Identifying risk factors for mastitis in postpartum bitches

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Mastitis in postpartum bitch is a disease that affects wellbeing of both dam and pups that rely on their mother's milk as the sole source of nutrition during a critical growth period. Risk factors for canine mastitis have not been well-studied and this information could help veterinarians and breeders identify females that may need closer monitoring. Goal of this study was to identify risk factors for postpartum mastitis in bitch. We hypothesized that age, breed, litter size, and whelping interventions are associated with risk of developing mastitis in postpartum bitch. A retrospective cohort study was performed using data from 2 guide dog colonies over a 13-year period. A total of 2,489 whelpings occurred during study period. All bitches were monitored closely and mastitis was diagnosed by colony veterinarian. Risk factors examined were: colony, breed of dam, litter size, neonatal pup loss, age (in years), parity, dystocia, use of oxytocin during whelping, use of calcium during whelping, and if cesarean section was performed. Risk factors were evaluated individually and offered to the model if $p < 0.20$. A final model was built using a backwards-stepwise method and colony was forced into the model to account for colony-specific differences. Logistic regression ANOVA was performed using JMP Pro v. 14 (SAS Institute). Mastitis incidence was 8.9% (222 cases). Three significant risk factors were identified and offered to the model built; all were retained in the final model. First, mastitis risk increased as litter size increased and ROC analysis identified ≥ 9 pups as the optimal threshold. Bitches that had ≥ 9 pups (120/1,048 or 11.5% versus 7.1% in litters < 9 pups) were more likely to develop mastitis (OR = 1.67, 95% CI 1.26 - 2.21; $p = 0.0003$). Second, there was an effect of dam breed; Golden Retrievers (12.9% OR = 2.81, 95% C.I. 1.32 - 5.99; $p = 0.0075$) and Labrador Retrievers (8.6% OR = 2.05, 95% C.I 1.03 - 4.11; $p = 0.0418$) were more likely to develop mastitis compared to German Shepherd Dogs (4.8%). Finally, bitches that whelped naturally (9.4%) were more likely to develop mastitis compared to bitches that had cesarean section (5.7%) performed (OR = 1.70, 95% CI 1.04 - 2.76; $p = 0.0332$); therefore, bitches that underwent cesarean section had lower rates of mastitis. Surprisingly, bitches with singletons (3%) had lower mastitis risk than bitches with large litters, and neonatal (> 3 days) loss of pups did not affect mastitis risk. The dogma of older bitches having higher risk of mastitis was also not observed in our population. There is an effect of dam breed on mastitis risk and further studies examining more breeds are warranted.

Keywords: Canine, mastitis, risk factors

Fungal growth is more likely to be affected by hormones in equine uterine isolates compared to isolates from nonreproductive sites

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Fungal endometritis is an important cause of infertility in mares. Studies indicated that whereas estradiol may have a stimulatory effect on fungal growth, progesterone may have an inhibitory effect. However, both studies only examined 1 fungal isolate. Hormone responsiveness of fungal isolates from mare reproductive tract has not been evaluated. Objective was to determine if clinical fungal isolates from mare uteruses are hormone responsive; if so, are uterine isolates more likely to be hormone responsive compared to fungal isolates from nonreproductive sites. We hypothesized that estradiol has stimulatory effects whereas progesterone has inhibitory effects on fungal growth and that fungal isolates from mare uteruses are more likely hormone responsive compared to fungal isolates from nonreproductive sites. Fungal isolates from mare uteruses (n = 7) and isolates from nonreproductive sites (n = 5) were evaluated for hormone responsiveness after being cultured in RPMI media for 48 hours at 30°C in 96-well plates. Absorbance was read at 600 nm at seeding times, 24 and 48 hours. Correlation between absorbance and fungal concentration was determined by hemocytometer. Dependent variable was percent change in fungal concentration from initial seeding. Estradiol treatment groups were: ethanol (vehicle) control and 10, 150, and 1,000 ng/ml whereas progesterone treatment groups were: ethanol control and 0.1, 5, and 100 μg/ml. Data were not normally distributed and nonparametric analyses were used. Effects of hormone treatment and site on fungal growth were determined by Kruskal-Wallis test and treatment effects were determined using multiple comparison rank sum Steel test with ethanol group as the control in JMP Pro v. 14 (SAS Institute, Cary, NC). Estradiol affected growth ($p = 0.0004$) at 48 hours with increased growth ($p = 0.0189$) in 10 ng/ml compared to control. Progesterone affected growth at 24 ($p < 0.0001$) and 48 hours ($p < 0.0001$) with decreased growth in 5 μg/ml ($p < 0.0001$) and 100 μg/ml ($p < 0.0001$) groups compared to controls. Estradiol affected growth ($p < 0.0001$), with 3/7 (at 24 and 48 hours) isolates from uterus and 0/5 (at 24 hour) and 1/5 (at 48 hour) isolates from nonreproductive sites affected by estradiol. Progesterone affected growth ($p < 0.0001$), with 6/7 (at 24 and 48 hour) isolates from mare uterus and 1/5 (24 hour) and 3/5 (48 hour) isolates from nonreproductive sites affected. Majority of fungal isolates from mare uteruses were hormone responsive to estradiol (that increased growth rates) and progesterone (that decreased growth rates) whereas most nonreproductive isolates were not hormone responsive. This study highlighted potential interactions between hormone status and fungal infectivity in equine uterus.

Keywords: Fungal, hormone responsiveness, horses

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A novel method to reduce egg laying in companion avian species using a hen model

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Reproductive-related diseases in female avian species are a common problem in zoological companion animal medicine. Of particular concern is a potentially serious condition called egg-binding where eggs are retained within female reproductive tract. In chronic cases, treatment involves preventing egg production with surgical removal of the ovary or administration of hormones such as leuprolide acetate, or deslorelin acetate. These approaches are problematic, as surgical gonadectomies in birds are difficult, if not impossible, and hormonal injections and implants are expensive, variably effective, and short-acting; therefore, an effective nonsurgical method of suppressing egg production is needed. We are developing a nonsurgical approach to reduce gonadal activity and germ cell production using an antiMullerian hormone receptor 2 antibody (AMHR2)-guided lipid nanocomplex carrying a cytotoxin (saporin) to induce apoptosis in gonadal support cells exhibiting AMH2 receptor. This method disrupted gonadal architecture and germ cell development in rats and pigs. We hypothesized that this unique technology would reduce hens egg production. Rhode Island Red laying hens (n = 12) formed control group (n = 6, 0.5 ml sterile intravenous saline) and treatment group ([n = 6], administered 0.5 ml of sterile intravenous saline containing 100 nmol of nanocomplex). Hens were housed separately, weighed twice weekly and photographed once a week. General health and number of eggs laid were evaluated daily starting 13 days prior to treatment until 28 days after treatment. Blood was collected prior to treatment and at end of study for concentrations of estradiol, progesterone and androstenedione. Hens were euthanized via intravenous pentobarbital and left ovary was collected, measured, weighed, and formalin-fixed for H&E histology. There were no differences between groups in general health, body weight, ovarian weight, or blood hormone concentrations. Histologically, all ovaries had evidence of follicular activity. Most obvious effect was cessation of egg laying in all treated hens starting 3 days postinjection and lasting at least 16 days in 4 of 6 treated hens. All injected hens eventually resumed laying eggs.

Eggs collected		**Preinjection**	**Days 0-2**	**Days 3-18**	**Days 19-28**
		(13 Days)	(3 Days)	(16 Days)	(10 Days)
Controls	Total eggs	59	12	52	31
	Eggs/bird/day	0.76	0.67	0.54	0.52
Nano injected	Total eggs	57	12	10	28
	Eggs/bird/day	0.73	0.67	0.1	0.47

Administration of nanocomplex resulted in short-term cessation of egg laying in all treated hens without impacting overall health of these birds supported the hypothesis. Further studies are needed to determine if a different dose and/or route of administration could increase the length of this cessation, or even lead to a permanent loss of egg production.

Keywords: Gonadal suppression, fertility, avian egg laying, egg-binding, nanocomplex

Sperm-bound antisperm antibodies are associated with poor cryosurvival of stallion sperm

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Semen freezing is a common practice in equine reproduction. However, ~ 30 - 40% stallions' semen does not survive cryopreservation. Reason for poor cryosurvival is not clearly understood. Antisperm antibodies (ASAs) are associated with poor cooling ability of stallion sperm. We hypothesized that presence of sperm-bound ASAs is associated with poor cryosurvival. Objective of this study was to evaluate ASA binding in stallions with semen with good versus poor cryosurvival. Ejaculates from stallions (n = 21) were extended in INRA96 to 40 x 10^6 sperm/ml and shipped overnight to laboratory in a passive cooling device (Equitainer®). After arrival, each ejaculate was divided into 3 aliquots, centrifuged, resuspended in Botucrio® (BC; Botupharma, Scottsdale, AZ), EZ Mixin Cryomax Modified French™ (MFR; ARS, Chino, CA) or EZ Mixin Cryomax Lactose-EDTA™ (LE; ARS) and frozen following manufacturer's instructions. Semen was stored in liquid nitrogen until evaluation. One straw from each aliquot was thawed at 38°C for 30 seconds and was assessed for total and progressive sperm motility (CASA). In addition, acrosomal integrity (FITC-PNA/PI), percentage of apoptotic and necrotic sperm (Annexin V/PI), and percentage of sperm with IgG and IgA binding (antiequine IgG and IgA) were evaluated with flow cytometry. Postthaw motility was considered acceptable if PM ≥ 30%. Semen considered as good cryosurvival if they had acceptable postthaw motility with at least 2 extenders. There was no difference in ASA binding among semen extenders and data were pooled. Semen with good cryosurvival (n = 13) had a lower percentage of IgG (4.1 ± 0.5%) and IgA bound sperm (2.9 ± 0.3%) compared to semen with poor cryosurvival (n = 8; IgG 13.5 ± 2.3%, IgA 11.2 ± 1.7%) ($p < 0.0001$; Student's t-test). None of the semen with good cryosurvival were ASA positive. However, 43.5% of semen with poor cryosurvival were ASA positive ($p < 0.0001$, Chi Square). There was a negative correlation between percentage of IgG and IgA bound sperm, and total motility (IgG: $p = 0.0045$, $R^2 = -0.36$; IgA: $p = 0.0003$, $R^2 = -0.45$) and progressive motility (IgG: $p = 0.0005$, $R^2 = -0.44$; IgA: $p < 0.0001$, $R^2 = -0.49$), and a positive correlation between percentage of IgG bound sperm and percentage of sperm with damaged acrosomes (IgG: $p = 0.049$, $R^2 = -0.36$). In summary, presence of ASAs was associated with poor cryosurvival of stallion sperm.

Keywords: Stallion, semen, antisperm antibodies, cryopreservation, freezing

Effect of energy substrates on cool-stored stallion sperm

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Commercially available equine semen extenders for cooled storage are typically formulated with glucose as an energy substrate. When incubated at 38.5°C, stallion sperm are reported to preferentially utilize oxidative phosphorylation (OXPHOS) substrates (lactate or pyruvate) for energy production. This study was conducted to determine the effect of 3 energy substrates (glucose, lactate, or pyruvate), individually or in combination, on sperm quality after cooled storage for 24 hours. We hypothesized that inclusion of OXPHOS energy substrates in extender would increase sperm quality parameters compared to glucose alone. Three ejaculates from each 1 of 6 stallions (n = 18) were processed by cushioned centrifugation using a milk-based extender containing various energy substrates, or their combinations, and 10% (v/v) homologous seminal plasma. Treatment groups were: no substrate added (Control); 40 mM added glucose (Glu-40); 2 mM pyruvate (Pyr-2); 2 mM lactate (Lac-2); 19.8 mM pyruvate (Pyr-19); 19.8 mM lactate (Lac-19); 40 mM glucose + 2 mM pyruvate + 2 mM lactate (Glu-Pyr-Lac-2); or 40 mM glucose + 19.8 mM lactate + 19.8 mM pyruvate (Glu-Pyr-Lac-19). Extended semen was stored at 2 temperatures, 10 or 20°C, for 24 hours. After storage, semen was analyzed for % total motility (TMOT) using CASA; and % viable/acrosome intact (VAI), and % viable/lipid peroxidation positive (VLPP) using flow cytometry. Data were rank-transformed prior to analysis using General Linear Model procedure. Statistical significance was set at $p < 0.05$. At both storage temperatures, %TMOT was similar ($p > 0.05$) among Glu-40, Glu-Pyr-Lac-2 and Glu-Pyr-Lac-19 (57, 58, 54%), and higher ($p < 0.05$) in these than other treatment groups (31 - 39%; $p < 0.05$). Percent VAI was lower ($p < 0.05$) in Control (68%) and Pyr-2 (69%), than in Glu-40 (73%). Percent VLPP was similar ($p > 0.05$) among Glu-40, Glu-Pyr-Lac-2 and Pyr-19 (22, 24, 22%, respectively), but higher ($p < 0.05$) in these groups as compared to the Control (17%). These results suggest that at reduced storage temperatures, addition of OXPHOS substrates in extender do not enhance sperm motility over that of glucose alone. Only slight differences on %VAI were observed, which might suggest that type of energy substrate does not have a substantial impact on stallion sperm membrane intactness. Higher % VLPP was observed in treatment groups with higher motility values, which may be related to a higher metabolic rate, and resultant production of reactive oxygen species.

Keywords: Stallion, sperm, low temperature, energy substrate, motility, plasma membrane

Effects of recent feeding on canine serum progesterone

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Serum progesterone values are commonly used to estimate luteinizing hormone (LH) surge in the bitch to appropriately time insemination. Known factors affecting serum progesterone values include type of sample tube used, size of dog, age of dog, anticoagulant, interval from blood collection to centrifugation (especially if refrigerated before centrifugation), time of day and stage of estrus. In cattle and humans, serum progesterone values have been reported to decline following a meal. Goal of this study was to determine the influence of a meal on serum progesterone in dogs. All procedures were approved by the OSU Animal Care and Use Committee. Client-owned female dogs (n = 7) of various breeds (body weight: 3.4 - 37 kg, mean: 17.65 kg) and ages (2 - 10 years, average 4.9 years) presented to OSU Lois Bates Acheson Veterinary Teaching Hospital for breeding management were enrolled. Dogs were fasted overnight prior to initial blood sampling between 0800 and 0900. Blood was collected into plain red top tubes with no anticoagulant and submitted to laboratory within 20 minutes after sample collection. Following initial sample, dog was fed a commercial dog food (a/d® Hills Pet Nutrition, Topeka, KS), 10 - 15 ml/kg body weight (average 13.3 ml/kg). All dogs consumed food readily and no adverse effects were observed or reported by owners. A second blood sample was collected 60 - 75 minutes after feeding (average 68.5 minutes). Serum progesterone was determined by a Siemens Immulite® 1000 (Siemens USA, Malvern, PA). Serum progesterone concentrations were lower ($p = 0.0005$) in post prandial sample in all 7 patients. Average reduction was 28.2% (11.3 - 68.4%), with an average decrease of 0.95 ng/ml (0.14 - 1.41 ng/ml). Two samples of special interest had fasting concentrations of 1.96 and 2.26 ng/ml that dropped postprandial to 0.62 and 0.93 ng/ml, respectively. Body weights of those 2 dogs were 5.8 and 14 kg, respectively. Since LH surge occurs when serum progesterone is in 1 - 3 ng/ml range, interpretation of pre and postprandial samples might be different if the dog was not fasted at the initial sample. However, neither of these 2 dogs tested positive for LH test (Witness®LH, Zoetis, Kalamazoo, MI) at first sampling. We recommend that samples for serum progesterone are collected from fasted patients to ensure most consistent results. Cause for decreased serum progesterone concentrations following a meal in dogs has to be determined.

Keywords: Progesterone, feeding, dog, ovulation

Effect of slide type to evaluate motility parameters of frozen-thawed equine sperm using computer aided sperm analysis

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In equine industry, motility assessment is the primary parameter currently used to evaluate sperm quality in an ejaculate. Motility is determined either by visual subjective assessment or objectively via computer aided sperm analysis (CASA) system. Various types of slides and coverslips are used for sperm motility assessment. Two main methods are a fixed coverslip slide (e.g. Leja® slide) or a drop coverslip slide (e.g. Cell-Vu® slide or glass slide). Both these slides can have a fixed chamber depth of 20 microns. Previous studies in our laboratory demonstrated a negative effect on total and progressive motility with fixed coverslip slides compared to drop coverslip slides for analysis of fresh semen using CASA. Objective was to determine if there is a difference in CASA derived motility parameters of frozen-thawed stallion sperm based on slide/chamber type. We evaluated the postthaw motility of 30 ejaculates of frozen semen. Samples were thawed at 37°C for 30 seconds and placed into a prewarmed 5 ml tube. Concentration was determined using a NucleoCounter and samples were diluted to 30 x 10^6/ml in 2 ml standard skim milk semen extender. Samples were incubated for 10 minutes and evaluated using the Ceros II CASA system. Then, 5 µl sample was placed in each chamber on either fixed coverslip slide or drop coverslip slide. To avoid any possible influence of an increased incubation time, the first slide type evaluated alternated with every sample. A minimum of 5 fields were selected for each sample and analysis continued until a minimum of 400 motile sperm were examined. Each field was limited to have a maximum number of 150 objects per screen in order to minimize the impact of collisions. Motility parameters evaluated were total motility (TM), progressive motility (PM), path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity (LIN), and straightness (STR). A sperm was considered progressively motile if it had a STR of $\geq$ 75% and a VAP $\geq$ 50 µm/sec. A Student's paired t-test was used to evaluate the difference in motility values between 2 slides and $p < 0.05$ indicated significance. There was a difference ($p < 0.0001$) for TM and PM between 2 slide types. Both motility values were higher in drop coverslip slides. Average TM for drop coverslip slides was 52% versus fixed coverslip slides was 35%. Average PM for drop coverslip slides was 38% versus fixed coverslip slides was 24%. There was no difference between cell types for VAP, VSL, VCL, STR, LIN, ALH, and BCF. These data agree with previous results in our laboratory obtained with fresh semen. Although drop coverslip slides and fixed coverslip slides utilized 20 micron chamber for motility analysis, these results indicate that slide type has substantial impact on sperm motility and comparison of motility measures between samples using various chamber types is not valid.

Keywords: Equine sperm, motility, CASA, fixed coverslip, drop coverslip

Effect of glycerol concentrations on a new extender for freezing dog semen

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Glycerol is the most commonly used cryoprotectant for frozen semen in several species, because it alters colligative properties of water, decreasing freezing point and increasing sperm survival. Many studies used various concentrations of glycerol for freezing dog semen, with no consensus regarding best glycerol concentration. Aim was to evaluate glycerol concentrations (6, 7, and 8%) in dog freezing extender. Two ejaculates from 12 adult dogs (2 - 5 years) of various breeds were used. Ejaculates were collected by digital manipulation and divided into 3 aliquots, centrifuged at 600 x g for 7 minutes and pellet was resuspended (100 x 10^6 sperm per ml) in an egg yolk-based extender BotuDog® (Botupharma®) with 3 glycerol concentrations: 6, 7, and 8% (G6, G7, and G8, respectively). Samples were packaged in 0.5 ml straws (IMV, France) and kept in a controlled temperature refrigerator (Minitub®) for 60 minutes at 5°C. Straws were placed in a Styrofoam box and kept 3.5 cm above nitrogen level for 20 minutes, and finally were immersed. Straws were thawed at 76°C for 8 seconds. Sperm kinetics were evaluated using the CASA system (Hamilton Thorne Research - IVOS 12, Beverly, MA). Integrity of plasma and acrosomal membranes, and stability of plasma membrane were determined by flow cytometry (BD LSR Fortessa - Becton Dickinson, Mountain View, CA), at 10 and 30 minutes (T10 and T30), after thawing with incubation at 37°C. Data were analyzed using statistical program Graph Pad 6.0 and Kolmogorv-Sminov test was used for normality. ANOVA followed by Tukey test were used and Friedman test followed by Dunns were used for nonparametric data. Total motility was not different ($p > 0.05$) among groups in T10: G6 (52.5 ± 5.3), G7 (58.7 ± 3.7), and G8 (57.3 ± 5.3) and in T30: G6 (35.1 ± 3.9), G7 (41.4 ± 4.1), and G8 (38.2 ± 3.8), neither progressive motility in T10: G6 (39.5 ± 4.9), G7 (45.4 ± 3.8), and G8 (44.1 ± 4.8) and in T30: G6 (26.3 ± 3.7), G7 (32.0 ± 3.8), and G8 (29.7 ± 3.6). Integrity of plasma and acrosomal membrane was higher in G7 (52.6 ± 3.4) compared to G6 (42.4 ± 3.7) at 10 minutes after thawing and after 30 minutes incubation in G7 (32.3 ± 2.8) compared to G6 (26.4 ± 2.7). We concluded that 7% glycerol in the Botudog® freezing extender yielded better results for plasma and acrosomal membrane integrity than 6%, whereas 8% had intermediate results.

Keywords: Cryopreservation, canine, CASA, plasma and acrosomal membrane integrity

Sperm protein reactive with antisperm antibody is immunoexpressed in equine primordial, primary, secondary, and tertiary follicles

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Sperm protein reactive with antisperm antibody (SPRASA) is present in all stages of ovarian follicles in humans, mice, cows, dogs, and cats,[1,2] and is localized to granulosa cells and ooplasm.[1,2] Objective was to determine if equine ovarian follicles expressed SPRASA. We hypothesized that SPRASA immune expression would be present in all stages of equine ovarian follicle, and localize to granulosa cells and ooplasm. Routine immunohistochemistry was performed on formalin-fixed paraffin-embedded ovarian sections (4 μm) from 3 adult grade Quarter Horse mares. Briefly, sections were deparaffinized and rehydrated before antigen retrieval was accomplished by heat activation in a sodium citrate buffer. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide and nonspecific binding was blocked using serum-free protein block (Dako #X0909, Carpinteria, CA). Polyclonal antiSPRASA primary antibody was applied to slides at a 1:200 dilution. Universal negative rabbit antibody (Dako #S3022) was applied to adjacent sections to serve as negative control. Horseradish peroxidase-conjugated antirabbit polymer secondary antibody (Immuno BiosCience, #IH-8064-OSU-15, Mukilteo, WA) was applied undiluted to all slides. Peroxidase activity was detected using Nova Red Kit (Vector Laboratories Inc, #SK4800, Burlingame, CA). Slides were counterstained with hematoxylin, dehydrated, and cover slipped. Digital images were captured at 10 and 40 x magnifications (QImaging #QIC-F-M-12-C). SPRASA expressed in pregranulosa cells of primordial, and in granulosa cells of primary, secondary, and tertiary follicles in equine ovaries. SPRASA was not expressed in equine ooplasm. There was no positive staining in negative controls. In US, wild horse and burro populations have drastically exceeded the carrying capacity of public lands where they are managed. With the exception of surgery (removal of ovaries), current methods for sterilizing wild female horses and burros (e.g. porcine zona pellucida or GnRH immunization) are temporary reversible solutions. Immunization against SPRASA may prove to be a permanent nonsurgical sterilant method because it targets all follicles including primordial follicles.

