embryo development rates

compared to conventional maturation. Bovine oocytes from slaughterhouse ovaries were collected and assigned to 2 groups: Conventional maturation (CONV) where oocytes were matured for 20 hours in maturation media (IVF Biosciences) or delayed maturation (HOLD) where oocytes were held for 24 hours in holding media (Vigro Holding Plus, Vetoquinol, USA) at room temperature (23°C) before in vitro maturation. After maturation, oocytes were stripped from cumulus cells and matured oocytes with visible polar body were treated with protease to remove zona pellucida. Oocytes were then manually bisected and the hemi-cytoplasts obtained were scanned under UV light after staining with bisbenzimide; halves containing the metaphase plate were removed and used as controls. Two enucleated halves were reconstructed with a single neonatal fibroblast and fused with 1 single electric pulse. Two hours after fusion, embryos were activated with ionomycin and 6- dymethylaminopurine. Embryos were cultured for 7 days in commercial culture media (IVF Biosciences) and evaluated for cleavage and blastocyst rates. Regular in vitro fertilized (IVF) oocytes and parthenogenetically activated oocytes were used as controls. Differences among groups were assessed using Logistic Regression ANOVA in JMP Pro v17. After 6 replicates, a total of 692 and 680 oocytes were placed in maturation for CONV and HOLD groups, respectively and the maturation rates were similar (CONV: 70.2 ± 3.2 versus HOLD: 67.2 ± 4.0). Cleavage (88.3 ± 10.4 versus 86.0 ± 8.1) and blastocyst (21.9 ± 12.6 versus 14.7 ± 8.9) rates for somatic cell nuclear transfer were not different ($p > 0.05$) between CONV and HOLD groups. Holding oocytes overnight did not affect ($p > 0.05$) development rates for conventional IVF, with similar cleavage (82.8 ± 2.9 versus 79.0 ± 3.5) and blastocyst (31.8 ± 5.5 versus 27.6 ± 5.6) rates for CONV and HOLD groups. Parthenogenetically activated, zona-free controls followed a similar trend where cleavage (91.6 ± 4.6 versus 97 ± 1.3) and blastocyst (38.6 ± 8.4 versus 40.5 ± 3.6) rates were not different between groups. In conclusion, oocytes held for 24 hours prematuration supported development of cloned, IVF and parthenogenetically activated bovine embryos.

Keywords: Bovine oocytes, maturation, cleavage, blastocyst, somatic cell nuclear transfer

Microfluidic and density gradient techniques improved frozen-thawed epididymal bighorn rams' sperm

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Gamete preservation of wild animals is a priority in conservation programs. Field techniques to recover sperm from deceased animals may impair its final quality. The variable degree of blood and tissue contamination may impact cryopreservation. To improve postthaw quality, postmortem-harvested and cryopreserved semen may need to be sorted before use. Sperm sorting techniques based on centrifugation can increase the risk of damaging sperm. In contrast, microfluidics uses hydrostatic pressure and capillary forces, avoiding the need for costly equipment and minimizing cell damage. This study aimed to compare sperm motion parameters, morphology, and acrosome integrity of cryopreserved-thawed epididymal bighorn ram sperm before and after sorting. Two commercial sorting techniques, VetCount Harvester® device (MFD) and Bovipure® density gradient centrifugation (DCG), were used. Our hypothesis was that semen motion parameters improve equally using either technique. Sperm parameters associated with quality were: progressive motile sperm (PMS), straight velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), beat cross frequency (BCF), amplitude of lateral head deviation (ALH,) and plasma membrane integrity (PMI). Computer assisted sperm analyzer was used for the semen analysis. Eosin/nigrosin and Spermac® stains were used to assess morphology and acrosome integrity, respectively. Total number of morphological normal sperm with intact acrosome was counted by 2 veterinarians. Data were normally distributed based on Kolmogorov-Smirnov and analyzed using a one-way ANOVA. Mean sperm concentration was higher ($p < 0.001$) before filtration (129.56 ± 53.9 x 106/ml) than after DCG (68.42 ± 29.18 x 106/ml) or MFD (52.94 ± 28.76 x 106/ml). PMS was higher ($p < 0.001$) after selection using the DGC (79.70 ± 11.11) or MFD (81.23 ± 5.64) compared to unsorted controls (45.36 ±14.67). Sperm motion parameters were higher ($p < 0.001$) for DGC treatment compared to MCD except ALH was higher ($p = 0.001$) for MCD. Sperm morphology and acrosome integrity were higher ($p < 0.001$) for DGC or MCD. Plasma membrane integrity was higher ($p < 0.001$) for DGC or MFD. In conclusion, microfluidics and single-layer centrifugation improved sperm motion parameters, viability, morphology, and intact acrosome of epididymal frozen-thawed bighorn sperm. Microfluidic device was comparable to density gradient centrifugation. Therefore, the use of VetCount Harvester® microfluidic device has potential as a sperm sorting technique when DGC is not an option. Microfluidics system does not require special laboratory equipment and may be advantageous for use in the field. Further research is needed to explore the effect of sperm sorted using this technique on artificial insemination, recryopreservation, and assisted reproductive technology outcomes.

Keywords: Microfluidic device, bighorn ram, sperm motion parameters

Investigation of an outbreak of papillomavirus and vaginal vesicular lesions in a dairy herd

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A Holstein dairy herd milking 150 cows in New York State was investigated for an outbreak of perianal warts (33% morbidity), vaginal warts (11% morbidity), and vaginal vesicles (78% morbidity); 11% of cows had no lesions. Cutaneous perianal warts surrounded the anus, perineum, or base of the tail and presented in 2 forms: papular and filiform. Mucosal vaginal warts affected the caudal vagina and also presented as papular or filiform. One cow had a large fibropapilloma on the clitoris that

was firm and pedunculated. Vaginal vesicles were 1 to 2 mm in diameter and filled with clear fluid with varying degrees of mucosal erythema. No lesions were observed elsewhere on the body, including head or back of affected cows. An initial clinical differential list included: bovine papillomavirus, bovine herpesvirus, parapoxvirus, ureaplasma, and mycoplasma. Samples from 3 affected cows were collected: 1. vaginal swab for ureaplasma and mycoplasma; 2. aerobic bacteriological culture; 3. biopsy of vaginal vesicles and warts, and cutaneous biopsy of filiform and papular perianal warts for PCR assays for bovine herpesvirus, pan-herpes, parapoxvirus, and bovine papillomavirus; 4. virus isolation; 5. in situ hybridization (ISH) for bovine papillomavirus; and 6. viral sequencing. All cultures and PCR tests were negative except the bovine papillomavirus ISH with positive signal restricted to dermal fibroblast-like cells. Virus sequencing identified 29.7 and 0.7% of nonhost reads with 99.9% identity with bovine papillomavirus 3 in 2 samples, and 17.4% of nonhost reads shared 99.5% identity with bovine papillomavirus 8. These results indicated the outbreak to be of bovine papillomavirus 3 and 8. A subset of affected cows was reexamined for clinical signs twice during 6-month period, where the proportion of unaffected cows remained 12 and 17%, respectively. Risk factors assessment using Fisher's exact text identified multiparous cows with risk ratio (RR) = 2.8 for perianal warts, RR = 1.8 for vaginal warts, and RR = 0.9 for vaginal vesicles compared to primiparous cows, and stage of lactation with cows > 200 days-in-milk with RR = 1.4 for perianal warts and RR = 1.9 for vaginal warts. Bioeconomic impact on affected cows at first examination revealed no significant differences in milk production; however, affected cows with vaginal vesicles tended to have higher ($p = 0.05$) milk production. Fertility tended to be higher ($p = 0.07$), in cows with perianal warts, whereas 21-day pregnancy rate, pregnancy per AI, and number of inseminations were not different ($p > 0.10$). between affected and nonaffected cows. Presence of lesions was not associated ($p > 0.05$) with risk of leaving the herd within 8-months after the outbreak This investigation of an atypical outbreak of bovine papillomavirus 3 and 8 in a dairy herd revealed a high prevalence of cows with cutaneous and vaginal lesions without a major impact on economic outcomes.

Keywords: Bovine papillomavirus, genital warts, genital vesicles, outbreak

Adverse effects of timed exposure to phytoestrogens on beef cattle conception rate

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Phytoestrogenic legume pastures in Australia have been observed to affect early conception rates in and early embryonic development in cattle. Early embryonic loss (up to 29%) has been observed in cattle grazing legume pastures. We investigated the effect of timed exposure to phytoestrogens resulting from grazing legume pasture on day 35 conception rate in multiparous cattle. A 10-week grazing trial was conducted using 68 multiparous Angus cows on mixed lucerne:fescue and ryegrass pastures, with timed grazing treatments applied. Cows aged between 3-9 years were allocated randomly to 1 of 4 experimental groups; preestablished annual ryegrass (control) and preestablished lucerne:fescue (60:40) treatment pasture. Cows were introduced to control (n = 17) and treatment (T1, n = 17) pasture, at grazing trial start. Another group of 17 cows were introduced at 2 weeks after start of grazing trial (T2, n = 17), and a final group were introduced at 4 weeks after start of grazing trial (T3, n = 17). Cows were subjected to a progesterone-based estrus synchronization program using a controlled internal drug release intravaginal device (Eazi-Breed CIDR®), estradiol benzoate (Bomerol™ 1 mg/ml) and gonadotropin releasing hormone (GnRH, Cattlemate™ 100 µg/ml). On day 25 after commencement of grazing trial, the CIDRs were inserted and cows were given 2 mg of intramuscular estradiol benzoate. On day 34, CIDRs were removed and cows were given 500 µg of intramuscular cloprostenol (Estrumate™ 250 µg/ml). Intramuscular gonadorelin (100) was given 24 hours after CIDR removal. Cows were subjected to timed artificial insemination (TAI) 16 hours after gonadorelin injection, using frozen semen. Pregnancy diagnosis was performed using transrectal ultrasonography on day 35 postTAI. Blood and pasture samples were collected biweekly, starting on day 0 of the trial and continued for 10 weeks. Samples were stored at –20 °C before extraction and analysis was performed using ultra high-performance liquid chromatography quadropole time of flight mass spectrometry (UHPLC- MS-QTOF) and high-performance liquid chromatography triple quadropole mass spectrometry (HPLC-MS-QQQ). Extraction and metabolomic profiling of pasture and blood plasma samples was performed using a preestablished method. Numerous phytoestrogens, including coumestrol, were present in plasma and pasture samples. Flavonoid apigenin was present in higher relative abundance compared to isoflavones and coumestans identified. Relative abundance of coumestrol increased over weeks 2 - 4, during introduction of T2 and TAI. Initial results suggested that phytoestrogens present in the treatment pastures may have contributed to a reduction in day 35 conception rates (CR). CR were different ($p < 0.01$) between timed treatments and were highest for T1 (47%) and lowest for T2 (29%). Cattle (7 and 4 years) had CR > 50%; however, there were no differences ($p > 0.05$) in CR depending on cattle age. This study demonstrated that grazing mixed lucerne pastures, with high abundance of phytoestrogens at least 22 days prior to AI, may significantly reduce day 35 CR > 70%. Development of a model to predict which phytoestrogens may be commonly associated with negative impacts on cattle reproduction is also underway.