Keywords: Antisperm antibodies, contraceptive, horse, immunohistochemistry, oocyte, SPACA3

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Comparison of two methods to induce acrosome reaction in stallion sperm

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Acrosome reaction (AR) failure in stallion sperm has been associated with pronounced subfertility. Acrosomal function in stallion sperm is typically assessed by sperm reaction to calcium ionophore, A23187 (CaI). This method is considered "nonphysiological," because sperm viability is greatly reduced. We have recently demonstrated that incubation of stallion sperm under "capacitating-like conditions" (presence of bicarbonate, bovine serum albumin [BSA], and calcium) using a Modified Whitten's medium containing only lactate as an energy source (Lac-MW) induces spontaneous AR in viable equine sperm (VAR). We compared rate of AR and VAR in stallion sperm induced by the CaI and Lac-MW methods. Two ejaculates each from 4 fertile stallions (n = 8) were collected. Sperm in Lac-MW treatment were washed, resuspended in this medium, and incubated at 38.5°C in 5% CO_2 in air. Sperm in CaI treatment were diluted in INRA-96 extender, treated with 10 μM CaI, and incubated at 38.5°C in air. At 1, 2, 4, and 6 hours of incubation, sperm aliquots were stained using FITC-PSA/propidium iodide (CaI), or FITC-PSA/Fixable Live-Dead Red Stain (Lac-MW), and analyzed by flow cytometry for percentages of viable (%V), total AR (%AR), and viable acrosome reacted (%VAR) sperm. FITC-PSA/Fixable Live-Dead Red stain was used in Lac-MW to reduce artifactual decrease in sperm populations due to agglutination after incubation. Previous data from our laboratory indicated agreement between techniques is high (Bias PSA-PI versus Fixable LD: -4.0%, SD: 5.3). Data were rank-transformed before analysis using a General Linear Model procedure. At all time periods, mean %V was higher in Lac-MW than in CaI (e.g. 1 hour: 62 versus 28%; 6 hour: 59 versus 0%; $p < 0.05$). Conversely, at all time periods, mean %AR was higher in CaI than Lac-MW (e.g. 1 hour: 40 versus18%; 6 hour: 95 versus 63%; $p < 0.05$). Mean %V-AR was different in Lac-MW than CaI starting at 2 h (2 versus 0%), and was notably higher at 4 hour (24 versus 0%) and 6 hour (29 versus 0%; $p < 0.05$). Use of Lac-MW appeared to be preferable for induction of AR in stallion sperm if viability is desired, since higher VAR were obtained, and treatment did not reduce sperm viability. This suggested that Lac-MW might create a more "physiological" condition for the AR. Differences in %AR and %VAR observed suggested that these 2 methods induce acrosome reaction via different mechanisms. Use of Lac-MW could be useful to investigate the mechanism associated with AR failure in stallion.

Keywords: Stallion sperm, acrosome reaction, viability, lactate, calcium ionophore

Sperm contact time in uterus and endometrial inflammation in mares bred by transrectally guided deep horn artificial insemination

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Sperm elicit an acute uterine inflammatory reaction that resolves within 24 - 48 hours in normal mares. Delay in uterine clearance results in persistent inflammation, alters uterine environment, and decreases embryo survival, resulting in substantial economic losses. To improve embryo survival, uterus is lavaged within the first 4 - 12 hours after insemination. Substantial reduction in pregnancy rate was observed if lavage is performed prior to 4 hours. Deep (uterine) horn transrectally guided artificial insemination (DHAI) is a technique that places sperm close to utero-tubal junction, ipsilateral to the ovulation side. Advantages of DHAI are reduction in volume and/or number of sperm inseminated, and reduction in sperm transport time, with faster oviductal sperm colonization. Since severity of endometrial inflammation is directly related to number of sperm and duration of uterine-sperm interaction, we hypothesized that uterine lavage prior to conventional 4 hours would have an effect on uterine inflammation. Objectives were to determine degree of inflammation at 1 and 4 hours after insemination in mares bred by DHAI and determine pregnancy in mares lavaged earlier than 4 hours. Four warmblood and 3 Thoroughbred mares with normal reproductive histories were used over 14 estrous cycles in a cross-over experimental design. Mares with obvious uterine edema and a dominant preovulatory follicle were induced to ovulate with 1 mg of Deslorelin IM. Within 0 - 6 hours after ovulation, mares were bred by DHAI with 1 x 0.5 ml straw of frozen-thawed semen from 1 of 2 stallions of proven fertility and containing 120 - 160 x 10^6 total sperm. On the first estrous cycle, mares were randomly assigned for lavage at 1 or 4 hours following DHAI. Mares were rested for a cycle and assigned to opposite group. At lavage, mares were examined for presence and degree of endometrial edema. Presence of uterine fluid and effluent samples were analyzed for presence or absence of PMN's, and PMN numbers. Pregnancy diagnosis was conducted at 12 - 14 days following DHAI. Uterine semen contact time of 4 hours versus 1 hour had a significant effect on degree of uterine edema ($p < 0.046$), presence or absence of PMNs ($p < 0.0009$), and PMN numbers ($p < 0.0006$), but not on amount of accumulated uterine fluid ($p > 0.5$) or pregnancy rate ($p = 0.28$). We concluded that presence of sperm in uterus of normal mare for 1 hour elicited lesser inflammatory reaction compared to 4 hours and pregnancy rate was not affected by early lavage in mares bred by DHAI.

Keywords: Endometritis, deep horn insemination, uterine lavage, pregnancy rate

Inflammatory proteins as novel diagnostic biomarkers for endometritis in mare

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Equine endometritis is a costly, prevalent, and challenging disease for equine practitioners to diagnose, with clinical implications ranging from subfertility to infertility. Currently, gold standard diagnostic test is endometrial biopsy subjected to Kenney-Doig grades; however, time to receive results can be variable. Objective was to test for inflammatory proteins that are previously validated as equine diagnostic biomarkers in uterine low volume lavage (LVL) fluid of mares. We hypothesized that these inflammatory proteins would substantially increase in LVL fluid of mares with documented evidence of endometritis or poor biopsy grade. Light breed mares (n = 29) 3 - 22 years old were used. Each mare had an LVL (250 ml) followed by endometrial biopsy. Endometrial cytology was performed from LVL and biopsy evaluation was graded by a board-certified veterinary pathologist. Mares were either assigned to endometritis group (n = 12), or healthy group (n = 17) based on endometrial cytology (> 1% PMNs), and/or poor endometrial biopsy score (IIB/III). LVL was utilized in a multiplex bead assay (Luminex Corp. Austin, TX) to quantify levels of following biomarkers: IFNγ, IL1β, IL10, IL17, sCD14, TNFα, chemokine (C-C motif) ligand 2 (CCL2), CCL3, CCL5, and CCL11. Data were analyzed by STATA (College Station, TX). Since data were not normally distributed, a Mann-Whitney U test was performed to compare levels of inflammatory markers between healthy and endometritis mares, with significance set at p value < 0.05. Following inflammatory markers were higher in LVL from mares with endometritis compared to healthy mares: IFN-γ (p = 0.0094), IL17 (p = 0.0296), CCL2 (p = 0.0196), and CCL3 (p = 0.0118). These 4 biomarkers are all proinflammatory cytokines, which orchestrate response of various leukocytes to endometrial changes. Further studies with a larger population are warranted; however, we suggest that proinflammatory markers identified may serve as potential diagnostic markers for endometritis in equine uterine fluid samples. Use of LVL samples for detection of inflammatory biomarkers could provide a less invasive and efficient diagnostic test to practitioners during busy breeding season.

Keywords: Biomarkers, endometritis, equine, low volume lavage

Oxytocin-induced secretion of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in mares with prolonged corpus luteum function

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Oxytocin treatment to prolong corpus luteum (CL) function for estrus suppression in mares is replacing use of intrauterine glass ball. Most common oxytocin protocol involves 60 IU of oxytocin treatment given intramuscularly once daily from days 7 - 14 postovulation (day 0). About 70% of treated mares had prolonged CL function lasting for 60 - 90 days. However, a longer duration of CL function would be more favorable. Serial treatment of human chorionic gonadotropin (hCG) during oxytocin-induced prolonged CL function would extend duration of CL function by having a luteotropic effect and/or by inducing ovulation of diestrous follicle(s) resulting in new CL(s) formation that would remain functional for additional 60 - 90 days (i.e. 120 - 180 days of CL function).[1] Although diestrous ovulations were documented during prolonged CL function period, there was no difference between control and hCG-treated mares in duration of CL function. Notably, in some mares, progesterone concentrations decreased abruptly after a diestrous ovulation, suggesting return of endogenous luteolytic mechanism responsible for terminating CL function. Objective was to evaluate the ability of endometrium to secrete prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during oxytocin-induced prolonged CL function. Oxytocin treatment was used to prolong CL function in mares that were subsequently assigned to 1 of 3 groups: 50 - 59 days ($n = 4$), 60 - 69 days ($n = 4$), or 70 - 79 days ($n = 3$); groups designated as 50s, 60s, and 70s, respectively). Day14 mares served as control group ($n = 3$). Endometrial secretion of $PGF_{2\alpha}$ was evaluated by measuring systemic concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) following a single 10 IU intravenous oxytocin bolus (time 0) treatment to mares in all groups. Blood samples were collected at -30, -15, 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 minutes. Area under PGFM curve was higher in 70s group compared to 50s ($p < 0.001$) and 60s groups ($p < 0.02$), with no difference between 70s and control groups. CL function was maintained after oxytocin bolus in all 4 50s mares, 3 of 4 60s mares, and 0 of 3 70s mares. These results provided clear evidence for return of uterine luteolytic mechanism between days 50 - 70 in mares with prolonged CL function.

Keywords: Equine, mare, estrus suppression, oxytocin, corpus luteum, PGFM

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Pharmacodynamics of clomiphene citrate in cyclic mares

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Clomiphene citrate (CC), a selective estrogen receptor modulator (SERM), is an orally administered reproductive drug that improved follicular recruitment and development primarily in human but also in bovine, ovine, and lagomorph species. Experimental data from mare after CC administration are slim; however, a 40 year-old, anecdotal case report describes some success after use of CC in 3 anestrous mares. We hypothesized that administration of CC in mare would result in an increased number of ovarian follicles. Objectives were to quantify AMH, LH, and FSH serum concentrations, to characterize endometrial edema, and changes in follicular dynamics between treated and control mares. Light-breed mares (n = 12) of 6 - 18 years were utilized from mid-July to early August. Mares (n = 6) had oral treatment of CC (500 mg) once daily for 5 consecutive days and control mares (n = 6) had oral placebo. Blood samples for AMH concentrations were collected on days 1, 3, 6, and 9. Blood samples for FSH and LH concentrations were collected prior to first dose of drug and then once daily through treatment period. Transrectal ultrasonography and follicle mapping were performed in both treated and control mares. Statistical analyses were done using two-way repeated measures ANOVA, with $p \leq 0.05$. Administration of CC did not induce changes in follicle numbers ($p > 0.05$). Plasma AMH concentrations did not differ between experimental groups; however, mean concentrations relative to time were different ($p = 0.009$). There were no differences for plasma FSH or LH concentrations, however, daily mean plasma LH varied ($p < 0.001$) with time within experimental groups. Endometrial edema scores were higher ($p = 0.041$) in mares treated with CC than in control mares, after allowing for time effect. In conclusion, daily administration of CC for 5 consecutive days did not affect follicle numbers or elicit substantial endocrine response. In humans, efficacy of CC treatment in correcting ovulatory dysfunction derives from its blocking effect on hypothalamic estrogen receptors. That, in turn, causes a significant release of FSH and LH. In contrast, in the present study, CC treatment had substantial estrogenic effects in uterus by inducing endometrial edema. Thus, it remains to be elucidated whether CC in mare has ability to modulate FSH and LH secretion. Pharmacokinetics analyses of blood samples are pending, and could provide a basis for revised protocols for CC administration.

Keywords: Equine, LH, FSH, AMH, estrogen, follicle dynamics

Effect of mycobacterium cell wall fraction on histological, immunological, and clinical parameters of equine uterine involution

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Maintaining yearly foal production is important for economic success of broodmares and requires breeding to occur as quickly postpartum as possible. First estrus occurs within 5 - 20 days postpartum, during uterine involution uterus (repair from tissue alterations of pregnancy and parturition). Attempts to hasten uterine involution have had minimal success. Mycobacterium cell wall fraction (MCWF), an immunomodulator, reduced bacterial growth and altered aspects of immune response to breeding; however, it is unknown if MCWF hastened uterine involution. Objectives were to: 1) investigate effect of MCWF on tissue remodeling; and 2) assess effect of MCWF on cell-mediated immunity of uterus. We hypothesized that MCWF treatment in postpartum would hasten uterine involution. Pregnant mixed breed mares (n = 10) were evaluated postpartum. Control mares (n = 4) received 1.5 ml LRS intravenously on day 1 postpartum and again 7 days later and experimental mares (n = 6) received 1.5 ml Settle® (MCWF) intravenously on day 1 and again 7 days later. All mares were assessed every 3 days for clinical, immunological, and histological parameters until day 15 postpartum. Clinical parameters were assessed via transrectal ultrasonography and included assessment of ovarian activity, uterine fluid retention, and uterine wall diameter, in addition to obtaining endometrial culture. Immunological parameters included endometrial biopsies for qPCR for expression of various cytokines (IL1β, IL1RN, IL4, IL6, IL8, IL10, TNF, IFNγ, and GM-CSF) and endometrial cytology. Histological parameters were assessed on formalin-fixed endometrial biopsies and variables included retention of microcctyledons, endometrial glands dilation, and inflammation of mucosa, stratum compactum, and spongiosum. Data were analyzed using SAS 9.4, utilizing a Mixed model for repeated measures, with treatment as a random effect. All posthoc analyses used Tukey's HSD test. Involution was considered complete by day 15 postpartum in all mares, and day postpartum had significant effect on all parameters investigated, indicating involvement of immunological process in uterine involution. Treatment with MCWF decreased severity of bacterial growth, in addition to time to obtain clean culture. MCWF treatment increased expression of proinflammatory cytokines, namely, IL1β, IFNγ, and TNF. Whereas treatment effect was minimal, there was histological evidence for decreased mucosal inflammation in MCWF-treated mares. In conclusion, uterine involution is heavily regulated by immune system. Additionally, MCWF had bactericidal effect on postpartum mare; this may be due to increases in proinflammatory cytokine expression. Further studies are needed to determine whether this immunomodulator will improve first estrous cycle fertility in postpartum mares.

Keywords: Mare, uterine involution, mycobacterium cell wall fraction, postpartum, immunomodulator

Transcriptomic analysis of equine chorioallantois reveals key regulators and pathways involved in ascending placentitis

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Although placentitis is the costliest disease in equine industry, its current standard treatment is not satisfactory and progress is slow in identifying efficient placentitis prevention, diagnostics, and treatment protocols. Therefore, a faster, more holistic view of molecular mechanisms underlying equine placentitis holds potential for development of new diagnostic tools and therapies to forestall placentitis-induced preterm labor. Our hypothesis was that characterizing transcriptome of equine chorioallantois (CA) during placentitis in comparison to pregnancy matched controls would elucidate key regulators and molecular mechanisms triggering equine placentitis. Chorioallantois samples were collected after euthanasia at ~ 290 days of pregnancy in mares with experimentally induced placentitis (placentitits group, n = 6) and uninoculated mares (control group, n = 6). Next generation RNA sequencing was performed with Illumina NovaSeq6000 and reads mapped to EquCab3.0 (STAR-2.5.2b). Differentially expressed genes (DEGs) were identified with Cuffdiff-2.2.1 (FDR < 0.05). Our study identified 2953 DEGs (2028 upregulated and 925 downregulated) in CA during placentitis compared to controls. Pathway analysis of DEGs revealed that these genes were involved in relevant pathways, such as inflammatory signaling (inflammation mediated by chemokine and cytokine signaling pathway, interleukin signaling, toll-like receptor signaling, T cell activation, and B cell activation), angiogenesis-related pathways (vascular endothelial growth factor signaling and angiogenesis pathway), cytostructural integrity (integrin signaling pathway), and apoptosis signaling pathway. Upstream regulator analysis revealed central role of toll-like receptors (TLR2, TLR3, TLR5, and TLR7) in triggering inflammatory signaling after placental infection, and this consecutively resulted in placental inflammation and immune cells chemotaxis. Increased leukocytic infiltration in CA was associated with upregulation of matrix metalloproteinase (MMP1, MMP2, and MMP9) with subsequent extracellular matrix (ECM) degradation and apoptosis, as reflected by upregulation of several apoptosis-related genes such as caspases (CASP3, CASP4, CASP7, and CASP10) that are believed to be implicated in placental separation during disease course. Additionally, activation of TLRs was associated with upregulation of a wide array of transcription factors and TLRs dependent downstream molecules in the inflammatory cascade. This in turn was associated with downregulation of transcripts coding for proteins essential for placental steroidogenesis (e.g. SRD5A1, AKR1C1), angiogenesis (e.g. VEGFA), nutrient transportation (e.g. GLUT12), and upregulation of hypoxia-related genes (e.g. HIF1A) that could explain placental insufficiency during placentitis. First time key regulators and mechanisms underlying placental inflammation, separation, and insufficiency during equine placentitis were characterized. Findings might lead to development of efficacious therapies by targeting key molecules and pathways.

Keywords: Pregnancy, placentitis, chorioallantois, mare, RNA-sequencing

Comparison of glucose and lactate concentrations between healthy equine and mule foals, and of an automated laboratory analyzer and Accutrend® Plus system

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Mules are hybrids resulting from breeding between female horses (*Equus caballus*) and male donkeys (*Equus asinus*). Information about physiological adaptation of newborn mules is scarce in literature. Profiles of blood glucose (GLUC) and lactate (LACT) concentrations are important to evaluate neonates, as they indicate metabolic dysregulations, disease severity, and prognosis. Thus, fast and affordable GLUC and LACT evaluations at farms using portable analyzers would be valuable. Additionally, it is important to know the pattern of variation of these parameters to differentiate healthy and compromised equid neonates. We hypothesized that portable devices measure glucose and lactate concentrations accurately, that these parameters differ between equine and mule foals, and vary over time. Aims of this study were to: 1) compare GLUC and LACT blood concentrations between equine and mule foals during their first 720 hours of life; 2) evaluate LACT and GLUC profiles from birth to 720 hours of life; and c) establish the correlation of LACT and GLUC concentrations using Accutrend® Plus system (ACP) with whole blood and Randox Daytona automated analyzer (AUTO) with plasma in both species. Healthy equine (n = 16) and mule foals (n = 15) were used and blood samples were collected immediately (T0), 1 (T1), 6 (T6), 12 (T12), 24 (T24), 168 (T168), and 720 (T720) hours after birth. GLUC and LACT concentrations were evaluated with an AUTO and with an ACP. Data were analyzed by repeated measures using PROC MIXED and intraclass coefficient correlation (ICC) between 2 analyzers was calculated. Overall, GLUC concentrations evaluated with AUTO were different between species; LACT concentrations were higher ($p < 0.05$) in equine foals when compared to mule foals (2.14; 1.01 - 5.99 and 1.83; 0.61 - 9.04 mmol/l, respectively). There was no difference in GLUC and LACT results evaluated with ACP for equine and mule foals. Pattern of variation of GLUC evaluated with both analyzers for equine and mule foals changed during first 720 hours of life, with an increase in GLUC concentrations soon after nursing and then a decrease to baseline levels. LACT pattern of variation was similar for all foals, with both analyzers detecting higher LACT values at T0 (4.05; 1.91 - 9.04 mmol/l), with a progressive decrease until T720 (1.21; 0.61 - 5.88 mmol/l). The ICC for equine and mule foals between AUTO and ACP for GLUC concentrations was low and was moderate for LACT concentrations (ICC < 0.90). We concluded that GLUC and LACT concentrations changed during first 720 hours after birth in mule and equine foals, with GLUC values differing between species. Additionally, we recommend standardization of the ACP with specific samples before clinical use for GLUC and LACT analysis.

Keywords: Neonate, hybrid, equine, hematological parameters, critical ill, prognosis

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Effect of ejaculation frequency, prostaglandin $F_{2\alpha}$ and cold storage on canine semen yield and postthaw quality

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Number of sperm obtained from a single semen collection can vary widely among dogs, often requiring multiple ejaculates to produce a single breeding dose of cryopreserved sperm. Alternative collection techniques and schedules have been reported to increase sperm yield, however, a direct comparison of these methods is warranted. Objective of this study was to determine most effective collection regimen to increase total number of sperm available for cryopreservation without compromising postthaw motility parameters. We hypothesized that multiple collections and administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) could be used to increase sperm yield without detrimental effects to postthaw semen quality. Sexually mature male dogs (n = 5) were all subjected to following 5 collection protocols in a random order: 1) 1 collection (control); 2) 2 collections 1 hour apart; 3) 2 collections 24 hours apart; 4) 2 collections 48 hours apart; and 5) 1 collection 20 minutes after $PGF_{2\alpha}$ (Dinoprost, 0.05 mg/kg, IM). Total sperm count, total motility (TM) and progressive motility (PM) were assessed for each ejaculate using computer assisted sperm analysis (CASA). Semen was either frozen immediately after collection (Protocols 1 and 5) or stored at 4°C, pooled with a second ejaculate (Protocols 2, 3, and 4), and then frozen using a Tris-egg yolk media. Dogs were allowed 7 days of sexual rest between protocols. Frozen samples were evaluated using CASA and flow cytometry to determine TM, PM, viability (VIA) and acrosome integrity (ACR). Data were analyzed using a general linear mixed model and Tukey's posthoc for pairwise mean comparisons. Significance was set at $p < 0.05$. All collection protocols resulted in a higher sperm yield compared to control. Postthaw semen parameters (presented in percent) were similar between control and Protocols 2 and 5 (TM: 50.4 ± 5.1, 49.4 ± 5.1, 53.8 ± 5.1, PM: 36.8 ± 4.7, 35.4 ± 4.7, 40.2 ± 4.7, VIA: 61.9 ± 3.4, 65.3 ± 3.4, 61.7 ± 3.4, ACR: 23.2 ± 2.7, 18.1± 2.7, 20.4 ± 2.7), respectively. Protocol 4 resulted in poor postthaw semen parameters (TM: 30 ± 5.1, PM: 20.4 ± 4.7, VIA: 49.8 ± 3.4, ACR: 29.8 ± 2.7). Protocol 3 had intermediate results for all semen parameters (TM: 37.2 ± 5.1, PM: 24.4 ± 4.7, VIA: 55.2 ± 3.4, ACR: 26.6 ± 2.7).
In conclusion, 2 collections performed 1 hour apart with 1 dose of $PGF_{2\alpha}$ dramatically increased sperm yield and also maintained postthaw quality. Cooled storage before cryopreservation was increasingly detrimental with increasing time between collections. We provided 2 alternative semen collection protocols to practitioners to maximize number of breeding doses cryopreserved in 1 visit. Further, this would enhance client satisfaction while also minimizing costs and veterinary visits.