Keywords: Phytoestrogens, beef cattle, conception rates, lucerne, fescue, coumestrol

Comparison of breeding soundness evaluation outcome using Society of Theriogenology and breed-specific standards in yearling Wagyu bulls

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Bull breeding soundness examination (BSE) is highly recommended for cow-calf operations, 2 to 3 months before

the breeding season. For a bull to be classified as a potential satisfactory breeder, a minimum age-specific scrotal circumference (SC), sperm motility and sperm morphology set by the Society of Theriogenology (SFT) must be met. Approximately 15% of tested bulls fail BSE because of smaller SC than SFT standards. The SFT SC standards are not breed-specific. American Wagyu bulls have a lighter body weight and reach puberty earlier than continental breeds; therefore, a different breed-specific SC standard has been suggested[1] for this breed. Our objective was to retrospectively analyze BSE results and compare the percentages of bulls failing BSE based on SFT and breed-specific SC standard for Wagyu bulls. We hypothesized that more yearling Wagyu bulls fail on the SFT standard than on the breed-specific standard. Records of BSE forms from 93 yearling (12-24 months) Wagyu bulls were analyzed using descriptive statistics and the Chi-square test. Bulls raised at the Washington State University Beef Center were evaluated for BSE from 2005 - 2023. Overall, a higher ($p < 0.0001$) percentage of bulls failed SFT standards (61.3%; 57/93) than breed-specific standards (22.6%; 21/93). Percentage of bulls failing due to small SC alone was higher ($p = 0.02$) if SFT SC standard was used (91.22%; 52/57) compared to breed-specific SC standard (61.9%; 13/21). Substandard SC and poor sperm morphology were the only reasons for bulls to fail in this study, whereas no bull failed BSE because of health or any abnormality on physical examination. A higher ($p < 0.0001$) percentage of bulls (55.9%; 52/93) did not meet SFT SC standard, whereas only 8.6% (8/93) of bulls failed breed-specific SC standard. Most (69.2%; 36/52) bulls failing SFT SC standard met the minimum required sperm motility and morphology standard. Among bulls failing based on breed-specific SC standard, a lower percentage of bulls (12.5%; 1/8) passed ($p < 0.01$) sperm motility and morphology standard. Percentage of bulls that failed BSE due to poor sperm morphology was similar (SFT 14.5 versus breed-specific 16.5, $p > 0.05$). Results stressed the importance of using breed-specific SC standard while evaluating Wagyu bulls for breeding soundness. Previous reports demonstrated that most bulls failed BSE because of poor sperm morphology. This was not the case for Wagyu bulls if SFT SC standard was used. However, when breed-specific SC standard was used, sperm morphology was the major reason for classifying bulls as unsatisfactory. Breed-specific standards for SC could be an important aspect of establishing guidelines for bull BSE, particularly in yearlings.

Keywords: Wagyu bulls, scrotal circumference, breeding soundness

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Effect of prematuration treatment on nuclear maturation in domestic cat oocytes

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We evaluated whether keeping freshly harvested immature cat oocytes at room temperature for 18 hours before in vitro incubation would affect nuclear maturation. Specific objective was to record and compare oocyte nuclear maturation in treated and control oocytes. Ovaries were collected from cats undergoing surgical sterilization at a local veterinary clinic. Immediately after surgical removal, ovaries were transported to the laboratory in 0.9% saline solution. Ovaries were dissected from adjacent tissue and placed in a Petri dish containing a heparinized oocyte handling media (OPU Medium, IVF Bioscience, UK). Only cumulus oocyte complexes (COCs) with a dark homogeneous cytoplasm and at least 3 layers of cumulus cells were selected. Shortly after collection, COCs were placed either into oocyte in vitro maturation (IVM) media (control) or into a serum-free HEPES-buffered media (Eq-Hold, IVF Bioscience, UK) and kept for 18 hours at room temperature before incubation for in vitro maturation (treatment). After 24 hours, COCs were removed from incubator, denuded by repeated pipetting, and fixed in 2% paraformaldehyde. Oocytes were washed 3 times in 75 µl drops of phosphate buffered saline with 20% bovine serum albumin and mounted on a glass slide with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, Thermo Fisher Scientific) to stain oocyte DNA. Confocal laser scanning microscopy was used to evaluate chromatin configuration. Differences in nuclear maturation between control and treated groups were analyzed by Chi-square test, with significance set at $p \leq 0.05$. Proportion of oocytes undergoing nuclear maturation after incubation did not differ ($p > 0.1$) between control and treated oocytes, (24 versus 28% and 30 versus 35%, respectively). A subset of oocytes held at room temperature for 18 hours were also analyzed by confocal microscopy, indicating that most remained at the germinal vesicle stage (37/80; 46.25%). Holding immature domestic cat oocytes for 18 hours at room temperature in a simple media did not affect their ability to undergo in vitro maturation once incubated. Domestic cat oocytes could potentially be kept at room temperature for 18 hours before arriving at an assisted reproduction laboratory for in vitro fertilization procedures.

Premature placental separation due to urine pooling in a late-term pregnant mare

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An 18-year, multiparous Quarter horse mare, 319 days pregnant, was referred to the veterinary teaching hospital, due to worsening of laminitis. On arrival, the mare was lame 5/5, tachycardic, normothermic, euhydrated and with excessive ventral edema. Despite daily treatment of phenylbutazone at home, mare's pain remained uncontrolled. Evaluation of mammary gland secretions was challenging due to marked ventral edema and discomfort during manual stimulation. Radiological examination revealed a 10° rotation of third phalanx in both front feet. Blood work indicated elevated blood urea nitrogen (BUN 58 mg/dl) and creatine (6.0 mg/dl) concentrations (normal: 12-27 and 0.9-1.9 mg/dl, respectively). Urine specific gravity of 1.018 revealed isosthenuria. Transabdominal ultrasonography revealed a live fetus with a heart rate of 82 beats/minute. Combined thickness of the uterus and placenta (CTUP) was increased at the uterine body and the cranial aspect gravid uterine (17.7 and 16.0 mm, respectively). On transrectal ultrasonography, the CTUP at the cervical star region was 9.2 mm, with no evidence of placental separation. Initial treatment consisted of intravenous fluids (lactated Ringer's solution at 3 liters/hour, twice maintenance rate), to increase urine output. Pain management included acetaminophen (20 mg/kg, PO, twice daily) and gabapentin (30 mg/kg, PO, twice daily). Oral Omeprazole was given to protect gastrointestinal mucosa. Altrenogest (0.044 mg/kg, PO, once daily) and pentoxifylline (7.5 mg/ kg, PO, twice daily) were given to support the pregnancy. Mare did not receive any antibiotics as there was no evidence of infectious placentitis. Mare received grass hay and a commercial low-protein diet (12.5%) in several meals per day. Urine production increased in response to treatment, and on day 7 after arrival (day 326 of pregnancy), urine dribbling was noticed. Vaginal examination revealed urine pooling with urine sediment. On transrectal ultrasonography, a 14-cm separation of placenta was evident at cervical region, with fluid accumulation between allantochorion and endometrium. Transcervical drainage of the fluid with a foley catheter was performed for 3 consecutive days. Fluid was confirmed to be urine. Creatine and BUN concentrations decreased slightly over time, so on day 10, fluid therapy was discontinued. On day 11, the amount of fluid between allantochorion and endometrium was markedly reduced. On day 332 of pregnancy, mare entered labor and had premature placental separation ('red bag'). A healthy colt was delivered. Due to economic constraints, the mare was discharged with elevated creatinine and BUN concentrations. Medical reevaluation and follow up were declined. A year later, the owner reported that the mare was apparently healthy. This case illustrated the importance of intervention by a

theriogenologist in the clinical management of a late-term pregnant laminitis mare. A vaginal examination and sampling of the fluid present are important in cases of placental separation and premature cervical relaxation. Premature placental separation increases the risk of 'red bag' delivery. Regular evaluation and monitoring are crucial for high-risk pregnant mares.

Keywords: Creatinine, blood urea nitrogen, fluid therapy, premature placental separation, laminitis

Cystic endometrial hyperplasia in a Jack Russell Terrier

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A 16-year, intact female Jack Russell Terrier presented for lethargy, loss of appetite, increased coughing, and head tilt. Medical history included chronic steroid-responsive tracheal collapse and bronchitis. Physical examination revealed left-sided head tilt, hyperreflexia in hindlimbs, a wide base stance in the forelimbs, respiratory dyspnea, and malodorous vaginal discharge. Vaginal cytology had large numbers of neutrophils with a mixed population of bacteria and a few superficial epithelial cells. Treatment with fluid therapy, antibiotics (intravenous ampicillin-sulbactam 30 mg/kg, every 8 hours, and enrofloxacin 10 mg/kg, daily), maropitant (1 mg/kg, daily), and methadone (0.1 mg/kg, every 4-8 hours) was initiated. Bloodwork and urinalysis results were unremarkable on presentation but severe neutrophilic leukocytosis with a left shift developed by day 3. Diagnostic imaging confirmed severe pulmonary disease and a fluid-filled uterus. Transabdominal ultrasonography findings included moderate to severe fluid distention of the uterus consistent with pyometra, multiple small cysts on both ovaries, and a focal, slightly bilobed, oblong centrally located luminal structure (6 x 3 cm) characterized by a thin wall and fluid-filled lumen in the right uterine horn. Ovariohysterectomy was recommended due to clinical deterioration, but the risk of anesthetic complications secondary to severe pulmonary disease prompted the owners to choose humane euthanasia and necropsy. Postmortem examination revealed mild to moderate hydrocephalus of the lateral ventricles bilaterally, explaining the recent head tilt, as well as severe bronchiolar inflammation consistent with clinical findings. Cervix, uterine body, and uterine horns were multifocally expanded by numerous fluid-filled cysts ranging from 0.3-6 cm in diameter. On cut section, the cystic structures exuded variable amounts of clear watery fluid. Ovaries contained multiple cysts ranging from 1-2 cm diameter. Further histological examination of the uterine horn revealed hyperplasia of endometrial glands that were moderately to severely dilated and contained small amounts of eosinophilic proteinaceous fluid and low numbers of neutrophils. All findings were consistent with advanced stage cystic endometrial hyperplasia. Cystic endometrial hyperplasia (CEH) has long been associated as a causative condition for pyometra in dogs due to degenerative endometrial changes and opportunistic bacterial overgrowth within the endometrium. This case presented findings both consistent with normal CEH-pyometra complex findings, but also several extraordinary circumstances. The presence of endometrial cysts throughout the endometrium is to be expected for an intact dog of that age. However, the patient's advanced age with still functioning ovaries is unexpected, as ovarian senescence can be observed in dogs as young as 7 years of age. Size of the largest cyst was also unexpected, as well as no previous history of pyometra in a dog that had not been bred for years, indicating that CEH can be present for years without causing clinical signs of disease.