Keywords: Canine, spermatozoa, yield, cryopreservation, prostaglandin

Seroprevalence of canine brucellosis in the southeastern United States

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There are four *Brucella* strains of zoonotic importance: smooth strains, i.e. B*rucella abortus* (*B. abortus*), *B. melitensis*, *B. suis*, and a rough strain, i.e. *B. canis*. Dogs can serve as hosts for all four zoonotic strains; however, standard serologic testing in dogs is limited to the identification of only *B. canis* antibodies. Well known to be transmitted venereally, *Brucella* are also readily shed in urine, feces, saliva, milk, and respiratory secretions, and can be easily transmitted through close contact. There is no approved treatment for the elimination of *Brucella* from infected animals. Human infection is a nationally notifiable condition, as treatment is not always effective in clearing the organism, resulting in recurrent infection. Therefore, preventing spread of disease is paramount for public health. The goal of this pilot study was to estimate the number of dogs with circulating antibodies to any of the four zoonotic strains of *Brucella* by sampling multiple subpopulations of hog hunting dogs, dogs presenting for routine spay and neuter, and imported animals throughout the southeastern United States. We hypothesized that the seroprevalence of smooth strains of brucellosis would be higher in imported and hog-hunting dog populations than dogs presenting for routine spay and neuter, and that the seroprevalence of the rough strain of brucellosis (*B. canis*) would be similar across all groups. To date, serum has been harvested from 65 client owned dogs (30 hog hunting and 35 companion dogs) and 19 shelter owned dogs of various ages, parities, and breeds. Reproductive tissues, semen and/or vaginal swabs were collected when available. Brucellosis serology was performed using the Canine *Brucella* Slide Agglutination test (SAT), *Brucella canis* Agar Gel Immunodiffusion II test (AGID), *Brucella abortus* Card Agglutination test (CAG), and *Brucella abortus* Fluorescence Polarization Assay (FPA). To date, among 84 samples collected and assayed, one shelter dog tested positive for a rough *Brucella* strain via AGID. An imported dog tested positive for both a rough and a smooth strain of brucellosis via SAT, AGID and FPA, and two client owned dogs tested positive for a smooth *Brucella* strain by a National Veterinary Services accredited laboratory via *B. abortus* plate and card agglutination. Three attempts at culturing the organism from reproductive tissue samples of seropositive animals yielded contaminant growth only. All other samples (n = 80) were seronegative. Although this project is still ongoing, at this time, 4 of 84 samples (5%) tested positive for at least one strain of *Brucella*, with 3 of 4 positive samples (75%) testing positive for a smooth *Brucella* strain. All hog-hunting dogs sampled were seronegative. Our preliminary results support an overall low seroprevalence rate for brucellosis, with most samples testing positive for a smooth *Brucella* strain, thus, supporting the need for a commercially available, validated test for the smooth strains in canines. To the authors' knowledge, this is the first study evaluating the seroprevalence of the smooth strains of brucellosis in dogs in the US.

Keywords: Canine brucellosis, rough strains, smooth strains, seroprevalence

Effect of polyacrylamide hydrogel injection into reproductive tract of mares

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Urogenital problems are common in older, multiparous broodmares. Vesicovaginal reflux (VVR) or "urine pooling" is a condition where urine refluxes from urethra and accumulates within cranial vagina. Currently, treatment for this condition involves medical management and surgery. Caudal relocation of transverse fold or extension of urethra are surgical procedures utilized to treat VVR. Currently, injectable therapies designed to bulk tissue surrounding transverse fold have not been utilized to treat VVR. Use of a polyacrylamide hydrogel to alter vestibulovaginal fold anatomy may be an adjunctive treatment option for VVR in mares. Polyacrylamide hydrogel is a nontoxic and nonimmunogenic biocompatible, nonabsorbable polymer gel that consists of 2.5% cross linked polyacrylamide and 97.5% sterile water.[1] This compound has been utilized safely for soft tissue augmentation and urinary incontinence in women for several years.[2] Objective was to evaluate Synamid® (Contura International A/S, Sydmarken 23, 2860 Soeborg, Denmark) injection safety into vestibulovaginal mucosa of normal mares. Ten mares (aged 7 - 15 years) were utilized. Prior to procedure, physical examinations and thorough reproductive evaluations were performed. Subjects were administered 3 ml of saline and 3 ml of Synamind® in separate sites, randomly assigned to 1 cm to either right or left of midline (urethra) in transverse fold. Mares underwent daily physical examination, transrectal palpation and ultrasonography, vaginal speculum examination, and digital examination of vagina for first 7 days, then at days 15 and 30. Daily physical examinations revealed no systemic abnormalities in all mares treated. Hematoma formation at injection site occurred in 7/10 mares, which was observed starting at 24 hours postinjection. Hematomas persisted for an average of 3 days. No pain was elicited on palpation of vestibulovaginal fold in any mares. A palpable bulge was present on Synamid® injection site for 30 days in 9/10 mares. In conclusion, no severe complications were observed with injection of Synamid® into transverse fold in mares. There is a potential for this long lasting product utilization in "urine pooling" mares to augment vestibulovaginal anatomy and in mares with issues of vulvar incompetence. Further research is needed to determine this product's efficacy in treating these conditions.

Keywords: Mare, urine pooling, polyacrylamide hydrogel

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Uterine expression of leptin, RhoA and Rho associated kinases in dogs with primary uterine inertia and with obstructive dystocia

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Primary uterine inertia (PUI) is the most frequent dystocia in the bitch, but its etiology is still unclear. Knowledge about underlying functional and molecular changes in the uterus is very scarce. Obesity and high concentrations of leptin (major adipose derived hormone) are associated with complications of labor in women, and obese bitches are at increased risk of dystocia. In pregnant dogs, leptin is also produced by the uterus and placenta, so it may exert a negative paracrine/autocrine effect on uterine contractility during parturition. We hypothesized that high uterine leptin concentrations would adversely affect RhoA/Rho kinase pathway, which is involved in calcium sensitization, leading to decreased uterine contractions in dogs with PUI. Bitches presenting with dystocia (n = 18) were assigned to PUI (n = 11) or obstructive dystocia (OD) group (n = 7). Dogs in OD group were still showing strong contractions and were designated as controls. Dogs that received ecbolic or tocolytic medication were excluded. During cesarean section, a full thickness uterine biopsy was collected at an inter-placental site. Relative gene expression (RGE) of Lep, Lep receptor (LepR), RhoA and its effector kinases (ROCK1, ROCK2) was analyzed by real-time TaqMan qPCR. Immunohistochemistry or *in situ* hybridization was used for protein or mRNA localization, respectively. One-way ANOVA was used to compare uterine RGE between groups with body weight as covariate. Statistical significance was $p < 0.05$. Dogs were not obese (body condition score recorded for 14 dogs 3-6/9). Lep expression did not differ between groups. LepR mRNA levels were below detection limit in 5 PUI dogs and in all OD dogs. RhoA expression did not differ, but uterine ROCK1 and ROCK2 mRNA levels were higher in PUI dogs ($p = 0.010$ and $p = 0.039$, respectively). Lep, RhoA, ROCK1, ROCK2 protein, and LepR mRNA were all localized in myometrium, and staining intensity appeared similar between groups. Protein and mRNA signals were also present in endometrium and blood vessels. In conclusion, despite similar uterine leptin gene expression, undetectable LepR mRNA levels in OD dogs might indicate decreased responsiveness to potentially negative effects of leptin. However, ROCK1 and ROCK2 gene expression was lower in OD than in PUI dogs, which may be a physiological result of long-lasting, strong uterine contractions, and as such, could be an indication of abnormal progression of labor and insufficient uterine contractions in dogs with PUI.

Keywords: Uterine inertia, parturition, uterus, contractility, canine

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Efficacy of a silicone Y design intrauterine device as horse contraceptive in captive breeding

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Due to continued population growth on western rangelands in US, there are now ~ 70,000 feral horses administered to by Bureau for Land Management (BLM) in an area where range ecologists have established 27,000 as desired population size. Therefore, need for an effective contraceptive for feral horses on western range remains a BLM priority. Goal was to find a means of reducing unwanted pregnancies within US feral horse population, while maintaining reversibility so that genetic diversity can be maintained within population. Multiple O Ring designs were deemed unsuccessful due to their inability to resist uterine expulsatory forces in face of immediate and subsequent breeding behavior. We hypothesized that coadministration of long acting progesterone at IUD insertion would increase IUD retention by preventing these immediate aftereffects. Furthermore, discovering that a fundus seeking design in human IUDs ameliorates normal uterine expulsion forces, we formulated an IUD 'Y' design to test for a desired minimum $\geq$ 75% retention rate. This current study compared 50 and 60 durometer (an indicator of material hardness) 'Y' design IUDs, using 2 breeding pods of 10 mares and 1 stallion in each group. In each pod, 5 mares received a 50 durometer IUD and 5 mares received a 60 durometer IUD. Every other mare received an IM injection of saline or long acting P_4. After early data indication that 60 durometer 'Y' shaped IUDs had a higher retention rate, 50 durometer IUDs were replaced with 60 durometer IUDs. Over next 2 years, all mares in both breeding pods were then monitored by transrectal ultrasonographic examination every 2 weeks to determine IUD retention and pregnancy rates for those mares that lost their IUDs. Endometrial biopsies were obtained, and a Kenney Biopsy grade assigned, before and after research period in efforts to assess IUD impact on endometrial health. Five of 20 mares expelled their IUD (75% retention rate) with each of these 5 mares becoming pregnant. Seven of 20 had no change in Kenney biopsy grade,
4 mares worsened by 1 grade, 5 mares worsened by more than one grade, and 4 mares improved by 1 grade. After 4 cycles following removal of IUDs, 12 mares became pregnant. Based on histopathologic and pregnancy results, mares that failed to become pregnant not due to a worsening endometrial grade but possibly due to involvement of other intrinsic factors. Y design IUD met intended parameters and achieved study goals.

Keywords: Intrauterine device, feral horse, Y design, endometrial grade, fertility

Canine brucellosis: serologically diagnosed positive cases

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Canine brucellosis is a devastating disease as it carries zoonotic potential and there are no effective treatments to clear infections in affected animals. Thus, animals that test positive are commonly euthanized. It is thought to primarily affect intact animals and can be readily transmitted through oral or venereal route. In past few years, number of inquiries for advice on managing positive animals has increased sharply. Goal was to evaluate serologically positive *Brucella canis* submissions. We hypothesized that number and proportion of positive cases would vary by year, source of submission, and patient demographics. Results from samples submitted for *B. canis* slide/Agar Gel Immunodiffusion (AGID II) tests to Cornell Animal Health Diagnostic Center from 2014 - 2019 from US were included in this retrospective study. Submissions were classified as either from a referral laboratory or from a veterinarian. Patient demographics (age, breed, and gender), source (referral laboratory or veterinarian, and state/region), and year were evaluated for association with positive serological diagnosis using logistic regression analysis SAS (v9.4) proc logistic. All variables tested were associated with positive serology ($p < 0.0001$) and were retained in final model. Number and proportion of positive cases were: 231 (16.8%) in 2014, 199 (10.3%) in 2015, 232 (11.4%) in 2016, 217 (11.8%) in 2017, 414 (14.8%) in 2018, and 293 (10.0%) in 2019. Referral laboratory submissions (n = 882; 17.4%) had more positive submissions compared to veterinarian submissions (n = 704; 9.0%). Animals between 1 - 6 years of age and mix breeds were more likely to be serologically positive compared to pure breeds. Most (83.0%) submissions were from intact animals, but proportion of positive animals was higher in spayed (33%) versus intact females (9%) and castrated (28%) versus intact males (8%). Number and proportion of serologically positive submissions were significantly different among South (14%), West (12%), Midwest (9%), and Northeast (3%) regions. Many submissions were likely prescreened by card agglutination test; thus, results should be interpreted with this in mind. Years 2018 and 2019 had higher number of positive submissions compared to 2014 - 2017. Submissions from young, mixed-breed animals were more likely to test positive, and intact animals had higher numbers but lower proportions of positive cases. Regional difference was also observed. We recommend testing breeding animals and semen to reduce spread of disease.

Keywords: Canine brucellosis, AGID II test

Use of intravaginal progesterone releasing device and estradiol 17β is equivalent to estradiol 17β and long acting progesterone in synchronizing acyclic embryo surrogate mares

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Long-acting progesterone (LA P_4) is the most common hormone used to synchronize acyclic surrogate mares for embryo transfer (ET). However, mares synchronized with LA P_4 not used for ET for various reasons (e.g. donor yielding a negative flush or nonviable embryo) do not respond to a resynchronization in a short time, likely due to residual LA P_4 in plasma. This results in economic losses to ET programs due to days that the surrogate mare is not being utilized. Intravaginal progesterone releasing devices (IPRD) for cattle have been used in transitional mares to hasten cyclicity. However, IPRD has not been tested to timely synchronize ET surrogate mares. This study aimed to assess serum progesterone (P_4) concentrations, pregnancy rates after ET and pregnancy losses of embryo surrogate mares synchronized with IPRD. In Experiment 1, crossbred acyclic mares (n = 12) received estradiol 17β ([17 βeta, Botupharma, Brazil] 10, 20 and 10 mg, respectively, on 3 consecutive days. At 24 hours after the last estradiol injection, an IPRD (Sincrogest, Ouro Fino, Brazil) containing 1 g of natural P_4, was inserted vaginally and kept for 9 days. Blood samples were collected daily for 11 consecutive days and P_4 concentrations assessed using RIA. Experiment 2 was conducted for 2 breeding seasons (2016 - 2018), with crossbred embryo surrogate mares, randomly assigned to 3 groups: 1) cyclic mares (n = 43) having ovulation confirmed after induction (follicle ≥ 35 mm) with histrelin acetate (250 μg, IM, Strelin, Botupharma); 2) acyclic mares (n = 57) treated with estradiol 17β for 3 days (as above) and then given a single dose of LA P_4 (P4-300, 1.5 g Botupharma); or 3) acyclic mares (n = 57) treated with estradiol 17β for 3 days and then given an IPRD (Sincrogest). Day-8 embryos were transferred 4 - 8 days after ovulation or P_4 treatments, using a nonsurgical, transcervical technique. Mares in Group 3 had IPRD removed immediately before ET, and immediately after ET, a new IPRD was inserted. Pregnancy diagnosis was performed at 5 days after ET, and 30 and 60 days later. Once pregnancy was first confirmed, mares in Groups 2 and 3 received weekly injections of LA P_4 (1.5 g) until 120 days of pregnancy. Mares in Group 3 had the device removed 3 days after pregnancy diagnosis. Data on P_4 concentrations were analyzed using ANOVA-RM and Tukey posthoc and data on pregnancy rates and losses were compared by multivariate logistic regression ($p < 0.05$). In Experiment 1, P_4 concentrations significantly increased from IPRD insertion (0.5 ± 0.2 ng/ml) to next day (15.8 ± 3.1 ng/ml), and then maintained satisfactory P_4 concentrations deemed suitable for successful establishment of pregnancy until day 9 (7.7 ± 2.3 ng/ml). After IPRD removal, P_4 concentrations were reduced significantly to baseline (0.78 ± 0.4 and 0.67± 0.3 ng/ml on days 10 and 11, respectively). For Groups 1-3, there was no difference in pregnancy rates (64, 66, and 69%, respectively) or pregnancy losses by 60 days of pregnancy (17, 13, and 10%). In conclusion, IPRD used herein resulted in a rapid increase in P_4 and sharp decline, upon its insertion and removal, respectively. In addition, this device can be used as a compatible alternative to LA P_4 to synchronize acyclic embryo surrogate mares.

Keywords: Equine, recipient mare, embryo transfer, fertility, hormonal therapy

Effect of age on follicle stimulating hormone receptor expression in ovine endometrium

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Follicle stimulating hormone receptors (FSHR) are present predominately in granulosa cells of secondary and preovulatory follicles. In addition, FSHR are present within ovine endometrium.[1] In humans, ovarian FSHR expression decreased with age.[2] Objective of this study was to compare endometrial FSHR expression among ewes < 9 months of age (prepubertal/peripubertal; n = 6), 1.5 - 2 years of age (mature; n = 4), and > 3 years of age (aged; n = 4). We hypothesized that similar to human ovary, ovine endometrial FSHR expression would decrease over time. Intracaruncular endometrial samples were collected from crossbred ewes (n =14) at slaughter. Age of ewe was determined by dental eruption and wear. Ovine ovary served as positive control. Samples were fixed in 10% formalin, embedded in paraffin, and sectioned (5 μm) onto charged slides. Sections were deparaffinized in xylene and rehydrated in graded ethanol series. Heat-induced antigen retrieval was performed with sodium citrate. Endogenous peroxidases were blocked with 3% hydrogen peroxide and nonspecific binding was blocked with a serum-free protein block. Rabbit antihuman FSHR (F-3929, Sigma Aldrich) was applied to tissues at 1:200 dilution and universal rabbit negative agent was applied to an adjacent tissue section to serve as control. Horseradish peroxidase-conjugated polymer antirabbit IgG was used as secondary antibody, followed by treatment with NovaRED™ peroxidase substrate, and counterstained with hematoxylin. Slides were dehydrated in graded ethanol series, passed through 3 xylene solutions, and then coverslipped. Slides were evaluated by a blinded observer (HH) at 400 x magnification with a Leica DM4000B microscope. Intensity of FSHR expression was quantified from no staining (score of 0) to very strong staining (score of 4) for various endometrium compartments from 3 randomly selected fields per ewe.[1] Data on FSHR expression between age groups were analyzed with one-way ANOVA. Significance was defined as $p < 0.05$. Although FSHR was expressed in endometrial luminal epithelium, superficial and deep glandular epithelium, superficial and deep blood vessels, and superficial and deep stroma in both age groups, there were no differences ($p > 0.05$) in expression among age groups. It is not clear what role FSHR plays in ovine endometrium, but it may regulate mucin-1 expression that is involved in early pregnancy establishment.[3]

Keywords: Immunohistochemistry, intracaruncular, sheep, uterus

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Acupuncture reduces milk N-acetyl beta-D-glucosaminidase in dairy cows with mastitis

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Acupuncture (AP) is used by dairy veterinarians as a complementary treatment for various conditions.[1] NAGase is a lysosomal glycosidase released from mammary epithelial cells during mastitis.[2] Objective was to determine if AP reduces mammary inflammation. We hypothesized that dairy cattle with subclinical mastitis treated with an intramammary antibiotic and AP would have lower somatic cell count (SCC) and milk N-acetyl beta-D-glucosaminidase (NAGase) concentrations compared to cattle treated with only an intramammary antibiotic. Lactating dairy cows at Oregon State University Dairy Research Center were selected (cows with SCC score of SCC > 500,000 cells/ml) based on Dairy Herd Improvement Association reports. A California Mastitis Test (CMT) determined the quarter that had high SCC (CMT score > 1). Pretreatment milk samples (pre) were collected for SCC scoring and NAGase concentrations. SCC was measured by DeLaval cell counter (Tumba, Sweden) and NAGase concentrations were determined by an ELISA kit (#MBS090625, MyBioSource, San Diego, CA). Cows were treated with an intramammary infusion of ceftiofur hydrochloride (Spectromast LC®, Zoetis, Parsippany, NJ) and randomly assigned to AP group (n = 7) and nonAP (control) group (n = 7). Both group cows were restrained for 30 minutes in a head catch 4 times, 12 hours apart. Six AP points were used (bladder [BL] 30, BL 30-1, BL 49, kidney [KI] 10, conception vessel [CV] 2, CV 3). Posttreatment milk samples (post) were collected 14 days after presample collection. Pre CMT scores, NAGase concentrations, and SCC were compared to post scores within and between groups. Significance was defined as $p < 0.05$. Both control and AP groups had reduced post CMT scores (pre: 1.41 ± 0.49; post: 0.42 ± 0.66; $p = 0.01$ and pre: 1.21 ± 0.39; post: 0.57 ± 0.79; $p = 0.039$, respectively) but there was no significant change in SCC in either group. In AP group but not in control group, NAGase concentrations were reduced (pre: 19.70 ± 3.65 U/L; post: 16.70 ± 2.05 U/L; $p = 0.04$ and pre: 25.60 ± 12.90 U/L; post: 20.40 ± 6.04 U/L; $p = 0.20$, respectively). Reduction in NAGase provided evidence for AP as a complementary treatment.

Keywords: Complementary treatment, California mastitis test, somatic cell count, subclinical

Acknowledgement

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Dominant follicle removal prior to superovulation

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Superovulation enables cattle producers to reach reproductive, financial, and genetic goals. Although knowledge of follicular development has improved, number of transferable embryos per collection has not been determined, leading to a high degree of unpredictability. Follicle stimulating hormone (FSH) is a major cost of embryo transfer, and treatment must occur coincident with an endogenous FSH surge for effective superovulation and embryo recovery, which has not improved substantially in many years. Following dominant follicle ablation, an FSH surge and associated follicular wave can be predicted and managed, which may lead to more consistent embryo collections and more transferable embryos. Aim of this field trial was to evaluate dominant follicle ablation prior to superovulation with a minimal dose of FSH. Cycling beef cattle, at random stages of estrous cycle, were subjected to transvaginal ultrasound-guided aspiration of all follicles (> 5 mm). Following aspiration, PGF_{2a} (25 mg) was administered and a CIDR was placed. Approximately 48 hours later, Folltropin-V administration began and was given twice daily (am and pm). On third day of FSH administration, PGF_{2a} was administered again and CIDRs were removed that evening. Cattle were inseminated at estrus. One week later, embryos were collected and corpora lutea (CL) were counted using transrectal ultrasonography. Data were analyzed using ANOVA. Neither number of follicles ablated, nor diameter of ablated follicles had any statistically detectable effect on embryo recovery; however, cattle (n = 24) with a CL > 20 mm at ablation, tended ($p = 0.107$) to produce more transferable quality embryos (mean ± SEM; 7.8 ± 1.1) than those with a CL ≤ 20 mm (5.6 ± 0.6). Cattle (n = 35) given ≥ 10 ml of FSH had similar number of total ova (11.3 ± 1.3), transferable embryos (6.2 ± 0.9), and CL (14.2 ± 0.9) compared to cattle (n = 28) given < 10 ml of FSH (12.3 ± 1.1, 6.3 ± 0.6, 15.1 ± 1.0, respectively). Top quartile of embryo donors (n = 15) had a CL diameter = 22.2 ± 5.0 mm, ≈ 8 follicles ablated per ovary, and largest ablated follicle was 11.5 ± 0.6 mm. Donors from this group yielded following numbers: total ova (13.1 ± 1.3), transferable embryos (11.2 ± 1.1), degenerate embryos (1.5 ± 0.4), and unfertilized (0.4 ± 0.2). Total dose of FSH for these donors was 9.9 ± 0.7 ml (i.e. 0.9 ml per transferable embryo). In conclusion, dominant follicle removal prior to superovulation required less exogenous FSH to achieve acceptable embryo recovery. This approach also facilitated acceptable results from consecutive embryo recoveries. These results indicated that ablation of follicles (> 5 mm) in cycling mid-diestrus beef cattle, prior to initiation of superovulation, may yield more consistent embryo production, perhaps due to a more tightly synchronized follicular wave emergence. Further, characterization of dynamics of this follicular wave may facilitate more consistent superovulation results and reduce costs.