Keywords: Pyometra, cystic endometrial hyperplasia, ovarian senescence

Cystic endometrial hyperplasia with segmental mucometra in a geriatric queen

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A 13-year, domestic shorthair nulliparous queen (4.5 kg), was presented for a routine ovariohysterectomy. Despite her advanced age, physical examination findings were within normal limits except for the presence of nonpainful, tubular masses in her abdomen. Complete blood count, serum chemistry, thyroid hormone concentrations, and urinalysis results were within normal limits. Transabdominal ultrasonography revealed an enlarged uterine body (2 x 1.5 cm) and horns (1.5 x 0.75 cm) with numerous anechoic mural cysts (0.2 cm). Right uterine horn was also focally dilated (0.5 cm) with fluid of mixed echogenicity. Two extra-uterine anechoic cysts (1 cm) were visible along the left uterine horn. Both ovaries appeared normal with numerous 2-3 mm follicles. Cat was sedated with oral buprenorphine (0.02 mg/kg) and intramuscular dexmedetomidine (0.01 mg/kg). Intravenous catheter was placed, intravenous maropitant citrate (1 mg/kg; Cerenia®) was given, and cat was maintained on intravenous isotonic fluids (14 ml/hour; PHyLyte®). Anesthesia was induced with intravenous propofol (3 mg/kg) and maintained on oxygen (1 liter/minute) with 1% isoflurane gas. Hair on the ventral abdomen was clipped and the skin was prepped with chlorhexidine scrub and alcohol. A ventral midline laparotomy incision was made. Right ovarian suspensory ligament was manually broken down, and the ovarian pedicle was ligated using a 2-clamp technique and transected. The process was repeated for the left ovary. Broad ligaments on both uterine horns were broken down, however, most of the uterine body was adhered to the colon and bladder. Efforts were made to gently breakdown these adhesions, but it was not possible. Uterine body was too thick to allow proper clamping, so a self-crushing ligature was used on both sides to include each uterine artery individually. Cranial uterine body, with both uterine horns and ovaries, were then excised. A routine 3-layer abdominal closure was performed, and cat recovered from anesthesia without complication. Intramuscular atipamezole (0.1 mg/kg; Antisedan®) was given to reverse dexmedetomidine and topical buprenorphine (20 mg; Zorbium®) was given for analgesia. Subcutaneous meloxicam (0.08 mg/kg) was given during recovery and dispensed for additional analgesia oral meloxicam (0.04 mg/kg; once daily for 4 days). A diagnosis of cystic endometrial hyperplasia (CEH) with segmental mucometra

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

with cystic mesonephric duct remnants was made based on gross examination and confirmed with histology and fluid cytologic evaluation. CEH is a common uterine problem in queens with an 88% incidence reported in cats > 5 years old.[1] Although CEH and mucometra can predispose to the development of pyometra, the current case illustrated that subclinical CEH can exist for several years in older queens and present as an incidental finding at the time of ovariohysterectomy.

Keywords: Cat, cystic endometrial hyperplasia, cyst, mucometra, uterus

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Use of anaerobic culture in an aborted mare with necrotic intrauterine tissue

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A 14-year, multiparous Thoroughbred mare, was presented for hysteroscopy after aborting at ~ 7 months of pregnancy. Mare was treated during 2023 breeding season for endometritis and during pregnancy for placentitis. During hysteroscopy, a small amount of uterine fluid with specks of debris was observed and sterilely aspirated for submission for culture and cytology. Towards right uterine horn tip, a discrete white-tan nodule was identified protruding from the endometrium that was ~ 1-2 cm in diameter. Endoscopic forceps were used to obtain samples of the nodule and ultimately to debride the entirety of the mass. On removal of fragments, the mass bled, suggesting vascularization and an intimate association with the underlying endometrium. Collected malodorous tissue fragments were submitted for culture to Hagyard Equine Medical Institute laboratory and to University of Kentucky Veterinary Diagnostic Laboratory for histopathology. Aerobic culture revealed no bacterial growth on neither the uterine fluid nor the tissue. Due to odor associated with the tissue, an anaerobic culture was requested for the fluid and the tissue. Uterine fluid grew moderate *Bacteroides* species, whereas the tissue grew heavy *Bacteroides fragilis* on anaerobic culture; the suggested treatment was systemic metronidazole. On histopathology, the diagnosis was neutrophilic inflammation and tissue necrosis; no evidence of a fungal component was appreciated. It is unknown what exactly this tissue originated from, whether placental or endometrial tissue, or another alternative. Location of the mass makes endometrial cup remnants unlikely, as those form at uterine horn base rather than the tip. In addition to systemic, oral metronidazole (15 mg/kg, 4 times daily) was given. Mare was treated for 5 consecutive days with deep uterine horn infusion (2 grams of ampicillin suspended in 120 ml of tris-EDTA). Mare is scheduled for an additional hysteroscopy to evaluate current endometrial status and will be bred again during the 2024 season. This case highlighted the importance of considering all diagnostic options, even those that may be less commonly utilized such as anaerobic culture of intra-uterine samples, particularly when those samples appear necrotic and/or are malodorous. Given the lack of microbial growth on routine aerobic culture in this case, the mare likely would not have received appropriate targeted treatment for the underlying organism that was revealed only on anaerobic culture.

Keywords: Mare, hysteroscopy, anaerobic, *Bacteroides*, endometrium

Management of pyometra in an aged Warmblood mare

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A 21-year, Warmblood mare, was presented for colic. Relevant history included a cervix that did not relax during estrus, as well as beta *Streptococcus* species endometritis that had been treated several months prior with sterile uterine lavage and infusion of 2 grams of ampicillin, in addition to oral Equisul® (24 mg/kg, twice daily). Mare had foaled once more than 15 years prior. The theriogenology department was consulted when it was discovered that a large, fluid-filled viscus was the uterus rather than part of the gastrointestinal system. On transrectal ultrasonography, the uterus was diffusely dilated with over 10.5 cm of echogenic fluid. Vulva was washed with dilute betadine scrub and rinsed with warm water. A sample was then sterilely aspirated from the uterus via insemination pipette and submitted for aerobic culture and sensitivity. Mare treated with oral sulfamethoxazole-trimethoprim (30 mg/kg, twice daily for 14 days) and intravenous gentamicin (6 mg/kg for 7 days) rather than intrauterine antibiotic infusion in an effort to prevent instillation of additional fluid into a uterus that was ineffective at accomplishing clearance. A flexible large- bore sterile tube was introduced through the cervix and 12 liters of purulent nonodorous fluid was spontaneously drained from the uterus. Fibrotic cervix would only permit 1 finger through the canal alongside the tube. After evacuation of fluid, a large-volume nonsterile lavage was performed. It was elected not to treat with an ecbolic as uterine wall integrity was unknown after being stretched to accommodate the expanding fluid volume. Further, with an abnormal cervix, promoting uterine contractility was unlikely to substantially aid in fluid clearance. Transrectal ultrasonography and large-volume uterine lavage was repeated daily/every other day as indicated by intrauterine fluid accumulation, and culture revealed growth of beta *Streptococcus* species. Mare remained comfortable and bright after the initial fluid evacuation. Options discussed with the owner included the conservative approach of monitoring the mare at home and draining the uterus as needed, placement of a stent, or cervical wedge resection. It was decided to first attempt cervical stent placement and if that was unsuccessful a wedge resection would be performed. Cervical stent was placed as described[1] and the mare returned to the farm. Three days after placement, mare was examined via transrectal ultrasonography. Approximately 2 cm fluid was present at the uterine bifurcation, and the cervical stent had fallen out of the mare the previous day. Stent was replaced twice over the following week due to repeated loss. After the third loss, it was decided to pursue cervical wedge resection. Surgery was successful and the mare was treated daily with topical application of antibiotic ointment to

the cervix to prevent closure of the created defect. Mare did not accumulate > 2 cm of anechoic fluid in the uterus at any time since initial hospitalization.

Keywords: Mare, pyometra, cervix, stent, colic, wedge resection

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Endometrial mineralization in a barren Thoroughbred mare

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An 11-year, multiparous Thoroughbred mare, barren from the previous season, was presented for hysteroscopy to evaluate hyperechoic irregularities observed via uterine transrectal ultrasonography. Six nearly circular hyperechoic structures ~ 5 mm in diameter were visible. These structures were distributed at the base of each horn and cranial uterine body. Mare had been treated with intrauterine infusion of kerosene on the presumptive diagnosis that these structures may have represented retained endometrial cups. No change in ultrasonographic appearance of these structures was appreciated after kerosene infusion. On hysteroscopy, the structures were directly visualized as tan, smooth, bulbous protrusions into the uterine lumen, with no appreciable disruption of the overlying endometrium. Standard uterine biopsy instrument was introduced into the uterine lumen alongside the endoscope; removed structures were submitted for culture, cytology, and histopathology. Culture and cytology results were unremarkable, with no evidence of inflammation or etiologic agents. On reevaluation ultrasonography (12 days after hysteroscopy), hyperechoic structures were again visible, and had proliferated in number (~ 14). Hysteroscopy and debulking of the structures in the above manner was repeated, and removed tissue was submitted for histopathology. Grossly, the interior of the removed tissue contained tan granular material. Histopathology of the nodules revealed 2 types of nodules; one had amorphous basophilic granular material (mineral) and a second type resembled endometrial polyps with no mineral component. Deposition of mineral was predominantly in the interstitium of the stratum spongiosum. No etiologic agents were identified. Mare became pregnant on second breeding attempt after hysteroscopy (~ 2 months from initial hysteroscopy) and maintained pregnancy through 7 months before being lost to follow-up. Histopathologic findings in this case most closely resembled reports of benign endometrial calcification in women, an uncommon finding associated with infertility and abnormal menstruation.[1] The current case bears some similarity to a case reported of calcified structures incidentally identified during cesarean surgery of a recipient sow that produced 5 cloned piglets with no gross apparent abnormalities.[2] To authors' knowledge, this is the first report of endometrial calcification in a mare.

Keywords: Mare, mineralization, hysteroscopy

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Application of reproductive sciences for conservation of America's last wild ocelots

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Historically, wild ocelots thrived across the southwestern US but because of habitat loss and fragmentation, overexploitation for fur and pet trade, and unregulated hunting, became listed as an endangered species in the US in 1982. Currently, ocelots only occupy a small region along the Texas coast in 2 known breeding populations within Laguna Atascosa National Wildlife Refuge and private ranches in Kenedy and Willacy Counties. The viability of these small populations is threatened by their proximity to low-lying coastlines with the risk of catastrophic weather-related disasters and their lack of interconnectivity promoting inbreeding and reduced genetic diversity. Proposed recovery actions include establishing new ocelot populations within historical ranges in Texas, introducing novel genetics into existing populations, and investigating the use of assisted reproductive technologies, such as semen cryopreservation and artificial insemination (AI), to establish a genetic resource bank and mitigate declining genetic variation. Our objectives were to compare the effectiveness of semen collection and cryopreservation methods in wild ocelots, evaluate the feasibility of laparoscopic oviductal artificial insemination (LO-AI) using frozen semen sourced from wild Texas ocelots to produce pregnancies in zoo-based females, and develop a reintroduction and monitoring plan for creation of a founding wild ocelot population in South Texas. Live-trapping efforts with wild ocelots were conducted each year from November through April. Individuals were anesthetized with a combination of intramuscular ketamine (200 mg/ml; 4-8 mg/kg) and medetomidine (10 mg/ml; 0.05-0.07 mg/kg). Semen samples were collected using either a urethral catheterization method and/or a controlled voltage electroejaculation procedure. Semen samples were cryopreserved using an ultrarapid freezing technique and/or standardized straw freezing methods and stored in liquid nitrogen until use. Postcollection, anesthesia was partially reversed with a single intramuscular atipamezole (25 mg/ml; 1 mg/5 mg of medetomidine) treatment. For AI, female ocelots in North American zoos were fed oral progesterone daily for 30 consecutive days to suppress ovarian activity. After 7 days of progesterone withdrawal, animals were injected with intramuscular equine chorionic

gonadotropin (eCG), followed by (82 hours later) porcine luteinizing hormone (pLH) or human chorionic gonadotropin (hCG) to stimulate follicular development and ovulation. Timed LO-AI procedures were conducted ~ 39 hours after pLH/hCG injection. Fecal samples were collected for 85 days postAI to allow analysis of fecal progesterone concentrations and determination of pregnancy status. From 2019 to 2024, semen was successfully collected from 11 wild ocelots. For collection and cryopreservation of semen samples, best results were obtained using a combination of electroejaculation with straw freezing. For ovarian synchronization of zoo-housed females, the majority (13/18, 72%) of attempts resulted in ovulation of 1 or more follicles followed by apparently normal luteal phases. However, no offspring were produced from LO-AI, likely due in part to suboptimal postthaw semen parameters. Our findings suggested that further refinement will be necessary for LO-AI with frozen semen to become a viable approach for promoting gene flow between in situ and ex situ ocelot populations and assisting with the recovery of extirpated wild Texas ocelots within their natural range.