Keywords: Bovine, superovulation, follicle, embryo

Effects of zinc on maturation and fertilization of bovine oocytes

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Zinc is an essential trace mineral in many species and is present throughout the body (highest concentrations in eye and prostate gland) having key roles in reproduction. 'Zink spark' (release of zinc) that occurs in oocyte following intracytoplasmic sperm injection or natural encounter with sperm influences embryo quality. In bovine oocyte, zinc is the most abundant transition element, with concentrations fluctuating occurring during maturation and fertilization events. Zinc has an important role in DNA stabilization during fertilization process. Objective was to determine the role of zinc in in vitro maturation and fertilization of bovine oocytes. We hypothesized that dose-dependent zinc supplementation would enhance oocyte maturation, whereas chelation of zinc would inhibit fertilization and early embryonic development. Bovine oocytes were obtained via follicular aspiration of postmortem ovaries harvested from an abattoir. Selected oocytes contained ≥ 3 layers of cumulus cells and a homogenous cytoplasm. Oocytes were separated into 4 maturation treatment groups, supplemented with 0, 5, 10, or 20 μM zinc. Supplementation doses where determined from analysis of zinc concentrations in adult cow plasma and follicular fluid (10.55 and 11.47 μM, respectively) and commercial maturation and fertilization medias (1.07 μM for each). Oocytes were considered mature if they had reached Metaphase II and had expelled their first polar body. Data were analyzed using Chi-Square. Rate of oocyte maturation was not different (78.1 $\pm$ 3.0, 59.5 $\pm$ 4.3, 69.8 $\pm$ 7.7 and 62.3 $\pm$ 3.2%, respectively). Oocytes matured in 0 μM zinc, fertilized with frozen-thawed bull semen, were assigned to 2 groups. A zinc chelated group (2.7 mM of TPEN (tetrakis [2-pyridinylmethyl]-1-2-ethanediamine) in fertilization media) and a control group with no TPEN. Following fertilization, presumptive zygotes were independently cultured for 7 days (no TPEN). Data analyzed using ANOVA. TPEN-treated group had lower ($p < 0.05$) cleavage rate compared to control group (46.1 $\pm$ 2.3 and 75.6 $\pm$ 3.4%, respectively). Embryo development rate was also lower ($p < 0.05$) in TPEN-treated group compared to control group (15.4 $\pm$ 0.03 and 37.8 $\pm$ 0.03%). Average embryo developmental scores were lower ($p < 0.001$) in TPEN-treated group compared to controls (2.2 $\pm$ 0.1 and 3.4 $\pm$ 0.2). Zinc supplementation had minimal effects on in vitro maturation of oocytes; however, removing zinc during in vitro fertilization significantly decreased cleavage rate and embryo development.

Keywords: Bovine, zinc, fertilization, maturation, oocyte

Bull sperm morphology analysis varies greatly by reader

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Sperm morphology assessments are important for selection of a herd sire, with acceptable fertility influencing bovine industry economics. Studies have evaluated effects of reader experience and evaluation method on sperm morphology in other species, but apparently no study evaluated bulls. Semen was collected from 35 yearling bulls at a university bull test station and from each bull, 1 eosin-nigrosin morphology slide (with a monolayer of sperm) was created by a board-certified Theriogenologist. Seven individuals (blinded to bull IDs) assessed slides: 4 board-certified theriogenologists (DACT) and 3 fourth-year veterinary students (VS) who completed an advanced reproductive elective course. One-hundred cells from each slide were evaluated (2018 Society for Theriogenology classification) in oil immersion (100 x) objective utilizing a light microscope in 1 sitting. A second evaluation was completed by each individual $\geq$ 1 week later. Three DACT's performed additional evaluations of 200 and 400 sperm from first 5 slides to determine if assessing a greater number of sperm would increase agreement of morphologic characteristics within and between reviewers. Data were analyzed using separate mixed model analysis of variance (Proc GLIMMIX, SAS v9.4) to test fixed effects of reviewer type, reviewer, slide number or number of sperm read, and interactions. There was an interaction ($p < 0.0001$) of reviewer type and slide (VS versus DACT), indicating some slide ratings were very similar between reviewer types and some differed significantly. Among slides that differed, VS identified larger number ($p = 0.0001$) of morphologically normal sperm compared to DACT. In addition, there were differences ($p < 0.0001$) in coefficient of variation between reviewers, but no differences ($p = 0.78$) between reviewer type. Within DACTs, there were no effects ($p = 0.96$) of numbers read on percent normal sperm, indicating that evaluating additional sperm did not affect outcomes of BSE. There was an interaction ($p < 0.0001$) of number of sperm assessed and reviewer on time to complete and for some reviewers, there were significant increases in time to complete increased sperm assessment. Standard cutoffs for adequate morphology assessment utilized for decades are questionable. Bulls not classified as satisfactory potential breeders are possibly due to fewer number of sperm evaluated and variation between assessors. Further investigation into slide preparation, microscope, and reader experience is important to validate this method of evaluating bovine sperm morphology.

Keywords: Sperm, morphology, bull, assessment

Effects of motility activation and cryopreservation on fish sperm glycocalyx

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Mammalian sperm are coated with a glycocalyx that is formed during sperm development, maturation, and contact with seminal fluid. In contrast, little is known about sperm glycocalyx of fish sperm. Our objective was to describe fish sperm glycocalyx characteristics in different sperm types (testicular, stripped, and cryopreserved), motility activation statuses (inactive versus activated), and determine its role in fertilization using sauger (*Sander canadensis*) as a model. Our hypothesis was that glycocalyx characteristics would differ among sperm types, activation statuses, and be related to fertilization. Stripped, testicular, and cryopreserved sperm were prepared using our laboratory's published methodology for sauger.[1] All 3 sperm types were assessed in both inactive state (in extender ± DMSO) and activated state (1:20 dilution in hatchery water, 35 mOsm/kg). Three fluorescent lectins commonly used in mammalian sperm analysis (Wheat germ agglutinin [WGA], concanvalin A [ConA], and peanut agglutinin [PNA]) were used to evaluate presence and distribution of specific sugar moieties in sperm membrane (N – acetyl glucosamine [GlcNAc], α-mannose, and β-galactose, respectively) using flow cytometry and fluorescent microscopy. Fertilization was assessed in each sperm type and in stripped sperm pretreated with WGA prior to insemination. Linear mixed models were used to compare staining patterns (%) and fluorescent intensity (a.u.) among sperm types and activation statuses ($\alpha = 0.05$, $n = 10 - 12$). Glycocalyx of sauger sperm contained GlcNAc and α-mannose but lacked β-galactose moieties. WGA staining patterns differed among sperm types and with activation. Apical staining of WGA increased ~10 fold in activated sperm, except that frozen sperm had fewest cells with apical staining post-activation (46% testicular, 50 % stripped, and 5% frozen). A majority (> 90%) of sperm stained homogenously with ConA independent of sperm type or activation status but fluorescent intensity was elevated (~ 2 - 3 fold) in frozen sperm compared to other sperm types. Activation did not affect fluorescent intensity of either WGA or ConA. Fertilization was similar for stripped (79.3 ± 4.2%) and testicular sperm (64.0 ± 5.7%); however, fertilization rates were significantly lower in both frozen sperm (17.4 ± 3.7%) and sperm pretreated with WGA (14.0 ± 1.4%). In conclusion, motility activation caused changes in glycocalyx of fish sperm. These changes, particularly in GlcNAc, were largely negated by cryopreservation, which could partially explain observed reduction in fertilization. Moreover, blocking GlcNAc moieties in sperm prior to insemination further demonstrated glycocalyx had a critical role in fertilization.

Keywords: Milt, sperm, cryopreservation, activation, glycocalyx

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Platelet-rich plasma reduces endometrial macrophages in postpartum beef heifers

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Postpartum endometritis in beef heifers can result in infertility and culling. Methods used by beef producers to treat endometritis can result in meat residues. Platelet-rich plasma (PRP) is an effective therapeutic treatment for endometritis in horses.[1] Therefore, we hypothesized that PRP would decrease endometritis in beef heifers. Objective was to compare endometrial responses of intrauterine PRP treatment to responses of platelet-poor plasma (PPP) or saline (SAL, 0.9% sodium chloride [pH = 5.0]) treatment in normal calving heifers. Fifteen normal calving crossbred beef heifers were randomly assigned to 3 intrauterine treatment groups. Heifers were examined at 14 and 28 days postpartum. Each examination included transrectal ultrasonographic measurements of cervical diameter, assessment of cervical discharge score (CDS) using a vaginal speculum, and quantitative aerobic bacterial culture and cytology of endometrial samples. After initial examination, heifers received 10 ml of either PRP, PPP or SAL via intrauterine infusion. PRP was prepared using routine methods previously described.[2] No treatment was administered after second examination. Cervical diameter, CDS, bacterial count, percentage of macrophages (%MAC), and percentage of neutrophils (%PMN) were compared between 2 time points using a Student's *t*-test. Groups were compared between time points using a repeated measures ANOVA, with a Tukey's post hoc test. Significance was defined as $p < 0.05$. Cervical diameter decreased in PRP and PPP groups, but not in SAL. CDS decreased in PRP and SAL groups, but not in PPP group. There was no difference in number of total aerobic bacteria count or %PMN among groups. However, %MAC decreased in PRP group, but not in PPP or SAL groups. PRP reduces in vitro responsiveness of bovine endometrial cells to bacterial endotoxins by downregulating several proinflammatory cytokines (e.g. interleukin-8).[3] Research is needed to determine if PRP downregulates endometrial pro-inflammatory cytokines in vivo.

Keywords: Cervical discharge score, endometrial cytology, endometrial culture, endometritis, neutrophil, transrectal ultrasonography

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Ultrafast cooling reduced oxidative stress in vitrified bovine oocytes

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Cryopreservation of gametes has been a revolutionary tool in assisted reproductive technology field. Traditionally, cryopreservation was performed by slowcooling (0.2 - 10°C/minute) method which induces ice formation in extracellular space and dehydration of intracellular space. Recently, fastcooling or vitrification-based approaches replaced slow cooling in human and equine gamete cryopreservation, with higher cryosurvival rates and superior clinical outcomes. Obstacles for cells to survive fastcooling include intracellular ice formation and exposure to high concentrations of cryoprotectants (CPAs) required to achieve vitrification. Increasing cooling rates should reduce ice formation, and ice formation during warming can be reduced by reducing volume of media around cell. Cryotop system is industry leader, achieving cooling rates of -3,000°C per minute. NANUQ™ Hyperquenching Cryocooler, designed to maximize cooling rate for protein crystallography, combines automated fast (< 2 minutes) plunging with removal of cold gas layer above liquid nitrogen, leading to ultra-fast cooling rates of ~ 600,000°C per minute. We hypothesized that ultrafast cooling rates would reduce oxidative stress from cryodamage of vitrified/warmed bovine oocytes. Bovine oocytes were collected from abattoir-derived ovaries, matured in vitro, partially denuded of cumulus cells, then assigned to 1 of 4 groups: 1) negative control group which is not exposed to CPA and not cryocooled, 2) CPA control group which is exposed to CPA but not cooled, 3) Cryotop group with CPA exposure and cryocooling on Cryotops according to manufacturer protocols, and 4) NANUQ group with CPA exposure and cryocooling on microloops using NANUQ hyperquenching cooler. Oocytes were warmed, completely denuded and cultured for 1 hour in holding medium. Effect of treatment on oocyte oxidative stress was determined by fluorescent signal measured from cells after coincubation with green fluorescent general oxidative stress indicator CM-H2DCFDA. Images were transformed in ImageJ to derive corrected total cell fluorescence (CTCF) values, and these values were compared using ANOVA with Tukey multiple comparison on RStudio. A total of 281 oocytes were included. There were no differences ($p > 0.05$) in CTCF values between negative ($n = 77$) and vehicle control ($n = 93$) groups. CTCF values for cryocooled Cryotop ($n = 65$) and NANUQ ($n = 46$) groups were higher ($p < 0.001$) than control groups. For cryocooled groups, microloops cooled using NANUQ gave a lower ($p < 0.001$) CTCF value compared to Cryotop group. Experiment was repeated and in vitro fertilization was performed, but vitrified groups had poor cleavage rates and no blastocysts were produced, whereas negative and vehicle control groups had similar ($p > 0.05$) acceptable embryo development rates. NANUQ™ reduced postthaw oxidative stress of vitrified bovine oocytes compared to an industry standard vitrification device. Exposure to CPA did not have a significant effect on development.

Keywords: Automation, vitrification, in vitro fertilization, bovine

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Cooled-transported epididymides for donkey semen cryopreservation

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In the event of death or unforeseen castration for medical reasons, epididymal semen harvesting represents the last opportunity to preserve valuable jacks' genetics. Objective was to compare 2 semen cryopreservation methods (centrifugation versus noncentrifugation) on donkey cooled-shipped epididymides. We hypothesized that both methods would result in equivalent postthaw semen parameters. Experiment 1: donkeys (n = 7) housed at Bureau of Land Management in Tucson, AZ were surgically castrated. Testes and epididymides were placed in a plastic bag containing skim milk-based extender (25 ml, Botusemen, Botupharma, Scottsdale, AZ), and cooled-shipped overnight in an Equitainer to University of Illinois for semen cryopreservation. Each pair of epididymides was submitted to retrograde flushing with 5 - 10 ml with a cooling extender Botugold (Botupharma), or a similar volume of Botucrio (Botupharma). Botugold group samples were submitted to cushion-centrifugation (1000 g × 20 minutes), supernatant was discarded, and pellet was resuspended at 100×10^6 sperm/ml in Botucrio. Botucrio group samples were resuspended to a similar final concentration. Semen from both groups was loaded in 0.5 ml straws, cooled at 5°C for 20 minutes, placed 5 cm over LN_2 for 15 minutes, and then plunged in LN_2 for storage. Experiment 2: donkeys (n = 20) housed at Peaceful Valley rescue in San Angelo, TX were surgically castrated and epididymides dissected away from testes and each pair was shipped similar to Experiment 1, except that a different shipping container was used (Botuflex, Botupharma). Each pair was submitted to retrograde flushing with either Botugold or Botucrio and processed exactly as in Experiment 1. For both experiments, 1 straw for each group was thawed at 38°C for 1 minute before sperm motility parameters assessments with CASA. Data were analyzed with R, using a mixed model Tukey's as post hoc. Motility parameters were affected by shipment method ($p < 0.05$) but not by cushion centrifugation ($p > 0.05$) processing (Table). Donkeys had poor postthaw sperm motility compared to what was expected for donkey ejaculates subjected to cryopreservation. This was apparently the first study involving epididymal semen in donkeys.

Table. Sperm motility parameters

		TM (%)	PM (%)	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)
Experiment 1	Centrifugation	32.7 ± 5.2	23.0 ± 4.4	115.9 ± 3.3	45.1 ± 1.8	55.3 ± 0.9
	Noncentrifugation	26.0 ± 4.4	18.5 ± 4.9	109.5 ± 2.3	42.7 ± 2.6	52.7 ± 2.5
Experiment 2	Centrifugation	20.4 ± 2.0	11.0 ± 1.5	87.7 ± 3.3	33.9 ± 1.6	41.9 ± 0.9
	Noncentrifugation	26.0 ± 1.3	10.6 ± 1.3	88.9 ± 3.9	36.3 ± 1.7	44.9 ± 1.9

TM: Total motility; PM: progressive motility; VCL curvilinear velocity; VSL straight-line velocity; VAP: average path velocity.

Keywords: Epididymis, storage time, testis

Intrauterine infusion of platelet-rich or platelet-poor plasma to modulate persistent breeding induced endometritis in embryo donor mares

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Goal was to assess postbreeding endometrial inflammatory response and embryo recovery rates in mares susceptible to persistent breeding induced endometritis (PBIE) treated with platelet-rich plasma (PRP) and platelet-poor plasma (PPP). We hypothesized that administration of PRP and PPP mitigate endometrial inflammation in mares susceptible to PBIE and platelets have an additive ability to modulate this response. Mares (n = 12) that had 3 cycles were classified as susceptible to PBIE, based on a challenge with killed sperm and randomly assigned in a crossover design. Mares received intrauterine infusions of 40 ml of lactate Ringer's solution (LRS, control), or autologous PRP or PPP at 48 and 24 hours before and 6 and 24 hours postbreeding. PRP and PPP were prepared from blood, using a standard double centrifugation method. Platelet count and viability were assessed with spectral flow cytometry (CD41/61 coupled with IgG-PE conjugated). Ovulation was induced (≥ 35 mm follicle) with a gonadotropin releasing hormone agonist (histrelin) and mares were bred on subsequent day with fresh semen collected from a single stallion. At 6 hour postbreeding, uterine lavage (2 liters LRS) was performed immediately before infusion with treatment. Mares had daily measurements (height and width) of intrauterine fluid accumulation (IUF) at uterine body for 96 hours. Endometrial cytology was performed until 72 hours postovulation, and number of polymorphonuclear cells (# PMNs) was counted. Embryo flushing was performed 8 days postovulation with LRS. Recovered uterine fluid was aerobically cultured. Plasma was collected on the day of ovulation induction, 72 hours and 8 days postovulation to assess P_4 concentrations. Statistical analyses were performed with ANOVA-RM and posthoc Tukey's (IUF, # PMNs, P_4 concentrations), whereas fertility and number of positive bacterial cultures were assessed with multivariate regression. Mean platelet concentrations were 608.7 ± 62 and 47.5 ± 12 × $10^3/\mu l$ in PRP and PPP, respectively. In addition, there were no differences in platelet-viability between groups (97 ± 0.7 versus 97.2 ± 0.6%). Both treatment groups significantly reduced the # PMNs on endometrial cytology from 24 - 72 hours postbreeding. Infusion of PRP and PPP resulted in a significant reduction in IUF postbreeding when compared to control. Estrous cycles assigned as control resulted in a higher percentage ($p < 0.05$) of positive cultures (42%), compared to PRP cycles (0%), whereas cycles treated with PPP were not different ($p > 0.05$; 17%) from other groups. P_4 concentrations substantially increased in both treatment groups on day 8 postovulation. Mares treated with PRP tended to have higher ($p = 0.08$) embryo recovery rates (83%) than mares in control group (42%), whereas PPP had intermediate embryo recovery (60%). In conclusion, plasma infusion can be used as an alternate method to modulate inflammatory response in mares susceptible to PBIE and PRP apparently had additional antimicrobial properties compared to plasma PPP.

Keywords: Embryo transfer, endometritis, reproduction, inflammation

Effect of pyruvate on lactate-induced spontaneous acrosome reaction in stallion sperm

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Stallion sperm metabolism is thought to be largely dependent on oxidative phosphorylation. Our recent study suggested that under "capacitating-like conditions" (presence of calcium, bovine serum albumin (BSA), and bicarbonate) only samples with pyruvate (as an energy substrate) had higher motility of stallion sperm. Presence of lactate alone was associated with substantial increases in spontaneous acrosome reaction rate in viable sperm, to almost half of all viable sperm at 4 hour incubation. However, motility was reduced in lactate treatment at 4 hours. We determined whether addition of pyruvate to a lactate-only-containing medium may increase stallion sperm motility while maintaining acrosome reaction rate in viable sperm. Fresh stallion ejaculates (n = 9) were washed and diluted to 30 x 10^6 sperm/ml using a Modified Whitten's medium (MW) containing 7 mg/ml BSA and 10 mM lactate (Control), or 10 mM lactate with 0.5, 1, 5, or 10 mM added pyruvate. To control the effect of added substrate treatments, additional lactate was added at similar concentrations. Diluted sperm were incubated under a 5% CO_2 atmosphere for 4 hours. After incubation, samples were analyzed for total and progressive motility, %TMOT, and PMOT by CASA. Samples were stained with a fixable live/dead stain, followed by FITC-PSA and assessed for viability and acrosome status by flow cytometry. Data were rank-transformed prior to analysis by General Linear Model. The % TMOT was higher ($p < 0.05$) in 5 and 10 mM pyruvate (47 - 50%) than in lactate-only media (30 - 37%). Similarly, % PMOT was higher ($p < 0.05$) in 5 and 10 mM pyruvate (16%) than in lactate-only media (10 - 11 %,). The % viable (55 - 57%) was not affected ($p > 0.05$) by any treatment tested. Addition of pyruvate was associated with a dose-dependent decrease in the proportion of viable sperm that were acrosome-reacted (AR; 42, 36, 31, 18, and 11% for 0, 0.5, 1, 5, or 10 mM added pyruvate), as compared to lactate (42, 40, 42, 39, and 40%, respectively). Results suggested that presence of pyruvate increased motility and decreased proportion of viable sperm undergoing spontaneous AR. Increases in motility and decreases in AR occurred at the same pyruvate concentration (5 mM added pyruvate). Increasing lactate concentration in the medium was not associated with an increase in AR. Findings may help to formulate media for stallion sperm capacitation, and highlighted the delicate energy balance required to support key functions of stallion sperm.

Keywords: Stallion sperm, acrosome reaction, energy substrate, pyruvate, lactate

Streptococcus equi subspecies *zooepidemicus* endometritis in mares: culture, cytology, and antimicrobial susceptibility tests

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Infectious endometritis is a significant cause of subfertility in broodmares, leading to devastating economic and emotional effects on horse owners and breeders each year. Aims of this observational, retrospective study were to report the percentage of positive uterine cultures and specific pathogens, evaluate the relationship between uterine culture and cytology results, and describe the results of antimicrobial susceptibility tests for Streptococcus isolates at an equine reproduction center in Colorado. We hypothesized that the most common bacterial pathogen would be Streptococcus sp. and that all Streptococcus isolates would be sensitive to beta-lactam antibiotics (i.e. penicillin G and ceftiofur), but not all isolates would be sensitive to other antibiotics. A double-guarded uterine swab and brush were used to collect 622 paired samples for culture and cytology, respectively, from mares at Colorado State University between 2017 and 2019. Swabs were plated onto Spectrum™ 4-Part (Colorado) plates (Vetlab Supply, Palmetto Bay, FL), which consisted of MacConkey agar, Tryptic Soy agar with 5% sheep blood, Chromogenic Gram-positive agar, and Chromogenic Gram-negative agar. Antimicrobial susceptibility tests were performed using the Kirby-Bauer disk diffusion method, incorporating amikacin, ceftiofur, ciprofloxacin, enrofloxacin, gentamicin, and penicillin G. Cytology slides were stained with a modified Wright-Giemsa stain (Astral Diagnostics Inc., West Deptford, NJ) and evaluated under 400 and 1000 x microscopy. A total of 513 cultures (82.5%) exhibited no growth of microbial pathogens, whereas 109 cultures (17.5%) had growth of 1 or more pathogens. Most common bacterial pathogens were Streptococcus spp. (73.4% of positive cultures), *Escherichia coli* (34.9%), Klebsiella spp. (5.5%), Pseudomonas spp. (3.7%), Staphylococcus spp. (0.9%), and *Enterococcus faecalis* (0.9%). A total of 20 positive cultures (18.3%) exhibited growth of more than 1 pathogen. A positive cytology was noted on 32.3% of samples collected from mares with a Streptococcus spp. culture and 42.1% of samples associated with an *Escherichia coli* culture. Antimicrobial susceptibility tests were performed on 38 isolates of Streptococcus spp. Percentages of susceptible isolates were as follows: amikacin (29.7%), ceftiofur (100%), ciprofloxacin (75%), enrofloxacin (61.1%), gentamicin (66.7%), and penicillin G (100%). In summary, Streptococcus spp. and *Escherichia coli* were the most common bacterial pathogens isolated. All Streptococcus isolates were susceptible to beta-lactam antibiotics. Use of antimicrobial agents to treat equine uterine infections should be based on antibiotic stewardship principles.

Keywords: Mare, uterine, culture, cytology, endometritis, antibiotics

Pregnancy rates in mares with pre and postovulation versus only postovulation frozen semen inseminations

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Mares may be bred by live cover or artificial insemination with fresh, cooled or frozen semen. Aim of this retrospective study was to compare pregnancy rates in mares after insemination with 2 doses of frozen-thawed semen (pre and postovulation) versus 1 dose (postovulation), in a commercial equine breeding program. Our hypothesis was that pregnancy rate will be similar. Reproductive records from mares bred with frozen semen at Colorado State University over a 3 year period (2017 - 2019) were evaluated retrospectively. Only first breeding cycle of the year was evaluated for any given mare. Mares were monitored by ultrasonography once daily when in estrus and administered 1 dose of 500 μg gonadotropin releasing hormone (GnRH) agonist (histrelin) at 8:00 pm when a dominant follicle of an appropriate size was present along with uterine edema. Mares were subsequently monitored 2 - 4 times daily to predict and subsequently confirm ovulation. Mares were bred by deep uterine horn insemination with either 2 doses of frozen-thawed semen, with 1 dose prior to anticipated ovulation plus a second dose after ovulation was detected, or only 1 dose of semen immediately after ovulation. Selection of the stallion, number of breeding doses allocated per cycle and number of straws per insemination were made by the owner of the mare and/or stallion. A mare was considered pregnant if an embryo was collected during a uterine lavage 8 days after ovulation, or if an embryonic vesicle was visible on 14 days postovulation via transrectal ultrasonography. Pregnancy rate was compared by Chi Square analysis. Records from a total of 156 estrous cycles were evaluated. Percentage of mares that ovulated within specific time frames after administration of histrelin were: ≤ 12 hours (8.3%), 13 - 24 hours (6.4%), 25 - 35 hours (2.6%), 36 - 40 hours (53.8%), 41 - 48 hours (19.2%), and > 48 hours (9.6%). Pregnancy rate of 54 mares inseminated prior to and after ovulation (46%) was not different ($p = 0.143$) from 102 mares inseminated with 1 dose of frozen and thawed semen immediately after ovulation (34%). There was also no difference in pregnancy rates based on mare age (3 - 10, 11 - 15 or 16 - 20 years) or reproductive status (maiden, barren, open or foaling). However, there was a difference ($p = 0.049$) in pregnancy rate between mares bred with semen of low progressive motility ($n = 8$; ≤ 15%) and high progressive motility ($n = 25$; > 60%). In summary, a numerical, but not statistical, difference in pregnancy rate was noted between mares bred with 2 doses of frozen semen (pre and postovulation) versus 1 dose of frozen semen, however, further studies are needed with more number in each group to confirm this finding. It is becoming increasingly common to breed mares with a single or partial dose of frozen semen, with veterinarians having little control over the quality and/or quantity of semen utilized.

Keywords: Equine, frozen semen, pregnancy, dose, motility

Use of serum amyloid A and other inflammatory markers to monitor inflammatory response in mares with periparturient complications

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Early recognition of excessive inflammation and infectious complications related to the peripartum period, leading to early institution of therapy, reduces postpartum discomfort and facilitates recovery. Because serum amyloid A (SAA) is a highly sensitive marker of inflammation, measurements of SAA and other inflammatory markers in postpartum mares may be valuable in assisting clinical assessment of periparturient complications. We hypothesized that mares with peripartum complications substantially altered inflammatory responses compared to normal postpartum mares. Aims were to: 1) determine if inflammatory markers (serum amyloid A (SAA), fibrinogen, white blood cell count (WBC), and iron are affected by normal parturition; and 2) investigate if parturition-related complications affect concentrations of WBC, SAA, fibrinogen, and iron. A retrospective case-control study included 118 postpartum mares, 72 clinically healthy (CH) mares accompanying sick foals and 46 mares with periparturient complications (PC) admitted to the University of Copenhagen Large Animal Teaching Hospital from 2008 - 2017. Periparturient complications were divided into 3 groups: metritis ($n = 9$), dystocia ($n = 13$) and others ($n = 24$). A multivariate linear regression analysis evaluated the effects of health status of mare (CH or PC), time after foaling (Day PP) and individual mare on blood parameters. Independent-samples Student's t test analyzed differences in inflammatory parameters between CH and PC mares at different days after foaling. CH mares had SAA, WBC, and iron concentrations within reference intervals for first week postpartum (PP). Mean fibrinogen concentrations increased above upper reference limit in both CH and PC mares during the first week PP, but PC mares had higher ($p < 0.05$) concentrations compared to CH mares. Health status of mares had substantial influence on concentrations of SAA ($p < 0.0001$), fibrinogen ($p < 0.0001$), and iron ($p = 0.009$), and day PP had an effect ($p = 0.02$) on WBC in both CH and PC mares. Fibrinogen concentrations increased ($p < 0.05$) on days 2, 3, and 7 and SAA concentrations on days 1 - 7 ($p < 0.05$), and WBC and iron concentrations decreased ($p < 0.05$) on days 1 - 3 ($p < 0.05$) in PC mares compared to CH mares. Iron and WBC concentrations were, however, within reference concentrations, for both groups of mares. Mares diagnosed with metritis had lower ($p = 0.008$) iron concentrations compared to mares with other periparturient complications. Inflammatory markers SAA and WBC were not affected by normal parturition and can be used to monitor inflammation and infection in mares with peripartum complications. Healthy postpartum mares had increased fibrinogen concentrations within first 7 days after parturition. Periparturient complications elicited, however, substantial higher fibrinogen concentrations compared to concentrations in normal postpartum mare. A major fibrinogen response, therefore, still indicates periparturient complications in a postpartum mare, and can, together with SAA and WBC, be used to monitor the inflammatory response related to periparturient complications.

Keywords: Acute phase response, serum amyloid A, periparturient complications, postpartum

Clinical effects of prebreeding intrauterine platelet-rich plasma in mare

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Platelet-rich plasma (PRP) is being used with increasing frequency in both human and veterinary medicine. By concentrating platelets, growth factors are recruited to aid in tissue healing and repair. In women, intrauterine PRP treatment improved endometrial receptivity. In mares, a limited number of studies focused on treating subfertile mares with intrauterine autologous blood products. Our aim was to examine the safety and efficacy of intrauterine administration of autologous PRP prepared with a commercial platelet isolation device (Restigen PRP®, [platelet buffy coat concentration device], Owl Manor, Warsaw, IN). We hypothesized that the treatment would be both safe and effective in addressing intrauterine inflammation, based on the frequency of adverse events, intrauterine fluid grade and pregnancy rate following treatment. Notably, we elected to focus on clinical end points rather than histologic or cytologic evaluations, and used frozen-thawed semen as opposed to fresh or cooled transported semen. In this clinical-based crossover study, inclusion criteria were failure to achieve pregnancy after artificial insemination with frozen semen, absence of clinical evidence of infectious endometritis, and normal physical exam parameters. Eighteen mares of various breeds, ages and parities were used. Each mare served as her own control and was bred to the same stallion as the previous cycle. Breeding management was performed in a routine manner. Autologous PRP was prepared by an experienced operator using Restigen PRP kits and uterine body infusion was performed 12 - 48 hours prior to artificial insemination. Postinsemination, intrauterine fluid was graded via transrectal ultrasonography using a predetermined scale based on volume and echogenicity. Pregnancy status was determined via transrectal ultrasonography from 13 - 16 days postovulation or via embryo flush performed 7 - 8 days postovulation. No adverse events were recorded in association with intrauterine infusion of PRP. Data were analyzed using nonparametric tests. There was no difference ($p > 0.05$) in post-breeding intrauterine fluid score between treatment and control groups. Postbreeding intrauterine fluid score improved in 7/18 mares (39%) and was unchanged in 6/18 (33%). There was substantial effect of treatment on pregnancy rate, as 11/18 mares (61%) became pregnant in the treated cycle as opposed to 0/18 (0%) in control cycle. Results indicated that intrauterine infusion of autologous PRP prepared with a commercial platelet isolation device in the periovulatory period was a safe procedure, and associated with improved breeding outcomes in this population of mares. To better determine efficacy, further studies are in progress to differentiate these results from those of second cycle pregnancy rates without PRP use.

Keywords: Mare, platelet-rich plasma, frozen semen, artificial insemination, safety, inflammation

Expression of prostaglandin E_2 and oxytocin receptors in stallion accessory sex glands

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It is unknown whether oxytocin and prostaglandin receptors are present in accessory sex glands. Furthermore, if receptors are present, their importance and distribution is unclear. Knowledge of normal receptor expression in intact and castrated males may help to improve semen collections in difficult stallions and may further our understanding of accessory sex gland physiology. Objectives were to characterize expression of prostaglandin E_2 (EP2, EP4) and oxytocin (OXTR) receptors' genes in equine accessory sex glands using immunohistochemistry (IHC) and to determine if localization varied based on age or reproductive status. We hypothesized that EP2, EP4, and OXTR are more strongly expressed in mature stallion glands than in gelding or fetal glands. At euthanasia, ampulla, prostate, vesicular, and bulbourethral gland tissue were collected from mature stallions (> 5 years old, n = 3), mature geldings (> 5 years old, n = 3), and male fetuses (280 days of pregnancy, n = 3). Fresh tissues were fixed in 10% neutral buffered formalin, then embedded in paraffin until processing. Tissues were sectioned in 5 μm sections and stained with rabbit antihuman polyclonal antibody for EP2 or EP4 (Santa Cruz Biotechnology), or mouse antihuman monoclonal antibody for OXTR. Slides were processed using an IHC Select HRP/DAB kit (Millipore, Burlington, MA). Protein localization of EP2, EP4, and OXTR was evaluated by IHC, and staining was characterized as absent, mild, moderate, or strong. Prostaglandin E_2 and oxytocin receptors' genes were expressed in all glands. EP2 expression was mild to moderate in luminal epithelium of all glands. EP4 was strongly expressed in luminal epithelium of all glands, moderately expressed in smooth muscle of ampulla and prostate, and mildly expressed in submucosa of vesicular gland. Presence of EP2 in lumen should not come as a surprise, since PGE_2/EP2 play an important role in secretory epithelium in other organs (e.g. lungs and stomach). Interestingly, upregulation of EP2/EP4 are associated with tumor development in accessory glands of humans and rodents and other tissues; however, neoplasia in accessory sex glands of stallions is extremely rare. EP2 and EP4 play an important role in contraction and relaxation in the esophagus and it is likely they play a similar role in the stallion accessory sex glands. Moderate expression of OXTR in epithelium and mild expression in glandular stroma, and its receptor presence suggested that oxytocin may help regulate glandular secretions. Presence in equine fetus suggested that these receptors may play a role in development of accessory sex glands. Lack of apparent variation in these receptors in castrated horses suggested a steroid-independent role for receptor expression in glands. In conclusion, expression of EP2, EP4, and OXTR was confirmed in all male equine accessory sex glands, including equine fetus. Relative roles of these receptors in stallion accessory sex glands physiology, during ejaculation, and disease remain to be studied.

Keywords: Prostate, horse, ampulla, seminal vesicle

Fungal endometritis caused embryo loss in a maiden mare

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A 6 year old, maiden, Standardbred mare was inseminated with chilled, extended semen (from same stallion) during 2 consecutive estrous cycles. Following first cycle insemination, traces (< 1 cm diameter) of fluid were occasionally detected in uterine body during routine transrectal ultrasonographic examination. Day after second cycle insemination, transrectal ultrasonographic examination confirmed ovulation and absence of uterine fluid. Thirteen days postovulation, she was reexamined for an early pregnancy diagnosis. Embryonic vesicle (9 mm) was detected in uterine body with no free fluid in uterus. At scheduled midpregnancy diagnosis (28 days postovulation), ~ 10 cm diameter, mainly hypoechogenic, fluid pocket with mixed echogenic contents was detected in uterine body. No uterine edema was detected and cervix was closed. Uterine lumen was initially lavaged with sterile saline, followed by 1 liter of sterile saline with 20 ml of white vinegar. Reflux from saline uterine lavage was turbid and included debris and round, "fluffy" white objects 1 - 3 mm in diameter. Samples from lavage were sent for laboratory identification. A smear was stained with 'Diffquick' and assessed in the examining area. Debris, epithelial and inflammatory cells, stout rod-shaped bacteria, and nonfruiting fungal hyphae were detected. Multiple drug resistant *Enterobacter aerogenes* was identified in culture. A fungus was cultured, but not definitively typed, although it was suspected to be Acremonium spp. Mare was treated with prostaglandin $F_{2\alpha}$ analogue (125 μg) and oxytocin (10 IU) IM twice daily for 2 days, and reexamined 4 days later. Minimal (< 0.5 cm diameter) intrauterine fluid was detected. A clitoral swab was collected for fungal isolation, but was unrewarding. A double-guarded uterine swab was collected prior to next insemination, but culture results were unrewarding. She was inseminated again 26 and 58 days after diagnosis of fungal endometritis. She was diagnosed pregnant 14 days following second insemination. Mare had good perineal conformation with no previous history of intrauterine antibiotics or immunomodulating pharmacological (corticosteroids or NSAID's) agents (considered risk factors for fungal endometritis). Putative fungus, Acremonium spp. is typically considered to be a plant associated symbiont or pathogen. Fungal endometritis is considered most likely reason for embryonic loss in this mare. To the authors' knowledge, there are no reports of fungal endometritis as a likely cause of embryo loss (prior to 40 days) in mare.

Keywords: Mare, fungal endometritis, embryo, embryonic loss

Pyometra associated with hyperammonemia in a mare

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Pyometra is an abnormal accumulation of purulent debris in uterine lumen. This accumulation may be due to cervical adhesions that interfere with drainage of uterine fluids. However, accumulation can also occur without cervical obstruction. In mares, clinical signs of systemic disease are rarely present. Etiology of pyometra in mares remains to be fully determined. A 20 year old multiparous Paint mare was presented for inappetence and lethargy. On physical examination, her mentation was obtunded with a low head carriage, head pressing, and facial grimace. She was underconditioned (BCS 3/9) with a distended abdomen. Her vital parameters were unremarkable except decreased (97.1°F) temperature. On abdominal palpation per rectum, a mass filling entire abdominal space was palpated which was confirmed to be uterus on ultrasonographic examination. Flocculent hyperechoic fluid (31 cm) was visualized. A complete blood cell count showed neutrophilia with toxic left shift, hyperfibrinogenemia and thrombocytosis. Chemistry revealed a metabolic acidosis associated with a high anion gap, mild azotemia, hyperglobulinemia, hypoalbuminemia and moderately elevated ALP, GGT, mildly elevated SDH, hypertriglyceridemia and hyperammonemia (300 µg/dl). Uterine drainage and lavage were performed. Thick, pale brown, and malodorous fluid (101 liters) was recovered from uterus. Uterine fluid sample was submitted for culture, which yielded *Escherichia coli* and anaerobes (*Fusobacterium necrophorum*, Prevotella sp, Bacteroides sp, *Fusobacterium nucleatum*). Mare received antimicrobials for a total of 27 days, including initial gentamicin (6.6 mg/kg IV once daily) and pencillin G procaine (22,000 IU/kg IM twice daily) for 3 days, followed by trimethoprim sulfamethoxazole (30 mg/kg PO twice daily) on Day 4 due to clinical improvement. Metronidazole (20 mg/kg per rectum every 8 hours) was initiated on Day 15. Flunixin meglumine 0.3 mg/kg twice daily was also administered for 3 days, and 5 IU of oxytocin was administered IM every 3 hours for 2 treatments. Uterus was lavaged daily for first 3 days, followed by every other day for remainder of hospitalization. Cervix developed adhesions, endometrium remained thickened, and fluid continued to accumulate between lavages (6 - 8 liters per lavage). A cervical wedge resection performed on day 27 of hospitalization resulted in full thickness vaginal tear. Mare was humanely euthanized. Clinical presentation of pyometra concurrent with signs of systemic disease observed in this mare provided new information regarding physiopathology of pyometra.

Keywords: Uterine infection, hyperammonemia, mare pyometra

Diagnosis of omphalocele in a Toggenburg goat fetus during pregnancy

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Multiple ovulation embryo transfer was performed on a 5 year old Toggenburg donor doe. Washed and trypsin-treated fresh embryos were transferred into 4 primiparous Toggenburg does. Pregnancy was confirmed via transabdominal ultrasonography at day 33 of pregnancy. Does were regularly monitored throughout pregnancy. A doe that received 3 embryos, lost 1 in embryo and 1 fetal (male fetus) stages, respectively, and gave birth to 1 live kid. At day 61, transabdominal ultrasonography of this male fetus identified an umbilical abnormality that was monitored until its death at day 104 of pregnancy. This male fetus was delivered as a mummified fetus with its unaffected, full term twin at day 149 of pregnancy. Omphalocele was diagnosed in this male fetus, based on ultrasonographic findings of a ventral abdominal wall defect with herniation of abdominal viscera into umbilical cord base. At parturition, an abdominal wall defect was apparent, but omphalocele was no longer observable, due to fetal autolysis. Significant postmortem findings included a multivessel umbilical cord, concurrent mummification and maceration of affected fetus, long bone abnormalities, and cleft palate. Infectious causes of caprine abortion were ruled out. This case demonstrates practitioner's ability to diagnose umbilical defects, including omphalocele, using transabdominal ultrasonography during routine pregnancy diagnosis. Transabdominal ultrasonography can identify omphalocele's bilaminar sac presence to distinguish omphalocele from gastroschisis, which has similar appearance. This sac may tear prenatally or during delivery, making perinatal differentiation of these 2 conditions more challenging.

Keywords: Omphalocele, mummification, maceration, pregnancy, umbilicus

Presence of *Tritrichomonas foetus* in a chronically infected bull's urethra

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A 5 year old Maine Anjou Angus cross bull was donated to Auburn University College of Veterinary Medicine. Bull's smegma was collected via preputial scraping with an artificial insemination pipette attached to a 20 ml syringe. Smegma sample was placed into a vial with Modified Diamond's media and submitted to Thompson Bishop Sparks Alabama state diagnostic laboratory (TBSASDL) for testing for *Tritrichomonas foetus (T. foetus)*. Bull was positive for *T. foetus* DNA, based on real time polymerase chain reaction (RTPCR). Bull was tested for *T. foetus* on numerous occasions annually over a period of 5 years and was deemed chronically infected with *T. foetus*. Prior to euthanasia, smegma was collected and placed in Modified Diamond's media. Bull and its smegma sample were submitted to TBSASDL for necropsy and RTPCR, respectively. Reproductive tract was dissected free; 1 x 1 cm sections were cut from prostate, right and left ampullae, right and left vesicular glands, and sections of urethra. The urethra was sectioned at specified locations; 5 cm proximal from distal end of urethra; 23 cm proximal from distal end of urethra; 5 cm distal to last bend of sigmoid; and 13 cm proximal to sigmoid and urethra at ischium level. All samples were placed in Modified Diamond's media and submitted to TBSASDL for culture and testing for *T. foetus* DNA via RTPCR. Reproductive tract was sectioned from most proximal portion to most distal portion. Gloves were changed between each collection of tissue sectioned. Instruments were cleaned following collection of each tissue section to prevent accidental contamination. A new sterile blade was used when sectioning each portion of reproductive tract. Prostate, vesicular glands, ampullae, and proximal urethra were all negative for *T. foetus* DNA. Smegma from preputial scraping and urethral sections 5 cm proximal from distal end of urethra, and 23 cm proximal from distal end of urethra were positive for *T. foetus* DNA via RTPCR with a cycle threshold (CT) of 30, 34.3, and 36, respectively. CT values from 30 - 37 are considered a positive reaction, indicative of moderate amounts of target nucleic acid present in this case. This is first documentation of *T. foetus* in a more proximal location in urethra. A few studies identified *T. foetus* presence in distal urethra of some bulls, but not in every bull. However, those studies were performed prior to availability of PCR; therefore, there might have been some false positives from bulls tested. This case provided important information for researchers assessing clearance of *T. foetus* from infected bulls, as topical treatments alone will not be curative to all bulls.

Keywords: Cattle, trichomonias, *Tritrichomonias foetus,* urethra

Hypertestosteronism in an intact female alpaca secondary to an interstitial cell tumor

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An apparently healthy, 10 year old female alpaca was presented for infertility and male-like behavior. She had failed to conceive after exposure to intact males (multiple observed breeding) since her last parturition. All female alpacas housed with her were determined to be pregnant. She had been regularly mounting other female alpacas for over 1 year. A month prior to presentation, she had elevated serum testosterone concentrations. Abdominal computed topography (CT) identified a soft tissue (attenuating mass like structure with multiple cystic areas) associated with right uterine horn and right ovary, consistent with a neoplastic process. Bilateral ovariectomy was performed; left ovary was 6 cm in length and had 2 firm nodules (0.9 and 0.8 cm in diameter) and cystic areas were identified in right ovary. Both ovaries were submitted for histopathologic evaluation. Pre and postop blood samples were submitted to endocrine laboratory (University of California, Davis, Davis, CA) for inhibin B, progesterone, and testosterone. Preop blood samples had following concentrations; inhibin β_B: 40.1 pg/ml; progesterone: 1.2 ng/ml; and testosterone 1538.3 pg/ml; and postop blood samples had: inhibin β_B: 4.9 pg/ml; progesterone: 0.11 ng/ml; and testosterone: 26.4 pg/ml. Blood samples submitted from a healthy intact female alpaca (to provide a reference range for normal values) had following concentrations; inhibin β_B: 41.7 pg/ml; progesterone: 0.01 ng/ml; and testosterone: 33.7 pg/ml. Preop hormone concentrations confirmed hypertestosteronemia and its ovarian source was confirmed by postoperation hormone concentrations. Lowering of inhibin β_B and progesterone in postop sample was consistent with expected changes that occur following removal of ovaries. Right ovary had multiple cysts lined with attenuated to ciliated cuboidal eosinophilic material and a diagnosis of cystic rete ovarii, whereas left ovary had 2 neoplastic structures composed of packed polygonal cells with indistinct borders and abundant eosinophilic cytoplasm. No mitotic figures observed (10 fields; 400 x magnification) indicating a benign nature of the tumor (interstitial cell tumor). Testosterone produced by left ovary was most likely was responsible for cystic condition of right ovary and cystic rete ovarii. Interstitial or Leydig stromal cell tumors are rare ovarian tumors that belong to sex cord stromal tumors group. These tumors account for < 0.1% of all ovarian tumors in women and are even more rare in camelids. Interstitial cell tumors are characteristically benign and unilateral and produce testosterone. Although interstitial tumors are considered benign neoplastic conditions, they have significant effects on fertility, often ending reproductive life, as in this case. It is important to determine hormone concentrations to diagnose hypertestosteronemiam, followed by histopathology of ovaries in females exhibiting male-like behavior and virilization, to assist in definitive identification of interstitial cell tumors.