Keywords: Ocelot, reproduction, semen banking, artificial insemination, conservation

Transcriptome analysis reveals gene expression changes in the equine myometrium during pregnancy

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In eutherian mammals, myometrial quiescence (relaxation) is maintained by the action of progestins through progesterone receptors (nuclear receptors; PGR and membrane receptors PGRMC), with consequent suppression of genes associated with the inflammatory cascade and uterine contraction. During late pregnancy, mares typically exhibit uterine contractions several nights before parturition, and these contractions increase in amplitude and frequency as parturition approaches. However, little is known about the molecular mechanisms triggering myometrial activation during the prepartum and partum periods in mares. We hypothesized that the myometrial transcriptomic profile during normal pregnancy exhibit different regulation of genes in a dynamic profile throughout pregnancy. Therefore, using RNA Sequencing, we sought to characterize the myometrial transcriptome of mares at various points in normal pregnancy. Myometrial samples were collected after euthanasia from mares at 4, 6, 9, 10, and 11 months of pregnancy (n = 4 mares/group), and RNA sequencing was conducted with the NovaSeq 6000 platform. Reads were mapped to the latest reference genome (EquCab3.0) using Hisat2 v2.0.5. A comparative analysis of gene expression was performed at 2 pregnancy lengths using the edgeR R package. The p value was corrected using the Benjamini and Hochberg method. Corrected p value < 0.05 and absolute fold-change (FC) of 2 were set to determine differentially expressed genes (DEGs). Database for annotation, visualization, and integrated discovery was used to functionally annotate DEGs based on gene ontology using the biological process. The highest number of DEGs was obtained in 6 versus 10 months (3937 DEGs) and 9 versus 10 months (3925 DEGs), suggesting that early changes occur in the 10-months myometrium in preparation for labor. Designation of the prepartum group (11 months) was based on a fixed pregnancy (330 days). Because normal equine pregnancy length varies widely (320-355 days) and many endocrine and physiologic changes occur immediately peripartum, this group might not capture the immediate peripartum changes in the equine myometrium. Nonetheless, in comparison to the myometrium collected from normal pregnant mares at 10 months of pregnancy, we identified 2990 DEGs (1411 upregulated and 1579 downregulated). Functional annotation of DEGs identified that these genes are involved in relevant biological processes such as prostaglandin biosynthetic process (*CD74*, *EDN1*, *PTGIS*, *MIF*, *PTGDS*, *PTGES*), muscle contraction (*ACE2*, *RGS2*, *KCNQ1*) apoptosis (*CASP3*, *CASP4*), inflammatory response (*CCL13*, *CXCL6*, *PTGER1*, *CXCL2*, *CX3CL1*, *CCL2*, *NLRP1*), and the maternal process involved in parturition (*OXTR*, *LDOC1*, *KALRN*), among others. Moreover, the 11-months myometrium was associated with functional withdrawal of progesterone that was indicated by the downregulation of *PGR* and *PGRMC1* in comparison to 4 and 6 months, respectively. This functional withdrawal might explain the activation of an inflammatory cascade in the 11-months myometrium. In conclusion, for the first time, equine myometrial transcriptome throughout pregnancy was characterized. We provided a comprehensive database of the key upstream regulators, hub genes, transcription factors, and gene coexpression networks associated with equine myometrial activation. This transcriptomic work improved our understanding of equine myometrial activation and provided a foundation for constructing new hypotheses.

Keywords: Mare, pregnancy, uterus, parturition, RNA Sequencing

Antioxidants expression increased in ejaculates obtained after standard teasing in stallions

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Abundant presence of certain proteins like caseins, annexinA2, and CRISP-3 in stallion semen proved beneficial for cooling and cryopreservation. Amount of seminal plasma affects their presence and expression; in a clinical setting, semen collection protocol dramatically affects the amount of seminal plasma present in each ejaculate. Our aim was to determine whether duration of teasing time affects the proteome of sperm and seminal plasma; we hypothesized that teasing time affects proteomic profile of stallion sperm and seminal plasma. Forty semen collections were performed from 10 stallions at 48 hours intervals. For each stallion, 2

collections were performed using standard teasing (i.e. a successful ejaculate was procured < 5 minutes after penile washing), and 2 collections included extended teasing (i.e. after penile washing, stallions were handheld for 10 minutes before allowing them to mount the dummy). Ejaculate was submitted to serial centrifugations (1,000 ´ g for 20 minutes) until seminal plasma was separated from sperm; an aliquot of both seminal plasma and sperm pellet was flash-frozen in liquid nitrogen and stored at –80°C until analyses. Proteomic analyses were performed by LC/MS-MS and then the data were analyzed with MaxQuant and Perseus. Protein groups were filtered, transformed (log2 and z- transformation), and normalized; expression of each protein group was compared among experimental groups with multiple-sample test, Fisher exact test, and principal component analysis. We identified 1,237 proteins within samples; protein database was restricted to include only proteins in at least 70% of the samples in each experimental group. Protein signature did not differ in sperm and seminal plasma in stallions after a standard and extended teasing. One protein (F6UJZ8) in the seminal plasma and 2 other proteins (A0A9LORWE3, A0A5FPLI4) in the sperm proteome had an increased ($p < 0.05$) expression in the seminal plasma of stallions collected after standard teasing compared to the extended teasing. Based on the UniProt *E. Caballus* database, the 3 accession numbers correspond to prolycarboxypeptidase, copper transport protein ATOX1 and aspartylglucosaminidase; the 3 proteins were inferred by homology (F6UJZ8, A0A5FPLI4) or had evidence at the transcript level (A0A9LORWE3). Those proteins were reported to be involved in ion transportation and regulation of oxygen radicals. Oxygen radicals are common byproducts of the OXPHOS metabolism typical of stallion sperm; presence of a higher abundance of antioxidants may translate into positive effects on sperm viability and longevity of the semen processed by stallions after a standard teasing time compared to an extended one.

Keywords: Stallion, teasing, proteomic

Morphodynamics of in vitro produced equine embryos: frozen versus cooled semen

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In vitro embryo production (IVP) by intracytoplasmic sperm injection (ICSI) in horses has become a more common procedure. However, despite modest advancements in technology and efficiency, there is large variability in embryo production, pregnancy, and early embryo loss rates. Some of the variability is due to mare's estrous cycle, stallion effects and even differences among cooled and frozen semen, and IVP culture conditions. We reported an increase in overall IVP efficiency and pregnancy rates when using extended cooled versus frozen semen.[1] To elucidate the differences, aim of this study was to evaluate the morphodynamics of embryo zygote first cellular division up to the blastocyst stage and determine the differences in embryo development time points obtained with frozen or extended cooled semen. We hypothesized that embryos obtained from cooled semen have a faster morphodynamic development rate. Embryos (n = 150) were obtained by ICSI using frozen (n = 86) or cooled semen (n = 64) of 40 and 23 different stallions, respectively, of 7 breeds. Injected oocytes were placed in a Time-Lapse incubator and cultured for 9 days. Embryo morphodynamics were annotated at the first cytoplasmic extrusion (1), 2, 4, 8, morula (M), compact morula (CM), blastocyst (B) and expanded blastocyst (EB) stages using Phototune Imaging Software. Furthermore, the first cleavage division was characterized as normal or abnormal (direct, explosive, or indirect) and blastocyst size was measured. Data were analyzed by ANOVA and Wilcoxon test for stage time, size and first cleavage normality, respectively, using SAS. Results indicated no differences ($p > 0.05$) between frozen and cooled semen for: developing times (hours) at 1 (19.8 ± 1.5 and 20.7 ± 1.8), 2 (28.3 ± 1.6 and 30.2 ± 1.8), 4 (41.3 ± 1.6 and 43.2 ± 1.8), 8 (63.1 ± 1.3 and 63.4 ± 1.6), CM (126.7 ± 1.4 and 127.5 ± 2), B (145.4 ± 1.4 and 146.3 ± 2.1) and EB (156.7 ± 2 and 156.5 ± 2.4) stages; normality of cleavage; or blastocyst size (156.5 ± 1.4 and 158 ± 1.6 µm). However, M formation occurred earlier ($p < 0.05$) in embryos produced with cooled (100.3 ± 1 hour) versus frozen semen (106.8 ± 1.3). In conclusion, morphodynamics of embryos obtained by frozen or extended cooled semen were very similar except at the M stage, the early formation of which has been reported to be a key for embryo developmental competence and most importantly, the fate of the inner cell mass and the trophectoderm cell lines. Further studies are warranted, including sperm decondensation parameters and PLCz content, and embryo assessment of mitotic aneuploidy and blastomere multinucleation, controlling for stallion and mare effects of cooled and frozen semen in the model.

Keywords: Mare, embryo, intracytoplasmic sperm injection, time-lapse

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Chronic placentitis and abortion caused by *Aspergillus fumigatus* in a mare

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Placentitis in pregnant mares commonly results in abortion, premature birth or compromised neonatal foal health if not addressed on time. Although the most common causes of placentitis are of bacterial origin, fungal placentitis can occur sporadically. A 12-year, Warmblood mare that had previously produced live foals became pregnant after embryo transfer. Following the farm's preventive health program, mare was vaccinated against equine herpesvirus type-1 (EHV- 1/4-Pneumabort-k) on day 150 of pregnancy. Routine reproductive examinations were performed throughout pregnancy, fetal cardiac activity, combined thickness of the uterus and placenta (CTUP) and cloudiness of fetal fluids. No abnormalities were noted on transrectal ultrasonography. Mare aborted on day 160 of pregnancy. No premonitory clinical signs of placentitis, such as premature mammary gland development or vaginal discharge, were observed. Abortion occurred

overnight and was unattended. Fetus and fetal membranes were kept for veterinarian evaluation. Uterine lavage was performed due to fetal membranes retention in nonfetal horn. Remaining fetal membranes were removed, and mare was treated with sulfadiazine/trimethoprim (30 mg/kg). Uterine samples were submitted for culture and cytology. Uterine cytology reveled polymorphonuclear neutrophils, some rod-shaped bacteria and rare yeast forms. *Klebsiella pneumoniae* was isolated. No fungal colonies grew after 120 hours in culture. Fetal membranes and fetus were submitted for postmortem examination and PCR. Fetal membranes weighed 3.3 kg. Approximately 90% of the chorionic surface from the fetal horn was thickened (up to 1.0 cm). Avillous areas around the lesions were overlaid by a thick milky grey caseous exudate. There was an invagination from the allantois to the chorionic side of the placenta, surrounded by a chronic inflammatory reaction and encapsulated. On histopathology, the lesions were characterized by chronic, fibrosing, necrotizing allanto-chorionitis with squamous metaplasia of the chorionic surface and adenomatous hyperplasia of the allantois. There were intralesional fungal hyphae with mixed bacteria. Hairless female fetus was covered with meconium. No internal organ abnormalities were observed. PCR revealed *Aspergillus fumigatus* as the main pathogen. On the third day postabortion to 'challenge' or stimulate an acute inflammatory response, mare was given intrauterine 400 µg of misoprostol. The induction of inflammation was aimed to increase the likelihood of pathogens isolation (authors' experience). Uterine lavage was performed 24 hours later and the effluent submitted for bacterial and fungal culture. *Klebsiella pneumoniae* and *Mycobacterium* spp. were isolated displaying a heavy colonies development. No fungus grew in culture after 120 hours of incubation. Mare was treated based on the laboratory sensitivity results. This case represented the need for a thorough pregnancy examination at any time in a broodmare. Bacterial and fungal culture with typification in cases of abortion should be routinely performed.