Keywords: Camelid, ovary, interstitial cell tumor, Leydig cell, hypertestosteronemia

Hemicastration for a suspected spermatic cord torsion in a dog

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A 4 year old domestic Black Mouth Cur dog, 32.5 kg, was evaluated for infertility. The dog had sired, with natural mating, 3 litters. Approximately 9 months prior, dog was reportedly febrile (40 - 41 ^{0}C) and had a stiff gait. Leptospirosis was suspected, based upon a pet-side antibody detection test. Veterinary care included subcutaneous fluid therapy and 3 week course of doxycycline. Two months prior to presentation, a bitch to which the dog was mated did not achieve pregnancy and upon subsequent recent semen collection, oligospermia and asthenozoospermia were evident. On initial presentation, at physical examination, dog appeared clinically systemically healthy, but scrotal content was abnormal. The left testis was normal in size and texture, whereas the right testis was firm and enlarged. Both cauda epididymides felt slightly firm and both testes apparently had normal orientation. Scrotal skin was normal. Prostate felt normal on transrectal examination and was nonpainful. On transcutaneous ultrasonographic examination, prostate had normal parenchyma, whereas right testis was abnormal, with mixed echogenicity. Blood flow was evident in both spermatic cords. Inhouse serum *Brucella canis* rapid slide agglutination test (RSAT) was positive and 2 mercaptoethanol-RSAT was negative, whereas New York State Diagnostic Laboratory agar gel immunodiffusion II and SAT results were negative. Semen had 120 million sperm with asthenozoospermia (< 10% total motile) and teratozoospermia (24% normal). Hemicastration of the right testis was recommended and performed 6 weeks later. Absence of testicular parenchyma (consistent with tissue necrosis) and structurally normal epididymis devoid of sperm were observed on histopathology. Prior spermatic cord torsion as the etiology of initial illness with subsequent complete testicular degeneration was postulated. Approximately 3 months postsurgery, an ejaculate contained 110 million sperm and showed significant improvement (50% progressively motile and 60% morphologically normal sperm). Determination of precise ovulation timing of bitch was recommended for future breeding to maximize chance of pregnancy. Hemicastration and reexamination at appropriate interval in management of unilateral testicular disease are necessary, as demonstrated by this case.

Keywords: Canine, infertility, testicular degeneration, spermatic cord torsion, hemicastration

Repair of a full thickness uterine tear via iatrogenic uterine prolapse in an anesthetized Thoroughbred broodmare

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A 9 year old multiparous Thoroughbred mare at 347 days of pregnancy was presented to Rhinebeck Equine, LLP for a delay in progression of stage II parturition. Farm foaling attendant reported that the foal was not present in vaginal vault and instead 2 feet and a nose were present within rectum. Mare was transported to clinic immediately. On presentation, there was no evidence of a full thickness rectovaginal tear; however, the foal was displacing rectal mucosa dorsally. Mare was induced under general anesthesia and a live foal was delivered via controlled vaginal delivery. Following recovery of anesthesia, mare was assessed, based on farm history. There was no evidence of a rectovaginal fistula. Transabdominal ultrasonography revealed presence of swirling echogenic free fluid. Abdominocentesis was performed and hemorrhagic, nonclotting fluid obtained. Fluid analysis revealed elevated total nucleated cell count (7.55 x 10^3/µl [reference range 1.5 - 5 x10^3 cells/µl]), elevated abdominal lactate (3.8 mmol/l [reference range 0 - 1.5 mmol/l]), and elevated total protein (2.6 g/dl [reference range < 2 g/dl]). Perineum was aseptically prepared. Digital manual transvaginal examination confirmed an ~ 15 - 20 cm full-thickness uterine tear on dorsal aspect of uterine body, to right of midline. Mare was induced under general anesthesia for uterine laceration correction. A ventral midline celiotomy was attempted, but due to caudodorsal location and size of the defect, surgical repair was not achieved. While in dorsal recumbency, uterus was manually prolapsed through vagina by placing gentle traction on attached fetal membranes. This approach allowed visualization and hand-sewn repair of laceration. The Dutch method (umbilical vessel water infusion) was used to facilitate removal of fetal membranes prior to uterus replacement. Recovery was uneventful. Rebreeding was delayed until the following season. Mare conceived on the second cycle and delivered a live normal colt at day 356 postovulation. To authors' knowledge, iatrogenic uterine prolapse and umbilical vessel water infusion have not been reported. This technique is used by authors with good outcomes in mares with large, miduterine body laceration.

Keywords: Mare, dystocia, uterine tear

Therapy and evaluation of early embryonic loss in a subfertile bitch

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A five year old intact female Cardigan Corgi was presented with a history of infertility and pregnancy loss. Interestrus interval reported was consistent (every 6 - 7 months), except for the last interval, which was reduced to 4½ months. Bitch was inseminated 5 times in 2 years. Surgical insemination using frozen semen was successful, and 2 puppies were whelped. Since then, bitch was inseminated with fresh or cooled semen 4 times (1 vaginal, 1 surgical, and 2 transcervical). It was suspected that pregnancy was either not established or lost in midpregnancy. Last insemination was performed 2 months before presentation. Local veterinarian diagnosed pregnancy via a relaxin assay and abdominal ultrasonography. Due to history of infertility, another abdominal ultrasonography was performed the following week, and early embryonic death was noted. On presentation, bitch appeared healthy and bright with normal physical parameters, and there was no vulvar discharge. Complete blood count and chemistry were within normal limits. Vaginal cytology had scant cellularity; noncornified parabasal cells, scant red blood cells, with few neutrophils and bacteria noted. Serum progesterone concentrations were 1.1 ng/ml. *Brucella canis* serology was negative. Vaginal samples were submitted for Mycoplasma and aerobic cultures. Scant growth was noted on Mycoplasma culture. Ultrasonographic evaluation revealed the presence of 8 mm echogenic fluid in right uterine horn and 2 mm fluid in left uterine horn. Both uterine horns contained thickened foci with a hypoechoic central region and hyperechoic luminal margins, consistent with necrotic placental zones. Uterus had multifocal cystic endometrial hyperplasia with several 4 mm cysts. Ovaries were normal and had luteal tissue. Renal cortical calcifications with normal abdominal content were noted. As bitch appeared clinically and systemically stable, 3 weeks of oral enrofloxacin (12 mg/kg) and Clavamox® (14 mg/kg) were prescribed for possible risk of fulminant pyometra. Two weeks of topical vaginal Misoprostol, 1 µg daily, was prescribed for cervical dilation. Future mibolerone administration was discussed to prolong anestrus, but was declined. Electro-acupuncture was performed several times and bitch was given an oral "lotus formula". One month later, ultrasonographic examination revealed an involuted uterus with no free fluid present and 2 small cysts. Two months later, no free fluid or cysts were present. Bitch will be bred at next estrus to a proven male, with results pending.

Keywords: Canine, infertility, cystic endometrial hyperplasia, early embryonic death

Generation of hormone-responsive organoids from fresh and cryopreserved equine endometrium: comparison between domestic and endangered Przewalski's mares

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Endometrium is responsive to signals associated with reproductive cyclicity and pregnancy. Organoids, 3-dimensional cultured cell structures, mimic in vivo tissue structure and function better than other cell culture methods and may improve understanding of endometrial physiology. However, there are no reports of endometrial organoids generated from equine tissues. Our hypothesis was hormonally-responsive organoids can be generated from fresh and cryopreserved endometrium from domestic (*Equus ferus caballus*) and Przewalski's (*E. f. przewalskii*) mares. Our objective was to compare histology, immunohistochemistry (IHC), transmission electron microscopy (TEM), and gene expression in organoids derived from fresh and frozen-thawed domestic horse and frozen-thawed Przewalski's horse endometrial tissues. Domestic (n = 11) and Przewalski's (n = 3) mare endometrial biopsies were collected, bisected, and either cryopreserved in 10% DMSO or dissociated enzymatically. Endometrial gland fragments were embedded in Matrigel and overlaid with DMEM/F12 (cell culture) plus supplements. Organoids (8 wells for each treatment) were cultured through 2 passages (6 days/passage) and then, for the third passage, incubated with no hormones (Control; C), or supplemented with: 1) 1μM progesterone (P_4) for 6 days; 2) 10 nM estradiol-17$_\beta$ (E_2) for 6 days; 3) E_2 for 2 days then P_4 for 4 days; 4) C for 5 days then 10^{-5}M oxytocin (OT) for 24 hours; or 5) C for 5 days then 10^{-6}M OT for 24 hours. At the end of culture, organoids were analyzed for histology, TEM, IHC, and gene expression. Gene expression was analyzed (R project) and Kruskal-Wallis and Dunn were used as posthocs. Organoids were derived from fresh and frozen-thawed domestic mare and frozen-thawed Przewalski's mare endometrium with histology and TEM revealing cystic structures of epithelial cells with microvilli and intact secretory apparatus. Expression (IHC) of estrogen receptor-α (ERα) and progesterone receptor (PR) was restricted to the nuclei and prostaglandin endoperoxide synthase-2 (PTGS2) to the cytoplasm of endometrial cells. Expression of ERα and oxytocin receptor (OXTR) decreased ($p < 0.05$) while PR and prostaglandin E synthase (PGES) increased ($p < 0.05$) in fresh-derived organoids exposed to certain treatments compared to C. ERα, E-cadherin, and PTGS2 expression decreased ($p < 0.05$) and PGES increased ($p < 0.05$) in cryopreserved-derived organoids exposed to selected treatments compared to corresponding C. Subspecies comparison revealed an increase ($p < 0.05$) in OXTR expression in the Przewalski's horse. This is the first report of equine endometrial organoid generation and long-term culture. This novel method of in vitro equine endometrial culture may lead to development of improved in vitro evaluation of normal reproductive physiology, pathological conditions, and potential therapies for uterine diseases in both domestic and endangered equids.

Keywords: Endometrium, organoid, mare, Przewalski's, in vitro

Semen parameters and fertility of cooled stallion semen extended with sodium caseinate and phosphocaseinate based extenders

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Aim was to compare 2 commercially available equine semen extenders based on phosphocaseinate (INRA 96 IMV, US) and sodium caseinate associated with cholesterol-cyclodextrin (Botugold, Botupharma, US), on sperm parameters and fertility of stallion semen after cooling. We hypothesized that use of a sodium caseinate cholesterol-enriched extender and seminal plasma removal would result in superior parameters in cooled stallion semen. In Experiment 1, 45 ejaculates collected from 9 mature stallions were extended to 50 x 10^6/ml (SP group) with INRA96 or Botugold and processed through cushion centrifugation (CC group) (1000 g x 20 minutes) before resuspension at 100 x 10^6/ml with respective extender. Noncentrifuged and CC samples were placed in 3 (Equitainer, Botuflex, and Equine-Express II) passive cooling devices for 24 or 48 hours. Seminal parameters assessed at 0, 24, and 48 hours, included total motility (TM), progressive motility (PM) with CASA (Spermvision, MOFA, Verona, WI), and membrane integrity and mitochondrial membrane potential with spectral flow cytometry (Zombie Green and Mitotracker Deep Red). In experiment 2, mares (n = 12 x 2 estrous cycles) were bred with 1 billion total sperm from 1 fertile stallion, 40 hours postinduction of ovulation with gonadotrophin releasing hormone agonist, histrelin. Semen was collected, extended in INRA96 and Botugold, and stored for 48 hours in Equitainer, and mares were randomly bred with 1 extender in an alternate order. Mares had transcervical embryo flushing performed on day 8 postovulation. Data were analyzed using mixed models (R project) and Tukey's as posthoc. Addition of cholesterol to sodium caseinate extender, resulted in superior ($p < 0.05$) semen parameters. There were no differences between container type. Cushion centrifugation increased ($p < 0.05$) sperm kinetics parameters and mitochondrial membrane potential and not ($p > 0.05$) plasma membrane integrity (Table). In Experiment 2, embryo recovery rates were identical ($p > 0.05$) between extenders (50%). In conclusion, we inferred that novel extender (Botugold) is a suitable commercially available product that can be used for stallion semen cooling with different containers, with superior sperm parameters than traditional INRA96 and at least with comparable fertility.

Table. Effect of cushion centrifugation on semen quality parameters

Time in hours		Total motility (%)		Progressive motility (%)		Sperm with intact plasma membrane (%)		Sperm with high mitochondrial membrane potential (%)	
		INR96	Botugold	INR96	Botugold	INRA96	Botugold	INRA96	Botugold
24	SP	70 ± 1^{aw}	75 ± 1^{bw}	63 ± 1^{aw}	67 ± 2^{bw}	72 ± 3.7^{a}	76 ± 2.3^{b}	23 ± 3^{w}	28 ± 2^{w}
	CC	74 ± 1^{ax}	76 ± 1.3^{bx}	67 ± 1^{ax}	69 ± 1^{bx}	68.4 ± 2.8	72 ± 2.2	28 ± 2^{x}	34 ± 2^{x}
48	SP	56 ± 2^{aw}	66.2 ± 1.8^{bw}	50 ± 2^{aw}	58 ± 2^{bw}	66 ± 2.2^{a}	69 ± 1.7^{b}	71 ± 3.3^{w}	71 ± 3^{w}
	CC	70 ± 2^{ax}	68.9 ± 1.6^{bx}	63 ± 1.8^{ax}	65 ± 2^{bx}	65 ± 2.3	66 ± 2.0	76 ± 2.7^{w}	76 ± 3^{w}

SP: before centrifugation, CC: after centrifugation

^{w,x}Within a column, means without a common superscript differed ($p < 0.05$)

^{a,b}Within a parameter, means within a row without a common superscript differed ($p < 0.05$)

Keywords: Stallion, semen, extender; cryopreservation, andrology

Sperm parameters after cushion centrifugation of stallion cooled-stored semen

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Clinical practice suggests that some stallions may benefit from semen centrifugation and re-extension after cooled storage and shipping. This study aimed to determine the effects of centrifugation and resuspension on stallion cooled semen. It was hypothesized that an egg-yolk based extender would improve sperm motilities parameters after cushion centrifugation (CC) of cooled-stored semen. Ejaculates (n = 25) from 5 mature stallions were extended to 25 million/ml with a skim milk-based extender (CST, Animal Reproduction Systems, Chino, CA) and stored for 24 or 48 hours in a passive cooling device (Equine-Express II; EE, Exodus Breeder, York, PA) or Equitainer (EQ; Hamilton BioVet, Ipswich, MA). After storage, semen was processed through CC (1000 × g, 20 minutes), supernatant was discarded, and pellet resuspended in 2 ml of INRA 96 (IMV, Maple Grove, MN) or Botucrio (Botupharma, Phoenix, AZ). Noncentrifuged aliquots of cooled stored semen served as controls. Motility parameters were assessed with a computer-assisted sperm analyzer (Spermvision, MOFA, Verona, WI) before and after CC and in control samples that were not processed. Assessment included total motility (TM) and progressive motility (PM). Data were analyzed using a mixed model (R project) accounting for fixed (containers, time, and extenders) and random (stallion and ejaculate) effects. CC did not affect ($p > 0.05$), TM at 24 or 48 hours, whereas PM tended ($p = 0.07$) to decrease after centrifugation and 24 hours of storage (Table). Additionally, after 24 hours, none of the other sperm kinetics parameters were affected ($p > 0.05$) by the type of extender, processing with CC or the type of container. These results could either suggest that: 1) CC cannot be used to enhance sperm kinetics parameters of cooled-stored semen; 2) or it could be possible that apparent benefits of CC cannot be seen on normospermic stallions (as used herein). Thus, it is reasonable to speculate that if stallions with poor semen cooling quality were used, results may be different. In addition, CC has another benefit that was not assessed here; upon arrival, semen needs to be concentrated in a small volume for deep uterine horn insemination. Additional studies are warranted to assess effects of CC on sperm motility parameters on stallions with poor-quality semen cooled semen.

Table. Motility parameters (mean ± SEM) after cushion centrifugation in cooled stallion semen

Time (hours)		Total motility (%)			Progressive motility (%)		
		Control	Inra96	Botucrio	Control	Inra96	Botucrio
24	EQ	64 ± 3^{xa}	64 ± 3^{xa}	70 ± 3^{xa}	54 ± 4^{xb}	52 ± 3^{xb}	58 ± 3^{xb}
48	EQ	54 ± 4^{yb}	61 ± 3^{yb}	64 ± 2^{yb}	45 ± 4^{yb}	50 ± 3^{yb}	53 ± 3^{yb}
24	EE	60 ± 3^{xb}	70 ± 2^{xb}	70 ± 3^{xb}	51 ± 4^{xb}	60 ± 3^{xb}	59 ± 2^{xb}
48	EE	55 ± 4^{yb}	61 ± 3^{yb}	64 ± 3^{yb}	45 ± 4^{yb}	51 ± 4^{yb}	50 ± 4^{yb}

EQ: Equitainer; EE: Equine-Express II

[a,b]Within a row and parameter, means without a common superscript differed ($p < 0.05$)
[x,y]Within a column, means without a common superscript differed ($p < 0.05$)

Keywords: Sperm motility, semen quality, horse, equine, andrology

Evaluating the impact of a systemic treatment protocol on uterine biopsy grade and uterine microbiome in mares with pretreatment Kenney grades of IIB-III

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Various treatment strategies have been utilized to correct endometritis in broodmares. Majority of described treatment protocols involve intrauterine administration of various pharmacologic compounds. One treatment protocol used by one of the co-authors, Dr. James Bailey, in embryo transfer donor mares and mares intended to carry their own pregnancies, involves a regimen of DMSO, antibiotics, and dexamethasone (all systemic treatments) administered at specific times during a course of 8 days. This treatment protocol has been anecdotally reported to produce positive results (successful embryo donors and also carrying their own pregnancies) in mares with uterine biopsy Kenney grades of IIB or III. It was hypothesized that this treatment protocol would: 1) improve Kenney biopsy grades IIB or III; and 2) alter uterine microbiome. Objectives were to evaluate the impact of this treatment protocol on each mare's: 1) endometrial biopsy grade; and 2) uterine microbiome. Mares (n = 13) with previous uterine biopsy grades of IIB-III were included. Eligibility was determined by first collecting an endometrial biopsy sample. Once mares with biopsies that were graded as IIB-III were selected and a pretreatment uterine microbiome sample was obtained using triple-guarded uterine culture swabs. Two swab samples were obtained from each mare. One swab was utilized for 16s-rDNA microbiome analysis. The other swab was submitted for aerobic and anaerobic bacterial culture. Once the pretreatment samples were obtained, the following treatment protocol began: intravenous administration of 250 ml of DMSO mixed with 750 ml of saline on days 1 and 3 (day 1 being the first day of treatment), a daily, oral antibiotic (Equisul-SDT, sulfadiazine-trimethoprime, 24 mg/kg, Aurora Pharmaceutical, Northfield, MN) on day 4 and continuing through day 8, and finally 80 mg of intramuscular dexamethasone on days 6 and 8 (day 8 was the last day of the treatment protocol). Thirty days after the completion of the treatment protocol, posttreatment uterine biopsies and microbiome swab samples were once again obtained. Results are currently pending (laboratory shutdown due to COVID-19), but will be available for presentation at the Society for Theriogenology conference in July. This study will provide objective data to characterize the impact of this treatment protocol on the endometrial grade and the microbiome of treated mares.

Keywords: DMSO, endometrial microbiome, 16s rDNA, embryo recipient, sulfadiazine-trimethoprim

Effect of platelet rich plasma lysate and fibroblast growth factor 2 on sperm motility in stallions

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Semen extenders are continually tested to improve sperm motility, longevity, and, consequently, improve fertility. Growth factors (GFs) modulate cell function, which could be advantageous to sperm by improving motility. In humans and mice, fibroblast growth factor 2 (FGF2) improved sperm motility. Platelet rich plasma (PRP), which is rich in growth factors (GFs) including FGF2, reduced postmating inflammatory response within the uterus when infused 24 hours before or after artificial insemination. Effect of PRP on sperm is not determined. Objective was to evaluate the effect of adding either pooled PRP lysate (PRPL) or recombinant equine FGF2 (reFGF2) at varying concentrations to semen extended in commercial equine semen extender (INRA 96). PRP lysate was used instead of PRP, since it is more purified and highly concentrated in GFs. Eight treatments were tested using concentrations of 1, 2.5, 5, and 10% of PRPL containing 1 IU/ml of heparin, and 0.1, 1, 10, and 100 ng/ml of reFGF2 and compared to control groups with and without 1 IU/ml of heparin. Heparin use was based on a previous titration to prevent gel formation from PRPL reaction with semen extender, precluding motility analysis. Motility parameters were evaluated with samples standardized to 50 million sperm/ml using computer assisted semen analysis (CASA) at hours 0, 0.5, 1, 1.5, 6, and 24 after treatment. For both PRPL and reFGF2 treatments, there were no differences ($p = 0.99$) in motility among groups at any time point, with ranges from 66 to 60% at hour 0 and 46 to 49% at hour 24. Interestingly, results concentrations of PRPL > 5% induced sperm agglutination via head-to-head attachment (HHA), starting at hour 1, whereas PRPL at concentrations below 2.5% did not induce HHA nor affect sperm motility. In addition, HHA was objectively detected by decreases in total number of cells counted per field (total cells) and estimated concentration measured by CASA, assuming that only free sperm were counted, whereas sperm entrapped by HHA not counted. Decreases in total sperm and concentration were different ($p < 0.0001$) for 5 and 10% PRP groups, being more pronounced for 10% PRPL group, suggesting a dose-dependent characteristic of HHA induced by PRPL. One important finding was that PRPL did not kill sperm, besides inducing HHA. Although no motility improvement was observed, present results suggests that direct addition of PRPL in semen extender at doses below 5% could be implemented to reduce postmating uterine inflammatory response, without substantially affecting sperm motility. However, further research on uterine inflammatory response is needed to test this hypothesis.

Keywords: Growth factors, sperm motility, endometritis, head-to-head attachment

Prevalence of and potential impact on fertility of pars pituitary intermedia dysfunction in a Thoroughbred broodmare population in England

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Prevalence of pars pituitary intermedia dysfunction (PPID) has been reported in general equine population as 21.2% in horses and ponies aged ≥ 15 years.[1] Aim was to establish the prevalence of PPID in a Thoroughbred broodmare population using seasonally adjusted cutoff values for basal plasma adrenocorticotrophic hormone (ACTH) concentrations and analyze the potential impact on fertility. Venous blood samples were collected from 79 Thoroughbred broodmares ≥ 15 years old (average 18 years) on first January 2019. Samples were analyzed for plasma ACTH concentrations using Immulite 1000 assay. Mares with plasma ACTH concentrations above the seasonally adjusted reference range for nonautumn months (> 29.7 pg/ml) were allocated into a followup group and underwent repeat sampling in 2019 autumn. Prevalence of PPID in January 2019 was 16.4% (13/79), with mean plasma ACTH concentrations of 38.0 pg/ml (30.2 - 61.4) in affected mares. On repeat sampling, 23% (3/13) mares remained positive (> 47 pg/ml; autumn reference range), whereas 46% (6/13) had a reduction in basal plasma ACTH concentration. Furthermore, 7% (1/13) had small increases in plasma ACTH that was still within normal limits for autumn, whereas 23% (3/13) were lost to followup. Mean pregnancy rate per cycle for 13 mares in the followup group was 52.4%. Five mares were not bred in the 2019 breeding season. Two pregnancies were lost between 15 and 28 days. One mare was lost to followup. Initial prevalence of PPID within this population was comparable to previous studies; however, followup data in autumn had a decrease. Prevalence of PPID in this study population was lower than general equine population, with elevated ACTH concentrations having no impact on fertility. Affected mares had consistent estrous cycles and were able to conceive and maintain pregnancy to 28 days at a pregnancy rate comparable to previous reports for aged mares. Furthermore, these results also highlighted the requirement for repeat testing and need for further investigation into use of single basal plasma ACTH concentrations as a conclusive test for PPID.