Keywords: Mare, chronic placentitis, fungal abortion

Equine endometrial organoids as an in vitro model to trial novel therapeutics for endometritis

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A healthy uterine environment is essential for equine fertility. Approximately 20% of Thoroughbred mares demonstrate persistent breeding-induced endometritis (PBIE), creating a uterine environment that is unfavorable for pregnancy. Despite extensive in vivo studies, the exact mechanisms and best strategies for resolving PBIE remains elusive. A potential therapeutic for PBIE is extracellular vesicles (EVs), which are a heterogeneous population of membrane-bound nanoparticles containing proteins and nucleic acids, secreted by equine embryo-derived mesenchymal stem cells (EDMSCs). We hypothesized that EVs secreted by EDMSCs contain antiinflammatory miRNAs that prevent PBIE in susceptible mares. Using organoids as physiologically-relevant 3D cell cultures, our objectives were to assess: 1. presence of specific antiinflammatory miRNAs in EDMSC-derived EVs; and 2. miRNA and mRNA of endometrial organoids (n = 6 mares; Kenney-Doig score IIb) pretreated with or without 40 x 10^6/ml EVs 24 hours prior to inflammatory stimulation with frozen-killed sperm (FKS), lipopolysaccharide (LPS), or PBS (Control) for 3 and 12 hours. Data were analyzed using ANOVA with Tukey's post- hoc test with organoids not pretreated with EVs and not stimulated with inflammatory reagents serving as Control. Significance was set at $p < 0.05$. Results demonstrated the presence of specific antiinflammatory miRNAs (miR-10a, miR-21, miR-24, miR-145, and miR-146a) in EVs secreted by EDMSC cell lines. However, these antiinflammatory miRNAs did not increase in organoids after EV treatment ($p > 0.05$). Treatment of organoids with FKS did not result in inflammatory gene expression differences ($p > 0.05$) compared to Control. However, organoids pretreated with EVs then stimulated with LPS exhibited increased ($p < 0.05$) expression of proinflammatory *IL1 β* at 3 hours and *PTGS2* at 12 hours after LPS exposure compared to Control organoids. Furthermore, organoids, with or without EV pretreatment, stimulated with LPS had increased expression of proinflammatory *IL6* (3 and 12 hours) and antiinflammatory *IL1RN* (12 hours) compared to Control organoids. These gene expression changes mirror reports describing inflammation of the equine endometrium associated with breeding in vivo.[1,2] In conclusion, these data indicated that: 1. EDMSC EVs contain antiinflammatory miRNAs; 2. pretreatment of organoids with EVs failed to alter miRNA expression in the recipient organoids under the conditions evaluated; and 3. LPS stimulated an inflammatory response in endometrial organoids similar to in vivo, demonstrating their utility as an in vitro equine endometritis model. Future studies will investigate the effect of reproductive steroid hormones on inflammatory cytokine protein and gene expression in this organoid model of equine endometritis.

Keywords: Mares, 3D culture, organoid, endometritis, inflammation

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Use of prostatic extracellular vesicles to improve viability and longevity of frozen-thawed canine sperm

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Reproductive success after artificial insemination (AI) with frozen-thawed dog sperm is highly variable. Even the most effective freezing methods results in compromised sperm function which negatively impacts fertility. Cryopreservation can damage sperm membranes, including plasma membrane (resulting in cell death or reduced longevity) and acrosomal membranes (that can cause premature acrosomal exocytosis). Adding prostatic fluid (PF) to frozen-thawed canine sperm benefits sperm motility, improves conception rates and results in larger litter sizes.[1] Extracellular vesicles (EVs) are nanoparticles secreted by all cells that mediate cell communication via their cargo. We hypothesized that EVs produced by prostatic cells have a physiological role in canine sperm function by modulating cryo-damaged sperm membranes, leading to enhanced sperm longevity, viability, and function. Study was conducted to determine if EVs, from PF, could ameliorate cryodamage to canine sperm. EVs were isolated from PF obtained from 6 young dogs (< 2 years) and were used to treat frozen-thawed sperm from semen collected from 3 young and 3 old dogs (> 6 years). After thawing, sperm were diluted in a capacitating media and treated with: 1. no EVs; 2. PF EVs; or 3. dilauroylphosphatidylcholine (PC12; + control). Sperm were incubated, at 37°C, and subsamples assessed for motility (subjectively and computer aided sperm analysis) and sperm viability, acrosome integrity, and membrane fluidity, using flow cytometry, over 24 hours. In addition, the ability of sperm to bind the zona pellucida was evaluated using a perivitelline membrane (PV) binding assay. Sperm from young and old dogs responded similarly to treatments; therefore, data were combined. Canine sperm treated with PC12 had higher percentages ($p < 0.05$) of live acrosome reacted sperm (> 6%) than control sperm (3%) and more sperm binding to PV membranes (10% higher binding). Sperm treated with PF EVs exhibited similar percentages ($p > 0.05$) of motile sperm, sperm with fluid membranes and live acrosome reacted sperm throughout incubation, but PF EVs did increase sperm binding to PV membranes (11% higher binding). In conclusion, the addition of PF derived EVs to frozen-thawed canine sperm may improve some sperm functions and help to improve reproductive performance of cryopreserved canine sperm.

Keywords: Extracellular vesicles, dog, frozen-thawed semen, fertility

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Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

Student Abstracts

Dr. Jimmy Alexander Student Case Reports

Chronic endometritis caused by uterine marble in a mare

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Uterine marbles are used in mares to suppress estrus; effectiveness of this device varies in studies from 11 to 41.3%.[1] Unfortunately, use of marbles has also been linked to chronic endometritis, uterine fibrosis, adhesions, and subfertility; those risks are exacerbated when they are left in the uterus for a prolonged period.[2] A 14-year, retired racing Standardbred mare, was presented for breeding management and subfertility workup. History reported that the mare failed to become pregnant during 2 years, despite multiple breeding attempts. Initial reproductive examination revealed signs of estrus and poor perineal conformation. Uterine culture and cytology were performed that had heavy growth of *Staphylococcus aureus* and severe inflammation. Transvaginal procedures revealed cervical adhesions. Treatment for endometritis included daily uterine lavages, infusions, antiinflammatory medication (dexamethasone), and oral antibiotic (sulfadiazine trimethoprim 24 mg/kg, every 12 hours). During one treatment, a hard foreign body was felt within the uterus, and it was removed and identified as a glass marble. During next estrous cycle, mare was inseminated with cooled semen. After AI, mare developed severe postbreeding induced endometritis that was managed with uterine lavages, ecbolics, and oral antiinflammatory medication (firocoxib 0.1 mg/kg, once a day); a double synchronous ovulation was confirmed after 24 hours. A Caslick's vulvoplasty was placed. Mare was confirmed pregnant 14 days later, with a singleton pregnancy visualized at the base of left horn. Progesterone concentrations were determined and were low to support pregnancy; exogenous progestin was recommended. This case highlighted the importance of a breeding soundness examination prior to breeding and the risks in using uterine marbles leading to subfertility and endometritis.

Keywords: Mare, subfertility, uterine marble, estrus suppression

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Fungal placentitis in a mare

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Placentitis causes 60% of pregnancy loss, periparturient disease and neonatal morbidity in horses.[1] Clinical signs include premature udder development, vulvar discharge and spontaneous abortion.[2] A 11-year, Quarter Horse mare was presented for premature udder development on day 290 of pregnancy, raising suspicion of placentitis. External examination revealed no signs of vulvar discharge with intact Caslick's vulvoplasty. Moderate mammary development with expressible yellow and cloudy secretions were observed. Ultrasonographic examination revealed increased combined thickness of the uterus and placenta (2.3 cm). Empirical therapies included intramuscular estradiol cypionate (10 mg, every 72 hours), oral sulfamethoxazole-trimethoprim (30 mg/kg, every 12 hours), oral pentoxifylline (8.5 mg/kg, every 12 hours), and oral firocoxib (57 mg, every 24 hours). Due to high risk of dystocia and other complications, mare was hospitalized for foaling, monitoring and assistance. Parturition occurred on day 327 of pregnancy with no complications. Examination of fetal membranes revealed a 20 x 20 cm avillous area of the chorionic surface surrounded by mildly raised edges with tan mucoid material at the base of gravid horn. Fetal membranes were submitted for histopathology and evaluation. Histologic analysis revealed severe, locally extensive necrosuppurative placentitis infiltration of the chorion by *Candida albicans*. Fungal treatment was performed during estrus (day 30 after parturition). Intrauterine infusion of 1% hydrogen peroxide (120 ml), followed with a large-volume uterine lavage, intrauterine fluconazole and nystatin (100 mg/0.5 MU, once a day for 3 days), and oral fluconazole (4 mg/kg, once a day for 21 days). It was hypothesized that nocardioform or ascending placentitis was resolved by long-term antibiotic treatment, allowing growth of opportunistic fungus. This case underscored the importance of thorough diagnostic evaluations and targeted treatment in cases of placentitis.

Keywords: Mare, placental disease, high-risk pregnancy

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Hemospermia in a stallion caused by a urethral rent

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Hemospermia in the stallion ranges from small to large (overt) amounts of blood that can result from penile trauma, infectious conditions, penile neoplasias, urethral rents, seminal vesiculitis/ampullitis, or urethritis.[1] A 10-year, American Quarter Horse stallion was presented in 2023 for acute hemospermia. During the 2022 breeding season, stallion bred ~ 150 mares with normal fertility. On presentation, stallion had a normal TPR with no visible lesions on the penis. A blood (overt) contaminated ejaculate, containing 10 $x10^9$ sperm was collected. Through a urethral endoscopy an 8-10 mm urethral rent was observed cranial to the bulbourethral gland openings and caudal to colliculus seminalis.[2] Rent was treated by laser therapy to stimulate healing in combination with a perineal corpus spongiotomy to relieve urethral pressure.[3,4] Following surgery, a large scrotal swelling developed. Urethroscopy was repeated 2 weeks later, and it was determined that the rent had reduced in size but had not healed completely, and a second laser treatment was performed targeting the rent lumen in an effort to stimulate healing from the inside. A third urethroscopy was performed to confirm rent healing. Subsequently, 2 ejaculates containing no blood and 2.88 and 0.87 $x10^9$ total sperm; 9 and 20% normal sperm and 44 and 22% abnormal DNA were collected. Based on his total testes volume of 271 ml^3 his predicted total sperm of ~ 5.5 $x10^9$ sperm was lower than expected, perhaps due to the scrotal swelling and it is yet to be determined if sperm quality improves for the 2024 breeding season. Overt hemospermia in this stallion was resolved using a combination of laser therapy and surgical intervention. Successful treatment of overt hemospermia is essential to maintain normal fertility[5] provided his semen quality returns to normal values.

Keywords: Stallion, hemospermia, urethral rent

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Unusual case of hemospermia and poor sperm quality in a stallion

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A 12-year, Quarter Horse stallion, was examined in mid-September 2023, on-farm, due to a history of a sudden reduction in sperm numbers and quality. Stallion had normal libido, large testes, and bilateral distention of the cauda epididymides. A presumptive diagnosis of plugged ampullae[1] was made and a recommendation of per rectum ampullar massage followed by daily semen collection was suggested. Several days later the referring veterinarian reported a brown discolored ejaculate. Stallion was referred for further evaluation. Complete breeding soundness examination was performed. Relevant findings included testicular volume of 410 ml, 1.5 x 10^9 sperm/ejaculate, 25% total motile and morphologically normal sperm and a thick, brown colored ejaculate. Culture of the discolored semen yielded no bacterial growth and no cytological evidence of inflammation or intact red blood cells. Ultrasonographic evaluation of the ampullae identified unusual hyperechoic areas in the glandular region. Semen collected in an open-ended artificial vagina localized the brown-tinged semen to the sperm-rich fraction of the ejaculate that originated from ampullae, ductus deferens and cauda epididymis. Potential differentials for hemospermia include seminal vesiculitis/ampullitis,[2] habronemiasis, or urethral rent[3] and were ruled out based on the ejaculate color (red versus brown) and negative bacterial culture and cytology, and ultrasonographic findings. In retrospect, the initial history (low sperm numbers and poor sperm quality) was likely a result of the prolonged elevated ambient temperature experienced in the State of Texas in the Summer of 2023,[4] rather than sperm accumulation. In this case, ampullar massage probably resulted in an unexpected ampullary hemorrhage event manifested as brown hemolyzed blood. During next 3 months regular ejaculation resulted in elimination of blood and improvement in overall semen quality.