Keywords: Equine, PPID, ACTH, fertility, prevalence

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Kisspeptin and RFamide-related peptide 3 neurons in bovine hypothalamus: estrogen receptor α expression and inputs to gonadotrophin releasing hormone neurons

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Fertility in dairy cattle is considered low, most likely due to selection for high milk production resulting in extreme metabolic demands and a period of negative energy status. In most mammals, there appears to be an inverse relationship between metabolic status and reproduction. To address and manage this relationship in dairy cows, a better understanding of brain control of reproduction is required. Final output signal of the brain is gonadotropin releasing hormone (GnRH), but it is evident that integration of internal and external signals, such as feed intake and metabolic status, occurs upstream of the GnRH neurons. The RF-amide neuropeptides, kisspeptin (Kp), and RFamide-related peptide 3 (RFRP-3) may be involved in this integration and relaying this to the GnRH neurons. In cattle, GnRH secretion is stimulated by Kp and inhibited by RFRP-3. We used immunohistochemistry (IHC) to map the distributions of Kp and RFRP-3 neurons in the hypothalamus of dairy cattle, determined their co-expression with estrogen receptor alpha (ERα), and whether they made contact with GnRH neurons. The heads of male and female calves (n = 3 each), steers, heifers, and lactating cows (n = 2/group) were perfusion-fixed (4% paraformaldehyde) and the brains dissected out and frozen. Dual-label IHC for Kp or RFRP-3 with ERα or GnRH was performed on 40 micron thick cryostat sections, using fluorescently labelled secondary antibodies. GnRH neurons were identified in the preoptic area and anterior hypothalamic area. Highest numbers of neurons expressing ERα were in the arcuate nucleus, ventromedial nucleus, preoptic area, and anterior hypothalamic area. Kp neurons were located primarily in arcuate nucleus, with some cells in the preoptic area (although few were observed in calves). Most Kp neurons in adult animals of both sexes, but few in calves, expressed ERα. Very few GnRH neurons, however, received close appositions from Kp neuron fibers. RFRP-3 neurons were localised in the dorsomedial hypothalamus and paraventricular nucleus, with fewer cells observed in cows than heifers and steers, whereas ERα was not expressed in these regions. Fewer than 20% of GnRH neurons received close contact from RFRP-3 neurons. In conclusion, we inferred that feedback actions of estrogen on GnRH secretion may, in part, be relayed via Kp neurons but unlike most other mammalian species studied, this is unlikely to be a direct action. RFRP-3 neurons likely have a small role in the regulation of GnRH neurons in cattle. Further studies are required to clarify the physiological roles of Kp and RFRP-3 in regulation of GnRH secretion in dairy cattle.

Keywords: Kisspeptin, dairy cattle, GnRH, hypothalamus, neuroendocrine signalling

Oviductal insemination by hysteroscopic hydrotubation in mares: a preliminary investigation

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Small numbers of sperm were recovered from the oviduct after natural mating or artificial insemination in mares. One advantage of oviductal insemination is that only small number of sperm may be required to achieve pregnancy in a mare compared to numbers required with standard intrauterine insemination. Objective was to investigate the potential for oviductal insemination, using hysteroscopic hydrotubation[1] with small number of sperm. We hypothesized that pregnancy can be established with a small number of sperm, obtained from frozen semen and infused into the oviduct directly through the uterotubal junction by hysteroscopic hydrotubation. Ten mares were used; 5 mares each were assigned into 2 groups and inseminated when preovulatory follicle size reached 35 - 40 mm. Ovulation was confirmed 48 hours after insemination. Progressively motile sperm were selected using the swim-up technique with frozen semen of known fertile stallions (A and B). One straw of 0.5 ml frozen semen was thawed and mixed with 0.5 ml Quinn's® Sperm Washing Medium and 1 ml of the same medium was floated onto the mixed solution and incubated for 10 minutes at 37°C. Number of sperm that swam up into the supernatant was counted with a Thoma cytometer. Progressively motile sperm concentrations were 1.3 and 1.2 x 10^5/ml in the supernatant of Stallions A and B, respectively. In Group 1 (5 mares) frozen semen of Stallion A was used. From 0.1 to 1.0 ml of the supernatant including sperm was mixed with 1.0 or 2.0 ml of the same medium to adjust the number of sperm (1.3, 2.0, 2.0, 4.1, 4.1, and 9.8 x 10^4) and total volume of medium (1.1, 1.7, 1.7, 1.9, 1.9, and 1.5 ml). Medium, including sperm, was infused into oviduct through uterotubal junction ipsilateral to ovulation site using hysteroscopic hydrotubation. One of 2 mares was pregnant in cases where 2.0 and 4.1 x 10^4 sperm were used, 1 mare was pregnant with 9.8 x 10^4 sperm. In Group 2 (5 mares), 1.9 x 10^4 sperm from Stallion B in 1.7 ml medium was infused into oviducts in 6 cycles of 5 mares by the same procedure used in Group 1. Three of 5 mares (3/6 cycles) in Group 2 were pregnant. In conclusion, oviductal insemination of small number of sperm to achieve pregnancy in mares using hysteroscopic hydrotubation may be a clinically applicable technique.

Keywords: Oviductal insemination, hysteroscopic hydrotubation, mare

Reference

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Effect of mid-diestrus oxytocin treatment on early pregnancy in mare

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Progesterone supplementation in early pregnancy is commonplace in equine reproduction. Recently, there has been some controversy questioning the beneficial effects of routine progesterone administration and rising concerns as an occupational hazard for humans. Although premature luteal regression and resulting low plasma progesterone concentrations is rare in the mare, select cases benefit from luteal support. Luteal function can be extended following oxytocin administration during mid-diestrus. Aim of this field study was to investigate the effects of oxytocin administration on early pregnancy in the mare. Thoroughbred mares (n = 39) were enlisted and bred over 55 estrous cycles. Prior to natural breeding, uterine cultures were obtained and mares were assigned to 1 of 2 groups: Oxytocin treatment (OT); oxytocin 60 units, IM, once daily, days 7 - 14 postovulation; or Control (CT), given no treatment. Mare reproductive cycles were monitored by transrectal palpation and ultrasonography. Ovulation was documented and defined as day 0. Pregnancy diagnosis was performed by transrectal ultrasonography 14 days postovulation. If pregnancy was confirmed, a blood sample was collected and assayed for plasma progesterone concentrations. Mares that did not conceive were rebred and treatment was determined by the stage in the study; therefore, some mares underwent the same treatment more than once. Subsequent pregnancy examinations were performed on days 28 and 45 of pregnancy. First and percycle pregnancy rates for OT and CT were 90.9 and 91.3% and 68.2 and 55.1%, respectively. Median progesterone concentrations for OT were 6.9 and 7.0 ng/ml for CT. There were no differences between OT and CT groups for first ($p = 0.165$) or percycle ($p = 0.289$) pregnancy rates, days 28 and 45 pregnancy rates ($p = 1.0$) or progesterone concentrations ($p = 1.0$). Oxytocin administration during mid-diestrus in early pregnancy did not induce a negative effect. Further investigation in this area may reveal potential beneficial effects of oxytocin administration in early pregnancy.

Keywords: Mare, oxytocin, corpus luteum, progesterone, breeding management

Combination of estradiol cypionate and altrenogest to control ovulation timing in mares

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Controlling ovulation timing in mares is used primarily for convenience when frozen or transported semen is used, or to provide recipients for an embryo transfer program. Luteal or progestational phase termination with prostaglandin $F_{2\alpha}$ or its analogs, results in too variable a time to ovulation to be of practical use (3 - 15 days). Progestogens alone, although capable of inhibiting ovulation, have no control on follicular development. Time from withdrawal of progestogen to ovulation is too variable to be useful in timed insemination with frozen semen or embryo reception. A combination of progesterone and estrogen (P & E) to inhibit both follicle stimulating hormone (FSH) and luteinizing hormone (LH) synthesis and release results in more precise control of follicular development and ovulation. This protocol requires daily intramuscular (IM) injections and use of compounded products. Estradiol-17β is reported to be the best product, because estradiol cypionate (ECP) and estradiol benzoate are too slowly metabolized and cause delayed or erratic return to estrus. Anecdotal reports dispute this claim. Goal of this study was to determine the usefulness of a combination of commercially available products and minimize the number of IM injections to control ovulation in the mare. Procedures were approved by Oregon State University Animal Care and Use Committee. Ten mares of variable age (17 - 23 years, mean 19.7 years) and size (515 - 626 kg, mean 570 kg) were given altrenogest liquid (Regumate®, Merck Animal Health, Madison, NJ) 0.044 mg/kg orally, once daily, for 10 days and estradiol cypionate (Depo Estradiol®, Pfizer, New York, NY) 0.011 mg/kg IM on day 1 and day 5. Study was performed during normal breeding season for the latitude. None of the mares were examined prior to treatment to determine stage of estrous cycle. Mares were evaluated by transrectal ultrasonography of reproductive tract, had serum progesterone concentrations measured, and received cloprostenol (Estrumate®, Merck Animal Health, Madison, NJ) 0.5 µg/kg IM on day 10. Reproductive examinations were performed daily or on alternate days, from days 15 or 16, to detection of ovulation. Nine out of 10 mares had progesterone concentrations < 1 ng/ml at the end of 10 day study periods. Largest follicle at cloprostenol treatment was 38 mm (< 20 - 38 mm) and second largest was 27 mm. Eight mares ovulated 7.25 - 7.625 days after discontinuation of altrenogest. Even a mare with 38 mm follicle did not ovulate until day 17, despite having < 0.2 ng/ml progesterone at cloprostenol treatment. Two mares did not ovulate within 14 days of discontinuation of treatment. It is concluded that ovulation timing can be controlled in mares using commercial compounds readily available to the practicing veterinarian, although fertility of those ovulations remains to be determined.

Keywords: Mare, ovulation, ECP, altrenogest

Prevalence of malignancy in canine mammary masses in a population of shelter dogs

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A retrospective study was carried out on 11 bitches with mammary masses that were surrendered to a municipal shelter in Florida from 2017 to 2019. Ten bitches had reached sexual maturity, and 1 did not have an indicated age. Objective was to determine the prevalence of malignancy of mammary masses in this population. Mammary masses were removed at ovariohysterectomy and submitted to a commercial pathology service for analysis. Ten bitches had solitary mammary masses and 1 had 2 mammary masses. A total of 12 mammary masses were submitted; 11 (92%) had no evidence of malignancy and 1 mass (8%) had low-grade neoplasia. Most common masses identified were adenomas (n = 7). Other masses identified included a parasitic granuloma, follicular cyst, lipoma, and mammary ductal ectasia. One neoplastic mass (grade 1 mammary carcinoma identified) was excised from a 7 year old Chihuahua mix with multiple mammary masses. Three (25%) masses in this patient were not mammary in origin. Chihuahuas and Chihuahua mixes (n = 6) were overrepresented in this study. In this small population, mammary masses were predominantly benign. Further studies are needed with expanded sample size and involving a reproductive pathologist.

Keywords: Mammary, neoplasia, canine, benign, malignant

Intravaginal progesterone releasing device to hasten first ovulation in mares: side effects and pregnancy rates in a commercial Thoroughbred farm

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Many protocols have been evaluated to hasten first ovulation of breeding season in mares, including use of progesterone releasing devices. However, use of these devices in clinical practice is not widespread. In the Thoroughbred industry, there is immense pressure to have mares cycling in the nonphysiologic breeding season. Thoroughbred mares (n = 20) over 2 consecutive breeding seasons in a commercial studfarm underwent treatment with a PRID™ Delta (1.55 g progesterone) inserted intravaginally for 10 days. Mares (5 - 23 years) were not under artificial lights. Mares at time of insertion were deemed to be in early transition via transrectal ultrasonography, having at least 1 follicle of 2.0 cm, no uterine edema, and no previous ovulation noted for that breeding season. On removal of PRID™ Delta, mares had a preovulatory follicle of at least 3.5 cm. On day 11 (1 day after removal), mares had a uterine edema score of at least 1 (on a scale 0 - 3) and were given deslorelin acetate to induce ovulation. All mares (100%) ovulated within 3 days after removal of PRID™ Delta (day 13). Eight mares (40%) cultured positive for endometrial *Streptococcus equi* subspecies *zooepidemicus*. Remaining 12 mares (60%) were inseminated via live cover, resulting in 10 established pregnancies (50%) diagnosed on day 14 by transrectal ultrasonography. No vaginal discharge was observed in any of the mares, although all mares were noted to have some increase in vascularization of the vaginal wall with some hyperemia (n = 20). No other complications were noted in the reproductive tract of any of the mares. Discomfort upon removal of the device, including straining, mild colic-like signs, and weight shifting, were noted in some mares (6/20) all of which were transient in nature, and required no medical interventions. It is concluded that PRID™ Delta use to hasten first ovulation in mare is effective with minimal side effects and results in a fertile ovulation. Use of intravaginal progesterone releasing devices along with other methods to manipulate mare's physiologic breeding season, such as artificial lighting regimes, can hasten time to first ovulation and reduce associated costs.

Keywords: Intravaginal progesterone, breeding management, mare, first ovulation

Individual variation of frozen-thawed sperm from stallions to survive dilution and cooling after thawing

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Within the equine industry, there has been interest in the ability to thaw frozen semen in a semen production laboratory, dilute further in extender and ship it as a cooled dose to the farm for insemination. Two previous studies concluded that acceptable retention of motility and fertility can be achieved using this technique, but was only done on a small set of samples. Goal of this experiment was to measure semen quality after thawing and cooling over 48 hours on a large number of stallions and illustrate the variability in the success among individual stallions. We evaluated 22 doses of frozen semen of variable post-thaw quality, all of which previously resulted in pregnancies. Motility analyses were performed using a Hamilton Thorne Ceros II computer assisted sperm analysis system to evaluate at least 400 motile sperm from each sample at each time point. Samples were analyzed for 40 video frames at 60 frames/second and progressive motility (PM) was defined as motile sperm moving with an average path velocity $\geq$ 50 μm/second and a straightness value of $\geq$ 75%. A full dose of eight (0.5 ml) straws per sample were thawed at 37°C for 30 seconds, then combined in a prewarmed 5 ml tube. Concentration was determined with a NucleoCounter SP-100 and an aliquot of the sample was added to pre-warmed cooling extender to a concentration of approximately 10 million/ml. After incubation for 30 minutes at 37°C, initial motility was determined. A second aliquot of thawed sample was incubated in modified Whitens medium with SYBR-14 and propidium iodide at a concentration of 5 million/ml. Membrane integrity analysis was completed on a Guava EasyCyte HT flow cytometer. Frozen-thawed samples were diluted to 30 million/ml in extender previously determined to be optimum for cooling semen of that individual stallion (INRA96® or a standard skim milk-glucose extender). Motility and membrane integrity analyses were performed initially (time 0), 6, 10, 24, 32 and 48 hours postthaw. Percent change in motility and membrane integrity was calculated from time 0 (initial) to 6, 24, and 48 hours after cooling. Average percent decrease in PM across all 22 stallions was 23, 41, and 67% at 6, 24, and 48 hours. Average percent decrease in sperm with intact membranes was 16, 16, and 23% at 6, 24, and 48 hours. Individual variation in the success of this technique was substantial. Decrease in semen quality after thawing and cooling for 24 hours, ranged among stallions from 2.5 to 77.6% for PM and 0 to 37% for membrane integrity. Decrease in PM for top 5 stallions at 6, 24, and 48 hours were 5, 13, and 33% respectively whereas for bottom 5 stallions there was a 47%, 71%, and 98% change in PM, respectively. It is concluded that although this technique is a viable option for some stallions, it is not appropriate for all stallions and therefore must be tested on every stallion.

Keywords: Equine sperm, frozen-thawed, cooled, membrane integrity

Comparison of pregnancy rates using new versus once used CIDR in 7 day estrus synchronization protocol during breeding season in ewes

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Small ruminant CIDRs are labelled for use in estrus synchronization protocols for up to 14 days. Protocols of shorter duration (5 - 9 days) include prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment at CIDR removal to induce luteolysis. Short duration protocols have led to anecdotal belief that a CIDR may be used a second time. Addition of gonadotropins to CIDR based protocols are known to enhance follicular growth and facilitate multiple ovulations. Our objective was to compare pregnancy status following natural breeding at a synchronized estrus using a 7 day exposure to either a new CIDR or once used CIDR, with PG600 treatment on day 6. Canadian Arcott ewes (mixture of lambs and mature) housed at 2 separate locations (Flocks A and B) were used. In Flock A, ewes (n = 61) were synchronized during 2018 - 2019 (n = 31) and 2019 - 2020 (n = 30) breeding seasons, whereas ewes (n = 71) in Flock B were synchronized during 2018 - 2019 breeding season. On day 0, ewes were randomly allocated to have either a new CIDR (nCIDR) or used CIDR (uCIDR) placed intravaginally. On day 6, all ewes were treated with PG600 (400 IU eCG + 200 IU of hCG/5 ml dose, IM). On day 7, CIDRs were removed and all ewes received a single dose of $PGF_{2\alpha}$ (cloprostenol, 125 mg, 0.5 ml, IM). On day 8, ewes were exposed to rams for 4 days. Rams were identified as satisfactory breeders using breeding soundness evaluation and previous breeding history. Ram to ewe ratio was maintained at 1:5 (Flock A) and 1:5 to 1:6 (Flocks B). Rams were provided 4 - 5 days of sexual rest before being used. Pregnancy diagnosis was performed between days 45 - 60 postbreeding via transabdominal ultrasonography. Fischer's Exact test was used to compare pregnancy rates between treatment groups. All ewes in Flock A were marked (ram marking harness) within 24 hours after ram introduction. Overall, pregnancy rates (nCIDR and uCIDR combined) were different ($p < 0.01$) between flocks (85%, 52/61 versus 58%, 41/71; Flocks A versus B, respectively); therefore, data were analyzed separately by flock. Within flock, pregnancy rates were not different between treatment groups (Flock A: nCIDR versus uCIDR, 25/30 versus 27/31, $p = 0.75$; Flock B: nCIDR 21/34 versus 20/37, $p = 0.63$). Lower pregnancy rates in Flock B were believed to be due to nutritional status (as a general observation, body condition scores of ewes in Flock B were lower than those in Flock A). In conclusion, a short duration (≤ 7 days) once used CIDR, can be successfully reused to obtain pregnancy rates comparable to new CIDR.

Keywords: Estrus synchronization, PG600, CIDR, ewes, fertility

Retrospective review of uterine prolapse in mares

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Uterine prolapse (eversion) is an infrequent but life-threatening emergency in mares. Since uterine prolapse is so uncommon, there is very little information published in scientific journals. Aim of this report is to describe uterine prolapse predispositions and case outcomes by reviewing clinical records from the UC Davis Veterinary Medicine Teaching Hospital (VMTH). Mare history and outcome data were analyzed for correlation to mare survival with Mann-Whitney U calculation and Fishers Exact Test. Significance level of $p < 0.05$ was set for all analyses. During 30 year period (1989 - 2019), 24 mares were presented with uterine prolapse. Age of mares ranged from 4 - 23 years (Mean ± SD; 11.1 ± 4.6) and parity ranged from 1st to 13th foaling (Mean ± SD; 3 ± 2.7). There was a high representation of maiden mares (7 out of 24). Investigation of interrelation between breed and uterine prolapse revealed that Arabians were overrepresented among affected mares compared to general breed distribution at the VMTH in the same time period. Uterine prolapse was associated with parturition and occurred within 1 hour after foaling in 82% of the cases. Fetal membranes were attached at the time of prolapse in 63% of the cases. Contrary to common belief that dystocia is the greatest predisposing factor to prolapse, only 33.3% of prolapses occurred after dystocia and 16.6% prolapses followed an abortion. Survival of the mares at discharge was 75%. Regarding fatalities, 5 mares suffered acute hemorrhage associated with prolapse and 1 died from secondary peritonitis. Fetal gender was overrepresented by males, 12 foals were colts and 5 were fillies (7 unknown). Fifteen foals survived. We were able to obtain follow-up history for 14 mares; 5 were rebred, and 4 foaled successfully. There were no correlations between mare survival and parity, age, sex of the foal, retained fetal membranes, or occurrence of sepsis. Based on the findings, we concluded that uterine prolapse occurred more after normal parturition (eutocia) than following abortions (16.6%) and dystocia (33.3%). Uterine prolapse may also have some breed predisposition to Arabians. Colts are overrepresented among affected mares. No characteristics were correlated with survival of mare, but severe hemorrhage was correlated with nonsurvival.

Keywords: Mare, uterine prolapse, normal parturition, abortion, dystocia

Alfaxalone crossreactivity affecting progesterone concentrations in cats

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Alfaxalone is a commonly used anesthetic agent in small animals. In cats, alfaxalone is used as an intramuscular agent to achieve clinically useful sedation or anesthesia, negating need for intravenous treatment, particularly in difficult patients. Molecular structure of alfaxalone is similar to progesterone (P_4) hormone allowing for activation of $GABA_A$ complex through a progesterone receptor. On clinical observation, intact female cats demonstrating signs of estrus, following alfaxalone treatment had serum P_4 concentrations suggestive of luteal activity. Concern that alfaxalone may be interfering with the assay, we hypothesized that alfaxalone would crossreact with progesterone assay. Eight domestic shorthair neutered male cats were administered 3 mg/kg of alfaxalone IM. Blood samples were collected at set time points (baseline, 30 and 60 minutes, and 3, 6 and 10 hours). Serum concentrations of P_4 immunoreactivity (IR) were determined using an automated immunoassay system (Immulite 1000). Data were analyzed using repeated measures ANOVA and a Tukey-Cramer multiple comparisons test, with $p < 0.05$ used for significance. Serum P_4 IR was elevated ($p < 0.05$) at 30 minutes, 1 hour, and 3 hours compared to baseline and returned to baseline at 6 hours. We concluded that intramuscular alfaxalone treatment in cats may interfere with immunoassay measurement of serum P_4 for up to 6 hours. Caution should be exercised when interpreting serum P_4 IR results in cats treated with alfaxalone.