Keywords: Heat stress, sperm accumulation, hemospermia

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Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

Successful treatment of fungal and bacterial endometritis in an embryo donor mare

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Fungal endometritis is a rare but potentially devastating cause of infertility in mares. Infection can result from a variety of causes and often presents together with bacterial endometritis; thus, treatment can be complex and often carries a poor prognosis for future reproductive performance. A case of a 13-year, multiparous mare, presented for artificial insemination and embryo transfer after pregnancy loss is described. Culture and cytology indicated severe inflammation and infection with *Candida* spp.; treatments included daily intrauterine antifungals (fluconazole 100 mg, nystatin 0.5 mu), uterine lavages (saline with iodine solution 0.05%, DMSO 10%) and oral fluconazole (10 mg/kg followed by 5 mg/kg, once daily). During the following estrous cycle, further tests confirmed the presence of a concomitant bacterial endometritis due to S*treptococcus zooepidemicus.* Treatments included daily uterine lavages and infusions (ceftiofur 1 gram, nystatin 0.5 mu), intramuscular ecbolics (oxytocin 20 ui), an oral antiinflammatory (firocoxib 0.1 mg/kg), an oral hemorheological (pentoxifylline 8 mg/kg, twice a day), and an oral antifungal agent. Mare was bred with cooled semen, and a double ovulation was confirmed within 24 hours. Two good- quality blastocysts were recovered 8 days after, thoroughly washed, and transferred into 2 recipient mares. With the concern of transmitting yeasts, recipient mares were also treated prophylactically with an antifungal (fluconazole 10 mg/kg followed by 5 mg/kg, once daily). Unlike other equine pathogens such as equine viral arteritis that is known to be highly transmissible through embryos, yeasts are not mentioned in the list of diseases and pathological agents by the OIE in the Terrestrial Animal Health Code 4.8.14.[1] Recipient mares recently delivered healthy foals, proving that the treatment protocol, in this case, was effective in controlling not only the endometritis of the donor mare but also in preventing the spread of infection to the recipient mares.

Keywords: Embryo transfer, pregnancy loss, fugal endometritis

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Pyometra and cystic endometrial hyperplasia secondary to cervical neoplasia in a doe

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An 11-year, Nigerian Dwarf cross-doe, was presented with a 3-day history of straining to urinate. Doe had never been bred but presented with an enlarged udder that had been present for ~ 3 years. Physical examination was unremarkable. On abdominal radiographs, a soft tissue structure filled with fluid was observed in the expected location of urinary bladder. An enlarged uterus with thickened walls and heterogeneous fluid was observed on transabdominal ultrasonography. Based on presenting complaint and examination findings, a uterine mass and pyometra were suspected, and ovariohysterectomy was scheduled. After surgery, uterus and ovaries were submitted for histopathology. A grossly pale tan-to-white mass measuring 3 x 2.5 x 1 cm attached to uterus and cervix and cystic endometrial hyperplasia were observed. Histology was performed, and findings were consistent with a leiomyoma with mild, multifocal, chronic lymphoplasmacytic endometritis with cystic endometrial hyperplasia. When doe returned for reexamination, there was minimal edema around incision site, and ultrasonography revealed no fluid in the uterine stump. Leiomyomas have been reported in small ruminants,[1,2] but it is not a common disease. This is likely due to does not living long enough to develop neoplasia. However, with the increased popularity of pet goats, we are noticing more cases because they are living longer. Presence of leiomyoma in the cervical area is likely the cause of the other clinical signs such as cystic endometrial hyperplasia, pyometra, and straining to urinate noticed by the owner. Cystic endometrial hyperplasia in ruminants is associated with hyperestrogenism,[3] that could be due to the increased steroid receptors present in leiomyomas.[4] Pyometra is commonly caused by contamination from bacteria in the vagina ascending into the uterus. The mass prevented the physiological closing of the cervix in the nonpregnant state and allowed an ascending infection that resulted in the pyometra.

Keywords: Does, cervical neoplasia, pyometra, cystic endometrial hyperplasia

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Dystocia associated with fetal monster in a mare

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An 11-year, Quarter Horse, mare presented with dystocia of ~ 8 hours. Estimated age of fetus was 268 days. Fetus was in posterior presentation, dorsosacral position with bilateral hock flexion. Fetus was retropulsed into uterus to allow repositioning of the hocks and placement of obstetrical chains for assisted vaginal delivery of the nonviable fetus. Fetus had numerous congenital abnormalities of the skull as well as umbilical torsion. After parturition, ~ 6 cm grade 2 vaginal tear was noted ~ 3 cm into the vagina on the right side. Uterus was lavaged with 7 gallons of isotonic, 0.05% betadine solution. Mare received intravenous lactated Ringer's solution with calcium, magnesium, phosphorous, and potassium at constant rate infusion of 1 liter/hour for the first 24 hours postpartum, antibiotics, antiinflammatory, oxytocin, and animax ointment. Uterus was lavaged twice daily for 4 days, then once daily for 2 additional days. Mare exhibited signs of mild colic 3 days postpartum that was medically managed with xylazine and buscopan. Mare expelled fetal membranes on the same day. Mare was discharged after 8 days of hospitalization. Necropsy of fetus revealed following congenital abnormalities: crania bifida, temporalis bone with aplasia cutis, palatoschisis, maxillary brachygnathism, anophthalmia right eye with incomplete cranial orbit, hydrophthalmia left eye, and campylorrhinus lateralis. There were no substantial internal abnormalities outside of the skull. Dystocia in mares is considered uncommon, but a recent retrospective study[1] reported the incidence of dystocia in healthy mares and mares that had previously experienced dystocia. Dystocia frequency was 9.7%;17.5% mares that had previously experienced dystocia compared to 6.7% in mares without a history of dystocia. Foal mortality was 1.9%, whereas maternal mortality was 0.5%.

Keywords: Mare, abortion, dystocia, umbilical torsion

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Dystocia and uterine rupture after cloprostenol treatment in a pregnant llama

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An adult female llama was presented to Theriogenology Service for retained fetal membranes and emaciation. Llama was rescued 1-week before alongside an intact male. A veterinarian was consulted and diagnosed the llama to be open but still gave an unknown dose of cloprostenol to terminate any potential early pregnancy. Next day, owners noticed fetal membranes hanging from vulva. Lama's health deteriorated during the following few days and lama was presented. Llama was dull and anxious, with normal vital parameters. A foul vaginal discharge was noted. Transrectal palpation and ultrasonography, transabdominal ultrasonography and vaginal examination revealed a retained fetus and a tight vestibular sphincter. A baseline CBC yielded left shift with toxic neutrophils and normal blood chemistry. Manual manipulation was elected as cesarean surgery was not an option. Vestibular sphincter was progressively dilated manually and cervix was fully dilated. Approximately 8- month fetus (anterior longitudinal presentation, dorsosacral position, head and both forelegs fully extended) was manually delivered. Subsequent uterine examination revealed a full- thickness dorsal uterine tear without fresh bleeding. Humane euthanasia was elected, and llama and fetus were submitted for necropsy. Necropsy confirmed a 10 cm full-thickness tear on the dorsal uterine body and lochia in the abdominal cavity. Uterine rupture possibly resulted from uterine contractions with a blocked birth canal due to nondilated vestibular sphincter, leading to subsequent peritonitis, left shift with toxic neutrophils, and clinical signs. This case highlighted the repercussions of misdiagnosis in theriogenology practice on animal welfare and the importance of theriogenology training in the DVM curriculum. Although camelid dystocia rates are low, failure of vestibular dilation has been reported to be a maternal cause of dystocia.[1] Dystocia is an emergency in camelids, and prolonged time to seek treatment contributes to poor prognosis.

Keywords: Camelids, dystocia, vestibular sphincter, abortion

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Effects of intrauterine ceftiofur on equine uterine microbiome

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Overuse of antibiotics is becoming overwhelming in veterinary medicine resulting in antibiotic resistance and dysbiosis of natural microflora. Antimicrobial therapy, such as ceftiofur, a third- generation cephalosporin, is a mainstay for treating reproductive diseases, including endometritis. We hypothesized that an infusion of ceftiofur disrupts healthy mare's uterine resident microbial community. University-owned mares (n = 8) underwent transrectal palpation and ultrasonography to determine the stage of estrous cycle. A double guarded endometrial swab (MOFA®) was inserted transcervically into uterus for characterizing estrual uterus after 3 days of sham (saline) and then subsequently 1 gram of ceftiofur (Naxcel®) intrauterine infusion. At third estrus, mares were bred to the same stallion via artificial insemination. The V4 region of the 16S rRNA gene was amplified for Illumina Miseq sequencing to examine core bacterial communities present before and after ceftiofur. Microbial community composition of sham and ceftiofur treated mares were not different (p = 0.321; [PERMANOVA with Bray-Curtis dissimilarity of 16S Amplicon Sequence Variant's relative abundance]). Alpha diversity was not significantly different after ceftiofur, meaning there were no differences in richness and evenness in the microbial communities after treatment. Only notable difference was the abundance of Christensenellaceae_R-7_ group was lower (p = 0.0428) after ceftiofur (0.14 ± 1.05% versus 2.89 ± 1.07% control) treatment. Finally, mares bred by artificial insemination achieved pregnancy. In conclusion, 3-day treatment of ceftiofur did not acutely change the microbial communities within mare's uterus when sampled directly after treatment. A limitation of this study was time of sampling. Ceftiofur may have a long-term effect on the uterine microbiome that may require sampling several weeks after treatment. In conclusion, ceftiofur did not change the healthy uterine microbiome during estrus and did not affect pregnancy rates; however, should be used judiciously because it may have long-term microbial effects.

Keywords: Mare, pregnancy, microbiome, antibiotics

Probable and actual degrees of homozygosity as a predictor of sperm quality in the domestic cat

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Important populations of purpose-bred cats - which include endangered nondomestics and closed research colonies - are bred selectively, representing reduced gene pools and high inbreeding. Parameters of sperm quantity and quality are widely regarded as relevant to an individual's breeding soundness, a relationship well established in many species but largely unexplored in domestic cats. The hypothesis is that poor sperm quality and increased homozygosity are correlated. This study compared semen characteristics with 2 measures of inbreeding, a pedigree-based coefficient of inbreeding (COI, a probable value indicating likelihood of homozygosity) and a genetic analysis (a measure of actual homozygosity), to determine their predictive power regarding ejaculate characteristics in the male domestic cat (*Felis catis*). Healthy adult male domestic cats (n = 18) were collected via electroejaculation under anesthesia (ketamine 10 mg/kg, dexmedetomidine 30 µg/kg) 1week after an initial collection. Semen characteristics were recorded: ejaculate volume, concentration, total sperm number, total and progressive motilities, and morphology. Inbreeding coefficients, calculated for each cat based on 12+ generations of archival records, ranged from 0 to 32.8%. DNA samples were then collected via buccal swab and submitted to UC Davis's Veterinary Genetics Laboratory for genotyping by sequencing using the International Society of Animal Genetics standardized panel of single nucleotide variant markers for the cat on their Ion GeneStudio S5 platform. Correlations ($p < 0.05$) were identified between COI and progressive motility, total motility, and percentage of sperm with coiled tails. Differences ($p < 0.05$) in both motilities and percent morphologically normal sperm in individuals with a COI below 10% when compared to those above 10% were also identified. Genetic analysis results are pending. Understanding correlations between genetic diversity and ejaculate characteristics will provide insight into the effects of inbreeding in felid populations, as well as a possible model for evaluation and selection of potential breeding stock.

Keywords: Domestic cat, inbreeding, fertility, sperm, coefficient of inbreeding

Comparison of domestic feline sperm vitality in TES-tris egg yolk, feline- optimized culture medium, and TRE-tris-citrate fructose

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Appropriate sperm handling medium is crucial to maintain sperm viability and ensure fertilization competence for downstream assisted reproduction procedures. However, little is done to optimize a sperm handling medium for the domestic cat. Objective was to compare the effects of 3 holding media (TES-Tris Egg Yolk [TEY], Feline-Optimized Culture Media [FOCM], and TRE-Tris-Citrate Fructose [TTCF]) on sperm morphology, total motility (TM), and progressive motility (PM). Adult male domestic cats (n = 4) were anesthetized and subjected to 1-2 rounds of electroejaculation for sperm collection. Semen was immediately split into 3 aliquots and diluted to a concentration range of 15.76-49.04 x 10^6 total sperm/ml. Computer assisted sperm analysis (CASA) system-IVOS II (Hamilton Thorne, Beverly, MA) was utilized for assessments of morphology, TM, and PM at the 0, 2, 4, 6, 24, and 48 hours timepoints. Data were analyzed with a mixed effect ANOVA model and reported as mean ± SD. Statistical significance was set at $p < 0.05$. Across all timepoints, TEY had a higher percent normal morphology (89.48 ± 7.25) than FOCM (79.27 ± 6.74); no other differences were observed (TTCF: 83.53 ± 5.29). At 0 hour, PM was lower in FOCM (34.2 ± 2.2) than TEY (53.3 ± 14.08) and TTCF (57.98 ± 5.95). At 2 and 4 hours, PM was lower in FOCM (2 hours: 31.28 ± 3.88; 4 hours: 24.95 ± 1.98) than TTCF (2 hours: 52.88 ± 1.72; 4 hours: 41.4 ± 2.68). However, at 24 hours, FOCM exhibited higher PM (20.15 ± 7.35) than TEY (3.8 ± 4.96) and TTCF (2.7 ± 5). Similarly, FOCM also demonstrated higher TM (56.15 ± 15.48) than TEY (13.88 ± 17.33) and TTCF (6.93 ± 11.3) at 24 hours. No other differences between treatments were observed for TM. In conclusion, although our findings revealed an initial reduction in PM and TM with FOCM, it demonstrated sustained PM and TM over a 24-hour period, surpassing the performance of TEY and TTCF. These insights contributed valuable information for optimizing sperm handling media in the domestic cat.

Keywords: Domestic cat, sperm handling media, motility, morphology

Multigenerational toxicity in progeny of zebrafish with developmental trichloroethylene exposure

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Trichloroethylene (TCE) is a legacy environmental toxicant used heavily up through the 1970's as metal degreaser and an industrial solvent. Due to a long half-life and persistence in soil and ground water, TCE toxicity is still a concern today. TCE is a known carcinogen and has been linked to spontaneous abortion, decreased birth weight, and developmental neurotoxicity. TCE is also suspected to cause epigenetic toxicity. This study used zebrafish biomedical model (*Danio rerio*) to test the hypothesis that developmental TCE exposure causes toxicity in the exposed group and their offspring. Wild-type zebrafish (F0) were developmentally (0-5 days after fertilization) exposed in groups to 0, 5, 50, and 500 parts per billion (ppb; µg/l) TCE. Reproductive toxicity in F0 zebrafish was assessed through success and fecundancy of dose-matched paired breedings. Developmental toxicity in the F1 progeny was assessed via video recorded and computer analyzed embryonic photomotor response and larval visual motor response behavior assays, heart rate analysis, and morphologic assessments. F0 reproductive assays demonstrated no significant alterations in embryo production or embryonic survival. In the F1 progeny, the 5-ppb embryos were hyperactive in the embryonic photomotor response test. On day 5 after fertilization, larval zebrafish had decreased movement and activity and altered turning behavior in all exposure groups. All exposure groups had altered morphologic parameters including body length, head length and width, eye diameter, and jaw length. Finally, the 500-ppb progeny demonstrated an increased heart rate (108.7 ± 17.0 bpm) as compared to the controls (100.9 ± 15.8 bpm). Despite no toxicity observed in the F0 parental generation, F1 progeny had significant behavioral, physiologic, and morphologic changes. Developmental TCE exposure in parental generations resulted in toxic effects in the offspring. Given the widespread contamination, TCE may pose a risk to people and animals living around contaminated sites.

Keywords: Trichloroethylene exposure, zebra fish, toxicity, developmental changes

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

Noncompetitive Student Posters

Malignant seminoma in a stallion

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A 22-year, Icelandic stallion, was presented with 1-year history of scrotal enlargement. Left testis was diffusely enlarged and soft on palpation compared to right testis. Scrotal ultrasonography identified a heterogenous multilocular, mass-like effect distorting entire left testis with minimal normal-appearing testicular parenchyma. Closed, left hemicastration was performed to conserve stallion's breeding potential. Left testis, on cut surface, contained only a thin rim of normal appearing testicular parenchyma surrounding an extensive multilocular, pale glistening mass. Histopathology identified diffuse sheets of round to polygonal cells effacing and replacing normal testicular parenchyma with marked anisocytosis and anisokaryosis with several multinucleated cells. Microscopic features were most consistent with seminoma. Aggregates of neoplastic cells were identified within the lumen of vasculature indicating lymphovascular invasion and potential metastasis. Owners declined additional diagnostics for identification of metastasis. Three weeks after surgery stallion was examined for unilateral, left-sided scrotal swelling, and was diagnosed with a subcutaneous incisional infection. Ceftiofur crystalline free acid and phenylbutazone were given and continued for 7 days. Incision was opened without necessitating suture removal, drained, and the site was copiously lavaged with lactated Ringer's solution under standing sedation. Hospital discharge recommendations were to increase movement with forced exercise. Seven months after castration, stallion was reported to be doing well with no known concerns. Seminomas are the most common equine testicular neoplasm arising from germ cells of spermatic epithelium and have been suggested to have increased metastatic prevalence compared to other domestic species (e.g. dogs). Future follow-up assessment of this stallion would allow assessment of right testis, local lymph nodes, and semen quality.

Keywords: Stallion, malignant seminoma, ultrasonography, histopathology, hemicastration

Presumed prolonged retention of fetal membranes in a mare

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Ensuring complete expulsion of fetal membranes is important to prevent complications including laminitis and metritis. A 17-year, Quarter Horse mare, was presented for a breeding soundness examination. Mare aborted twins at 6-7 months of pregnancy, 2 years prior and failed to become pregnant during subsequent season. On presentation, mare had multiple small right ovarian follicles, no uterine edema, and poor tone. Endometrial biopsy was Grade IIA and no growth resulted from uterine culture. Findings were consistent with mare's age and seasonal transition; breeding was recommended. Mare was presented again 20 days later, presumably just after ovulation. Owners gave $PGF_{2\alpha}$ after 14 days. Four days later during artificial insemination, a soft tissue mass (~ 6 x 4 x 3 cm) was palpated within the uterine body. Mass was retrieved after misoprostol-assisted manual cervical dilation. Grossly, it was nonodorous and putty-like in consistency. Histopathology identified amorphous dense aggregates of erythrocytes, amorphous to fibrillar beaded eosinophilic material, and rare pyknotic poorly preserved cells. Tissue was presumed to be retained fetal membranes, having observed no other gross pathology and inconclusive histopathology. After mass removal, mare's uterine body was artificially inseminated and lavaged after 4 hours. Mare was inseminated with a second breeding dose at ovulation after 2 days. Mare was diagnosed pregnant on day 14 day and a heartbeat was confirmed on day 34. Mare was infertile possibly due to the uterine mass, presumed to be fetal membranes retained from the mid-term abortion 2 years before. Reproductive prognosis for future fertility was considered good. During breeding soundness examinations in subfertile or infertile mares, evaluation of entire uterus is critical not to miss potential foreign objects or material. Furthermore, complete fetal membranes' examination is necessary, especially of both uterine horn tips, after foaling or abortion, and reduces the chance of undetected, retained fetal membranes.

Keywords: Mare, retained fetal membranes, artificial insemination, breeding soundness examination

Schistosomas reflexus and uterine tear in a heifer

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A 2-year, Hereford heifer was referred for dystocia after a failed attempt by referring veterinarian. On physical examination, heifer was visibly uncomfortable and 2 of fetus' feet were protruding from vulva. Heifer was confined in a hospital stall and was restrained in a head gate for further examination. As heifer's vulva and perineum were cleaned and prepped for manual vaginal examination, heifer collapsed into sternal recumbency. Halter was placed on heifer's head to release from head gate while maintaining adequate restraint. Halter was tied to the lowest pipe on the head gate and heifer was assisted into left lateral recumbency. Vaginal palpation was reinitiated and it was identified that fetus was an anatomical anomaly known as a schistosomus reflexus; uncommon congenital malformation, occurring in 0.01-1.3% of all dystocia cases that is primarily characterized by exposed abdominal and thoracic viscera and extreme spinal inversion producing prominent ventral convex curve. Fetotomy was elected; caudal fetal portion was removed and during further removal of fetal parts, entire uterus prolapsed. Once reduced, it was noted that there was roughly 300° tear of uterus at cervix level with only a small attachment at the ventral aspect that allowed uterus to protrude through and out of vulva. Due to tear's size and prognosis, owner was consulted and it was decided to humanely euthanize heifer via captive bolt and intravenous KCl.

Keywords: Uterus, dystocia, heifer, schistosomas reflexus, fetotomy

Dystocia as a consequence of bicornuate pregnancy in a mare

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An 8-year, Friesian mare, was presented for dystocia of unknown duration, and with an unknown reproductive history. Mare was in the field and determined to be in stage 2 labor. On physical examination, mare was recumbent, mildly hypothermic, tachycardic, and tachypneic. Foal's right front hoof was protruding from vulva; vaginal examination identified that fetus was in transverse presentation, dorso-ileac position, with left forelimb and neck extending into left uterine horn. Mare was referred to the veterinary teaching hospital. Initial diagnostics on presentation revealed hypocalcemia, hyperglycemia, hyperlactatemia, decreased oxygen saturation, elevated total solids, and respiratory acidosis. Transabdominal ultrasonography confirmed absence of fetal heartbeat and fetal death. Controlled vaginal delivery under general anesthesia was attempted. First fetotomy cut at left carpus level was achieved; however, it was not possible to reach neck for a second fetotomy cut. Ultimately, decision was made to perform cesarean surgery, confirming a bicornuate pregnancy, where fetus was present in both uterine horns. Fetus and fetal membranes were successfully removed through hysterotomy, and mare had an uneventful recovery. Mare was initially treated with intramuscular procaine penicillin G (22,000 IU/kg, twice daily) and intravenous gentamicin (6.6 mg/kg, once a day), before transitioning to intramuscular ceftiofur (6.6 mg/kg) at discharge. Fetal membranes evaluation confirmed a 'T shape' form with 2 gravid horns and a type II vascular pattern. Bicornuate pregnancy is rare, with transverse presentation resulting in dystocia and increased fetal mortality due to a reduced placental perfusion and rapid placental separation. This case highlighted the importance of reproductive ultrasonography throughout pregnancy to detect abnormal positions that would benefit from in hospital monitoring and attended parturitions, enabling early interventions when dystocia occurs.

Keywords: Mare, bicornuate pregnancy, dystocia, fetus, pregnancy

Premature lactation in a recipient mare

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A recipient mare, carrying an embryo transfer pregnancy (7 months), was presented for premature udder development. Mare had slight vaginal discharge and an increased placental thickness on transrectal ultrasonography. An area of placenta around cervix was detached and fetus could not be palpated. A diagnosis of placentitis was made. Placentitis is defined as inflammation or infection of placenta. Within equine breeding industry, it has become the leading cause of reproductive loss, with either abortion or delivery of a small, ill-thrift foal. In mare's placenta, amnion surrounds fetus and chorioallantois attaches to endometrium. These structures protect fetus and provide nutrients and gas exchange for growth. Most commonly affected site with placentitis is chorioallantois, leading to a compromise of the attachment site between placenta and endometrium, or thickening of placenta due to inflammation, both of which cause harm to fetus. In this case, ascending infection was the most likely cause, as evidenced by placental separation at cervix. This prevents adequate nutrient and gas exchange in that specific area, leading to fetal death and thus, abortion. Septicemia, placental insufficiency, or prostaglandin release from inflammation leading to uterine contractions can cause an abortion.[1,2] Mare was initially treated with intravenous flunixin meglumine (1.1 mg/kg, twice daily), oral trimethoprim sulfa (30 mg/kg, twice a day) and oral altrenogest (0.088 mg/kg, once daily). Fetal heartbeat was not present and no fetal movements were appreciated after multiple attempts. Fetus was determined to be dead and medications were discontinued to allow mare to abort on her own. This case depicted the devastating outcome (dead fetus) of a mare with placentitis.

Keywords: Mare, vaginal discharge, placentitis, udder development

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

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Successful treatment of 28-hour retained fetal membranes in a mare using umbilical vessel water infusion

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Expulsion of fetal membranes in mares should occur within 2 hours after parturition, after which, considered retained. Complication ranges from asymptomatic to life threatening conditions in as little as 8 hours. Several methods are described to treat retained fetal membranes, including oxytocin treatment, manual removal, and water infusion into allantoic cavity and umbilical vessels. Umbilical vessel water infusion quickly and gently separates chorioallantois from endometrium by interstitial swelling of membranes.[1] An 8-year, Clydesdale mare, in dystocia for 4 hours with a dead foal at 307 days pregnancy, was referred. Foal was delivered via assisted vaginal delivery and mare retained fetal membranes. Mare was started on oral trimethoprim/sulfamethoxazole (30 mg/kg, twice daily), intravenous flunixin meglumine (0.5 mg/kg, 4 times a day) and intramuscular oxytocin (20 IU, 4 times a day). Due to broken water line in the hospital, umbilical vessel water infusion could not be performed, and uterus was lavaged twice a day with dilute betadine solution. After 4 lavages, fetal membranes in non-pregnant uterine horn remained tightly attached to endometrium. Biochemistry and CBC revealed leukopenia, elevated fibrinogen, decreased segmented neutrophils, and hyperglycemia, placing the mare at high risk of toxic metritis and septicemia. Water line was restored 28 hours after foal delivery and fetal membranes were successfully removed intact using umbilical vessel water infusion alternating between 2 available umbilical vessels. Mare was discharged next day morning and recovered uneventfully. This case highlighted that even in prolonged retention (> 12-24 hours) of fetal membranes, umbilical vessel water infusion can still be used successfully and it is worth attempting to avoid damage to endometrium and mitigate risks (hemorrhage, impaired uterine involution, tearing of fetal membranes and damage to endometrium) associated with other manual methods to remove retained fetal membranes.

Keywords: Mare, retained fetal membranes, umbilical vessel water infusion

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Pharmacologically induced ejaculation in an aged stallion

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A 13-year, Quarter Horse stallion, was presented for chemical ejaculation due to musculoskeletal limitations that hindered phantom collection. There are 2 protocols for chemical ejaculation that target an α-adrenergic response: combinations of oral imipramine (1.3 mg/kg) and intravenous xylazine (0.3 mg/kg) or oral imipramine, intravenous oxytocin (20 IU), and intravenous detomidine (0.02 mg/ml, imipramine-oxy-det). Imipramine was given 1.5 hours prior to other agents, and additional stimulation was provided via estrous mare urine used ~ 15 minutes before ejaculation. Chemical ejaculation was first attempted in this stallion on April 11th using imipramine-xylazine; collection was unsuccessful. Next day, imipramine-oxy-det yielded 32.0 ml semen with 5.02 x 10^9 sperm. Computer assisted sperm analysis revealed 34% total motility (TM), 12% progressive motility (PM), and 44% normal morphology. Stallion was collected again on May 30th, and the same protocol produced 18.6 ml of semen with 5.01 x 10^9 sperm, 16.4% TM, and 7.9% PM. Collection with imipramine-oxy-det failed once on May 31st, then twice on June 16th, with urination in second attempt. Six attempts from June 17-20th were also unsuccessful: 5 attempts with increasing doses of detomidine (0.2-0.8 ml) and 1 with xylazine-imipramine reattempt on 20th. On June 21st, stallion was given imipramine-oxy-det with 0.8 ml detomidine and produced 88.4 ml of semen with 15.40 x 10^9 sperm, 14.1% TM, and 7.7% PM. On August 6th, stallion was collected for the final time using imipramine-oxy-det with 0.8 ml detomidine that yielded an ejaculate volume of 30.3 ml with 14.14 x 10^9 sperm, and total and progressive motilities of 20.5 and 13.7%. Semen was cushion centrifuged and 3 mares were bred during 4 estrous cycles via deep uterine horn artificial insemination, with first and third collections resulting in pregnancies. One mare conceived on first estrous cycle, 1 mare on its second, and 1 mare was not pregnant. Ultimately, although challenging due to poor reliability and decreased motility, chemical ejaculation presents a viable option for stallions that are unable to be collected through traditional means. In combination with other semen processing techniques, it is possible to have offspring from stallions with poor semen quality.

Keywords: Stallion, chemical ejaculation, imipramine, oxytocin, sperm motility

Iatrogenic pneumoperitoneum following vaginoscopic investigation of chronic vaginitis

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A 2-year, spayed Pitbull mix dog, was referred for evaluation of chronic vaginitis that was diagnosed 1 month prior by the primary care veterinarian via vaginal cytology and culture. Primary veterinarian performed vaginal douche with gentamicin and prescribed systemic antibiotics. Following appointment, hemorrhagic vaginal discharge and abdominal pain were observed and a second veterinarian was consulted. Purulent vulvar discharge was noted, and dog was referred to Theriogenology service for further diagnostics and treatment. On presentation, dog appeared depressed, lethargic, and pyrexic. Abdominal ultrasonography results were unremarkable. Vaginoscopy, using a transcervical insemination scope, was performed to investigate the possibility of stump pyometra and evaluate vaginal mucosa. Vaginal mucosa was hyperemic, and a tract was observed on the vaginal dorsal aspect, seemingly terminating in fascia. During the procedure, it was difficult to maintain sufficient vaginal insufflation. Immediately after vaginoscopy, dog became tachycardic, vomited several times, and gradually abdomen increased in size. Dog was transferred to hospital's Emergency and Critical Care service; pneumoperitoneum was diagnosed via radiography. Dog was stabilized by removal of 500 ml of air from the abdomen and supportive therapy prior to emergency exploratory celiotomy. A 1 cm necrotic perforation of the cranial vagina, communicating with the abdominal cavity was diagnosed and accounted for pneumoperitoneum after vaginoscopy. Uterine stump and cervix were removed, and a cranial vaginectomy was performed, utilizing vaginoscopy (transcervical insemination scope) it was ensured that the vaginal necrotic perforation was completely removed; dog recovered uneventfully. This case highlighted the importance of vaginoscopy in the evaluation of dog presented with vaginitis. Although rare, clinicians should consider that vaginal cytology, douching, manipulation, and endoscopy can lead to vaginal perforations.

Keywords: Vagina, pneumoperitoneum, vaginoscopy, vaginitis, vaginal perforation

Left ventrolateral cesarean surgery in a heifer carrying schistosomus reflexus

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A 2-year, Aberdeen Angus heifer, was presented with 10-hour history of dystocia. Owner reported (via phone) that manual manipulation was attempted and viscera were protruding from vulva. Euthanasia was discussed, suspecting maternal evisceration after uterine rupture with poor prognosis. However, photos from the owner confirmed protrusion of dark pink tubular tissues were fetal viscera; schistosomus reflexus was suspected. Transrectal and transvaginal palpation revealed an abnormal fetus in uterus and dilated cervix with engaged fetal viscera. Vaginal delivery was unsuccessful and fetotomy was not indicated due to unknown extent of fetal malformation. Left ventrolateral cesarean surgery was chosen, as heifer went recumbent. A dead, malformed fetal monster of undefined sex, consistent with schistosomus reflexus, characterized by visceral exposure and spinal inversion, was successfully extracted under intramuscular sedation with acepromazine and butorphanol, followed by intravenous xylazine every 15 minutes. Uterus, abdominal musculature, and skin were closed by Utrecht, simple continuous, and simple interrupted pattern, respectively. Heifer was given subcutaneous ceftiofur crystalline free acid and intravenous flunixin meglumine. Heifer was clinically normal at discharge after 2-day hospitalization. Ten days later, heifer returned for treatment of surgical site infection and dehiscence of several skin sutures. Intravenous flunixin meglumine, subcutaneous ceftiofur crystalline free acid, and intramuscular procaine penicillin were given for 3 days. Incision was lavaged and allowed to heal by second intention during 17-day hospitalization. Heifer recovered uneventfully and was discharged. This case illustrated an important differential for viscera protruding from vulva after attempted manipulation, importance of photos from owners, an approach to cesarean surgery in a recumbent cow, and a known disadvantage of this approach (an increased risk of surgical site infection and wound dehiscence).

Keywords: Schistosomus reflexus, dystocia, cattle, cesarean surgery

Severe preputial hemorrhage in a neutered male dog

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Substantial, frank hemorrhage from canine prepuce is considered an emergent situation that warrants further evaluation. A 10-year, neutered Jack Russell Terrier, was presented at the emergency service of the hospital with severe hemorrhage from prepuce. Approximately 2 months prior, patient was presented to the primary veterinarian with similar signs. A urinary tract infection and/or prostatitis was suspected, and patient was treated with amoxicillin/clavulanic acid and carprofen that appeared to resolve the problem. Physical examination was normal except for intermittent hemorrhage from prepuce. Dog was sedated to allow for a more detailed examination of penis and prepuce; exteriorized penis was normal. However, a pedunculated mass was located on preputial mucosal surface and was confirmed to be the source of hemorrhage. Under sedation, prepuce was manually everted allowing access to base of the mass. Mass was 1 x 1.5 cm and was

Citation line: Clinical Theriogenology 2024, 16, 11054, http://dx.doi.org/10.58292/CT.v16.11054

removed with suture ligation and subsequent excision with no complications. Dog recovered uneventfully and was discharged with gabapentin, codeine sulfate, and carprofen. Tissue was submitted for histopathology that identified the mass to be an extramedullary plasmacytoma. Tumors occurring on canine penis and prepuce are uncommon and are most likely due to venereal disease conditions (e.g. transmissible venereal tumors).[1] Extramedullary plasmacytomas are normally observed in dogs aged 5-11 years and the growth is usually 1-2 cm in diameter.[1–3] Most common location for this neoplasm was skin, with nearly ⅓ occurring in mucous membrane sites in oral cavity or rectum.[1] Extramedullary plasmacytomas are reported in locations such as uterus, third eyelid, and penis.[1] Surgical excision achieving complete margins is generally curative for these tumors and the prognosis is excellent.

Keywords: Extramedullary plasmacytoma, preputial mass

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