Keywords: Alfaxalone, progesterone, immunoreactivity, cat

Influence of extender, temperature, and equilibration time on postthaw sperm motility in ram semen

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Objective was to investigate effects of extender, loading temperature, and equilibration time on postthaw sperm motility. Ejaculates were collected with an artificial vagina from 4 rams from May - July. Each ejaculate was split into 3 aliquots and extended in a 1 step protocol to a concentration of 200 x 10^6 sperm/ml with a liposome-based extender (OPTIXcell™), an egg yolk-based extender (Triladyl®), and a soy lecithin-based extender (AndroMed®). Half of each extended aliquot was loaded into straws at ambient temperature (23°C) and left to equilibrate for either 2, 4, or 12 hours at 4°C before cryopreservation in liquid nitrogen. Remaining half was loaded at 4°C after equilibration before freezing. Concentration and motility were measured with iSperm® Semen Analyzer. Linear mixed models were used for statistical analysis. Results are expressed as least square means ± SEM. Postthaw sperm motility differed ($p < 0.0001$) among OPTIXcell™ (37.1 ± 1.5%), AndroMed® (26.3 ± 1.8%), and Triladyl® (30 ± 0.8%) extenders. Ambient loading temperature (32.6 ± 1.5%) also had appreciable positive effects ($p < 0.0147$) on postthaw motility compared to 4°C (29 ± 1.1%). Samples with an equilibration time of 2 hours had lowest ($p < 0.0001$) postthaw sperm motility (23 ± 1.2%) compared to 4 hours (33.8 ± 2.3%) and 12 hours (35.6 ± 1.5%) equilibration. However, sperm postthaw sperm motility between 4 hours and 12 hours equilibration was not different ($p < 0.4270$). In conclusion, it is optimal to extend ram semen with OPTIXcell™ to avoid risk of microbial contamination that comes with animal-based extenders, load at ambient temperature which is less labor intensive and may limit possibility for temperature fluctuations, and to equilibrate at 4°C for 4 hours which could lengthen the window of AI postthaw. This protocol is ideal for efficiency and attractive to those without experience or cuttingedge equipment to freeze semen.

Keywords: Extender, cryopreservation, ram semen, motility, iSperm, temperature

Sonographic appearance of late pregnancy sheep fetal intestine and kidney

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Objective was to identify changes in sonographic characteristics of late pregnancy ovine fetus associated with fetal maturity. Ewes were synchronized, ram bred, and confirmed pregnant (n = 18) for potential cesarian section (c-section) at day 145 of pregnancy (d 0). Transabdominal ultrasonography was performed at days (d) 22, 15, 10, 7, 4, 1, and 0 prepartum to assess fetal heart rate (HR), abdominal diameter, renal volume, corticomedullary distinction (CMD), and gastrointestinal peristalsis (GIP). At day 1, 12 ewes were induced with 20 mg dexamethasone IM and underwent elective c-section on day 0. At birth, lambs were assigned a vigor score (1 - 10) based on HR, respiratory rate, muscle tone, irritability reflex, and mucous membrane color. A total of 19 live and 3 dead lambs were delivered with an average vigor score of 7 ± 0.6. Statistical analyses were performed using R. Mean days prepartum were assessed using ANOVA. Pearson's correlation analysis was used to assess associations. Both CMD and GIP were strongly correlated with days prepartum ($r = 0.7$, $p < 0.001$), whereas low correlation was observed with renal volume ($r = 0.27$, $p = 0.006$) and abdominal circumference ($r = 0.25$, $p = 0.009$). Days prepartum differed for CMD categories (absent: 22 ± 0, indistinct: 9.1 ± 0.6, distinct: 0.5 ± 0.1; $p < 0.001$). Days prepartum also differed for GIP categories (no peristalsis: 12 ± 0.7, occasional peristalsis: 2.3 ± 0.4, frequent peristalsis: 0.6 ± 0.1; $p < 0.001$). Days prepartum did not differ when fetal HR measured < 130 bpm (7.1 ± 0.7) or 130 - 149 bpm (8.8 ± 1.1; $p = 0.43$), but differed when fetal HR measured > 150 bpm (15 ± 1.5; $p < 0.001$). Fetal HR prior to induction (day 1) was moderately correlated with vigor scores at birth ($r = 0.55$, $p = 0.07$). In summary, qualitative sonographic characteristics, such as onset of fetal GIP and CMD were superior to quantitative measurements in determining fetal maturity. Additionally, ultrasonographic assessment of fetal HR before induction may help to predict neonate survivability.

Keywords: Ewe, pregnancy, GI peristalsis, renal development, ultrasonography

Efficacy of deslorelin acetate in advancing ovulation in timed artificial insemination in goats

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Objective was to determine the efficacy of deslorelin acetate in advancing ovulation for use in timed artificial insemination (TAI) protocols in goats. Does received an intravaginal CIDR containing 0.3 g progesterone and 10 mg dinoprost tromethamine IM at day -5. After CIDR removal (day 0), does received 0.2 mg deslorelin acetate (n = 9) or saline (control, n = 10) IM. Transrectal ultrasonography was performed twice daily to measure follicular growth and determine time to ovulation. Subsequently, does were synchronized similarly and left untreated (control, n = 42), administered 0.2 mg deslorelin IM (n = 42), or administered 5 ml P.G. 600® IM (n = 42). TAI was performed 48 - 56 hours after CIDR removal using fresh semen from 1 of 3 bucks, randomly distributed among treatments. Does were then housed with assigned bucks for natural breeding. Transabdominal ultrasonography was performed at 50 and 90 days after TAI to assess pregnancy status. Statistical analyses were performed using R. Time to ovulation was analyzed using a Kruskal-Wallis test and number of ovulations was analyzed by Student's t-test. Pregnancy rates were analyzed by ANOVA using treatment and buck as variables. Deslorelin-treated does had increased ($p < 0.01$) number of ovulations (2.9 ± 0.4) over control does (1.5 ± 0.2). Hours to ovulation tended to be less in deslorelin-treated does (53 ± 11) than in control does (61 ± 11; $p = 0.06$). Pregnancy per AI was affected by both treatment ($p < 0.01$) and buck ($p = 0.04$). Control does bred by Buck 1 had higher ($p = 0.02$) percent pregnancy rates (50) than those treated with deslorelin (7) or P.G. 600® (14). Deslorelin-treated does also had decreased ($p < 0.01$) in overall pregnancy rates across all bucks. It is concluded that deslorelin may have superovulatory effects impacting does' cyclicity and ability to achieve pregnancy. Further research is needed to investigate use of lower doses of deslorelin in goat estrus synchronization programs.

Keywords: Artificial insemination, caprine, deslorelin, estrus synchronization, pregnancy

Monozygotic twins in a Thoroughbred mare bred by natural cover

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Twin pregnancies in mares are undesirable, with a majority resulting in abortion or neonatal death.[1] Most twin pregnancies are dizygotic and originate from 2 oocytes rather than division of an embryo after fertilization (monozygotic). The exact etiology of monozygotic twin occurrence is not well understood in humans[2,3] or horses.[4] It is known, however, that although the natural occurrence of monozygotic twins is rare in both horses and humans (only 0.4% of in utero conception pregnancies in humans), the rate more than doubles when using assisted reproductive technologies.[2,5,6] A 13 year old Thoroughbred mare was evaluated by transrectal ultrasonography while in estrus and was bred naturally (natural cover) the same day a 40 mm follicle was identified. A single ovulation was detected by ultrasonography on the following day. At 14, 15, 16, 20, 26, and 30 days postovulation, a single embryo presence was observed. On day 33, 2 heartbeats (apparently from 2 embryos) were noticed and the vesicle measured 36 mm. Presence of 2 embryos was confirmed on day 34. Due to the inherent risks of twinning, her uterus was lavaged to remove and recover the embryos for analysis. Two distinct embryos were present within a single chorionic membrane. Tissue samples from both embryos were analyzed for genetic markers, specifically 13 microsatellites, and both embryos had identical genotypes. This case of monozygotic twins was unusual in that it was not associated with assisted reproductive technologies, but with natural cover.[1,7] Unlike the diagnosis of dizygotic twins, which can be determined around 14 days postovulation, monozygotic twins are often not diagnosed until around 30 days postovulation.[8] It is therefore important that mares diagnosed with single vesicles early in pregnancy, be re-examined ~ 30 days postovulation to ruleout the presence of monozygotic twins.[8,9] Even though monozygotic twins have been successfully reduced later in pregnancy,[10] most likely both embryos will be lost.

Keywords: Mare, Monozygotic twins, natural cover

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Pregnancy toxemia in the bitch

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Canine pregnancy toxemia can be a life-threatening condition for the dam and fetuses. The condition is characterized by hypoglycemia, ketonemia, ketonuria, and hepatic lipidosis during late pregnancy and clinical signs include weakness, collapse, seizures, and coma. Pregnant bitches bearing a large litter are more predisposed, especially if they develop anorexia during the last 2 weeks of pregnancy. The diagnosis is made by association of clinical signs, hypoglycemia and, importantly, high concentrations of ketone bodies in the blood and urine.[1-3] Measurement of ketone bodies (β-hydroxybutyrate) is commonly performed in food animal medicine; however, not often in small animal practice, which may impair the diagnosis of pregnancy toxemia. If diagnosed early, the condition can be resolved with enteral and/or parenteral glucose supplementation. However, in severe cases, pregnancy termination may be required. A 2 year old female Labrador retriever presented with 1-week history of vomiting and severe lethargy. The bitch had been accidentally bred 65 days prior to presentation. Tachypnea, distended abdomen, and engorged mammary glands were noted initially on physical examination. Hypoglycemia (38 mg/dl) and hypocalcemia (9.1 mg/dl) were observed. Serum ketone measured 1.0 mmol/l (normal range, 0.0 - 0.1 mmol/l). Plasma progesterone concentration was 1.07 ng/ml. Twelve fetal skeletons were seen radiographically. On ultrasonography, fetal viability and maturation were confirmed by fetal heart rates, presence of well-defined fetal renal corticomedullary distinction and intestinal peristalsis. Based on those findings, cesarean section was elected. Surgery was performed without complication and 12 healthy puppies were successfully delivered. Dam received Vetivex® (lactated Ringer's with 2.5% dextrose) and 22 mg/kg calcium gluconate. This case highlighted the importance of maintaining proper nutrition and energy balance in late-term pregnancy and measuring ketone bodies in an anorexic pregnant bitch.

Keywords: Canine, pregnancy, hypoglycemia, ketonemia

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Dystocia due to congenital hydrocephalus in a miniature Dachshund

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Congenital hydrocephalus (CH) is an active distension of the ventricular system that develops because of either an interruption of cerebrospinal fluid (CSF) flow or absorption. Chihuahua, Pomeranian, Pug, Pekingese, English Bulldog, Lhasa Apso, Yorkshire, Boston, and Maltese Terrier breeds are predisposed to CH.[1] Although Dachshunds have a relatively low frequency of congenital defects compared to other breeds,[2] CH has been previously reported.[3] However, there have been no published reports of CH resulting in dystocia in this breed. An 8 year old multiparous miniature Dachshund presented with a dystocia persisting for > 8 hours. The bitch was clinically stable on physical examination and a digital vaginal examination revealed no fetal parts. Transabdominal ultrasonography of uterus revealed 4 fetuses with heart rates between 150 - 250 bpm. A catheter was placed in the right cephalic vein and the bitch was anesthetized with propofol. Anesthesia was maintained with sevoflurane. A routine hysterotomy was performed and all 4 fetuses were delivered alive, including a fetus with a large, dome-shaped head that was lodged in the cranial vagina. No other congenital defects were present during gross postnatal examination of pups. Puppies were followed to 9 weeks of age. The pup with the CH developed normally and displayed no neurologic signs other than a consistent lack of menace reflex. Transfontanelle ultrasonography at 4 weeks of age revealed the lateral ventricles were greatly distended with anechoic fluid and lined by a thin wall of cortical tissue. At 9 weeks of age, the pup (2 kg) began acutely and inconsolably crying and began circling with head pressing. Euthanasia was performed and necropsy findings revealed 5 open fontanelles, occipital dysplasia, and symmetrical ventriculomegaly with periventricular cortical atrophy. Prior to ossification of the cranial sutures, CH may cause abnormalities of skull development such as a thinning of the bone structure, a dome-shaped appearance to the head, and/or persistent fontanelles.[1] In the current case, each ventricle contained approximately 10 ml of cerebrospinal fluid. Concurrent congenital abnormalities of the brain (e.g. intracranial arachnoid cyst, Dandy-Walker syndrome, Chiari-like malformation) without CE were previously reported in dogs.[4] No cerebellar or other abnormalities were present grossly and there was no histological evidence of neuronal degeneration or inflammation. The most common reported malformation in CH is a stenotic mesencephalic aqueduct,[4] which was the presumed cause in the current case, since no other lesions were present. In the current case, the formation of CSF must have equilibrated with absorption, resulting in a compensated hydrocephalic state, until an acute blockage of the ventricular system occurred, resulting in rapid decompensation.

Keywords: Canine, cranial deformity, dog, fetus, occipital dysplasia

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Fetal mummification and abortion secondary to umbilical cord torsion

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A 14 year old, Quarter Horse, mare presented, having aborted the previous day. Mare was bred by natural service and confirmed pregnant at ~ 30 days of pregnancy. Fetal crown rump length measured 24.8 cm and fetus weighed 318 grams. Estimated fetal age was 4 - 5 months. Fetus was partially mummified and visceral organs were autolyzed and appeared tan. There were > 20 counter-clockwise twists in the umbilical cord, extending from umbilicus to allantois. Umbilical cord torsion is considered a major cause for noninfectious abortion in mares. Reported frequency of abortion secondary to umbilical cord torsion varies, likely reflecting regional differences, as well as management differences leading to increased reporting. According to a retrospective study in UK, umbilical cord torsion accounted for 35.7% of all abortions and stillbirths.[1] Some umbilical twisting were normal, averaging 4 - 5 twists.[2] Excessive torsion occludes umbilical vessels and the urachus, resulting in decreased perfusion and fetal death. Umbilical edema, hemorrhage, thrombosis and fluid-filled sacculation were commonly observed along umbilical cord.[1,3] Long umbilical cords predisposed cords for excessive torsion, with cords > 84 cm having a higher risk.[1,4] Etiology of abnormally long umbilical cords is unknown.[2] Mares that aborted due to umbilical cord torsion had successful subsequent pregnancies.[2] Fetuses were not expelled immediately after death and generally had autolytic changes.[3] Abortions were most common at 6 - 8 months.[2] Infectious causes should be ruled out in all abortions to reduce transmission. In this case, fetus was tested for Equine Herpes Virus 1 and *Leptospira interrogans* using PCR and both were negative. A needle aspirate of lungs had only mucus. Allantois was unremarkable. Diagnosis of abortion secondary to umbilical cord torsion was confirmed.

Keywords: Equine, mare, abortion, umbilical cord torsion, noninfectious

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Left testicular rupture in a Red Angus bull

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A 16 month old, Red Angus, bull presented with a history of bilateral scrotal swelling. On presentation, his scrotum was swollen and firm on palpation. There was a line of demarcation at the ventral aspect of scrotum, suggestive of trauma. Pain was elicited on palpation of scrotum. Ultrasonography of scrotum revealed a loss of normal homogenous echogenicity of testicular parenchyma and disruption of testicular architecture. No blood flow was detectable on the affected side with Doppler examination. Inguinal herniation was ruled out by ultrasonography of scrotum and transrectal palpation; however, diagnosis remained undetermined until surgery was performed. Scrotal enlargement most commonly results from fluid accumulation in the vaginal cavity.[1] Typically this fluid can be readily observed by ultrasonography, but was not apparent in this case. Traumatic rupture of testis has been reported to occur occasionally and can be diagnosed on palpation and confirmed by ultrasonography. Unilateral castration was performed to conserve the function of the unaffected testis. It was readily apparent after incising the scrotum that the bull had suffered a traumatic rupture of left testis. There was a large blood clot contained within the scrotum and disruption of vaginal tunics and tunica albuginea. Bull recovered uneventfully from surgery and was discharged with follow up appointments for suture removal and 90 days postsurgery for Breeding Soundness Examination (BSE). Following unilateral castration, remaining testis hypertrophied and produced ~ 75% of normal sperm capacity.[2] Although these bulls have been successfully used for breeding, they will not pass a BSE with 1 testis. This bull returned to service and was maintained as a breeding bull for several years after his procedure.

Keywords: Angus, bull, testis, hemicastration

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Successful treatment of ascending placentitis in a Thoroughbred mare

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Ascending placentitis in mares is 1 of the leading causes of late-term abortions, premature delivery, and neonate morbidity and mortality.[1] A 5 year old nulliparous Thoroughbred recipient mare presented on the 306th day of pregnancy for vulvar discharge without mammary development. Fetal viability was confirmed by transrectal and transabdominal ultrasonography. An increase in the combined thickness of the uterus and placenta (CTUP [17 mm]), was detected via transrectal ultrasonographic examination, although no discharge was evident on vaginal speculum examination. Based on examination findings, treatment for ascending placentitis was initiated using an established treatment protocol.[2] Antimicrobial (24 mg/kg oral sulfadiazine and trimethoprim twice daily), antiinflammatory (8.6 mg/kg oral pentoxifylline twice daily), and tocolytic (0.088 mg/kg of oral altrenogest daily) agents were given. In addition, 10 mg of estradiol cypionate (IM, once every 3 days, 3 doses total) was given, as it had improved clinical outcome of ascending placentitis cases.[3] A decrease in CTUP was observed on transrectal ultrasonographic examination 8 days after initial presentation. However, all measurements did not return to normal limits; therefore, treatment had to be continued. All measurements of CTUP were within normal limits on transrectal ultrasonographic examination 16 days after initial presentation and the ascending placentitis was considered resolved. Transrectal and transabdominal ultrasonographic examination continued throughout pregnancy to monitor fetal health and screen for recurrent ascending placentitis. On 350th day of pregnancy, the mare delivered a healthy colt and grossly normal fetal membranes. The colt stood and nursed without assistance < 2 hours after parturition. Histopathological examination of fetal membranes demonstrated no evidence of placentitis. This case demonstrated that successful outcomes are possible in cases of ascending placentitis with early detection and aggressive treatment.

Keywords: Mare, ascending placentitis, parturition

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Anasarca in a neonatal bulldog puppy, secondary to a ventricular septal defect

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A 2 year old primiparous English Bulldog presented for fetal monitoring prior to elective cesarean section. Bitch was bred via transcervical insemination and her singleton pregnancy was confirmed via transabdominal ultrasonography and radiographs. Due to risk of primary uterine inertia and fetal oversize with 1 fetus, and due to being a brachycephalic breed, a cesarean section was scheduled. First fetal monitoring was performed at 64 days post LH peak; at this visit, bitch had colostrum present within caudal mammary glands, and her progesterone concentration was 6.32 ng/ml. On ultrasonography, although the fetal heart rate was normal (226 - 232 bpm), fetus appeared to have pleural effusion and subcutaneous edema, consistent with an anasarca puppy. Cesarean section was performed on the following day. A single pup with severe subcutaneous edema (confirmed ultrasonography findings) was removed. Euthanasia was elected due to poor prognosis and necropsy followed. Gross examination revealed a ventricular septal defect, a finding that has been associated with hydrops fetalis in human and veterinary species.[1] Renal tubules and renal pelvis were mildly dilated, suggestive of urinary obstruction presumptively due to severe anasarca. On histologic examination, hepatocellular necrosis and congestion of various organs were noticed (lesions secondary to decreased left-sided cardiac output). In summary, ultrasonography was diagnostic (anasarca fetalis and pleural effusion) and findings enabled us to decide on a timely surgery.

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Ejaculatory failure in a halter stallion due to dorsal displacement of soft palate

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Exercise-induced dorsal displacement of soft palate (DDSP) commonly occurs in racehorses, characterized by decreased performance;[1] however, it has not been identified as a cause for failure of ejaculation. In May 2019, a 5 year old maiden American Quarter Horse halter stallion was presented for failure of ejaculation. During breeding soundness examination, stallion demonstrated normal libido and mounting behavior during repeated mounts on phantom, but never ejaculated. While mounted, stallion expressed labored breathing that owner had noticed during previous breeding attempts. Manual palpation of scrotum, accessory sex glands, and penis were normal. An echocardiogram and chemistry analysis for lactic acid, alkaline phosphatase, and creatine kinase were normal. Static endoscopy of larynx was normal, but dynamic endoscopy during stallion's mount on phantom revealed 50 - 70% of airway became blocked by DDSP. To correct this condition, a laryngeal tie-forward procedure was performed. Following 2-week postoperative recovery under owner's supervision, stallion was able to ejaculate normally and repeatedly. Ejaculation disorders in stallions were attributed to physical, musculoskeletal, psychogenic, or neurologic causes;[2] apparently, DDSP has not been reported before as a cause. Halter-type stallions with ejaculatory dysfunction have been observed to exhibit labored breathing during and following breeding and was assumed to be a "common" breed characteristic. Occurrence of DDSP is associated with exercise intolerance; however, exact etiology is unknown.[1] In this stallion, increased activity was associated with breeding-induced displacement of soft palate above epiglottis. As a result, stallion was unable to complete ejaculation, due to blockage of airway. Following surgical correction, stallion was able to ejaculate. Ejaculatory dysfunction in stallions can be difficult to assess; therefore, DDSP should be considered as an underlying cause, especially when abnormal respiratory sounds are heard during breeding.

Keywords: Halter stallion, ejaculatory failure, DDSP

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Priapism in a Quarter Horse gelding

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Priapism is an uncommon finding in domestic animals; however, it is reported in horses. It has multiple etiologies, including use of phenothiazine tranquilizers for routine procedures such as sheath cleaning and dentistry.[1] Priapism results in a selective engorgement of corpus cavernosum and corpus spongiosum that ultimately leads to venous occlusion.[2] Exact mechanism for initial failure of detumescence is not known, but ultimately, red blood cells become sickle shaped in response to increased partial pressure of CO_2.[3] This deformation in red blood cells can lead to permanent obstruction of venous drainage. A 25 year old, Quarter Horse gelding was presented with paraphimosis (noticed 2 days prior to presentation) secondary to priapism. His teeth had been floated 1 week earlier, under acepromazine sedation. Initial efforts to address priapism included placement of 12 gauge trocar needles, 1 dorsally and 1 ventrally within corpus cavernosum for irrigation with heparinized saline. Egress fluid from ventral trocar needles were thick, dark, and serosanguineous. As flush continued, fluid consistency changed to thin, bright red, serosanguineous to sanguineous, suggesting intact arterial supply. Phenylephrine was also administered directly into corpus cavernosum to promote vasoconstriction and reduction of priapism. Complete detumescence was not achieved by flush or phenylephrine treatment. A vascular shunt was established from corpus cavernosum to corpus spongiosum to relieve venous obstruction. Two days after the procedure, there was minimal improvement. Humane euthanasia was performed due to quality of life and financial constraints. At necropsy, vascular shunt (communication between corpus spongiosum and corpus cavernosum) appeared intact. Chronicity of the disease might have prevented full resolution and recovery, despite creation of a functional vascular shunt.

Keywords: Equine, gelding, priapism, acepromazine, corpus cavernosum, corpus spongiosum

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Changes in milk pH, milk protein, and udder development in late-pregnant ewe and effects on lamb serum total protein concentrations

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Objective was to describe changes in milk pH, milk protein concentrations, and udder development in late-pregnant ewes. Ewes were synchronized, ram bred, and confirmed pregnant (n = 18) for potential cesarean section at 145 days of pregnancy (d 0). Udder score was assessed on days 22, 15, 10, 7, 4, 1, and 0 prepartum. Milk pH (digital pH meter) and protein concentrations (Brix refractometer) were monitored once daily from days 6 to 0 prepartum. Twelve ewes were selected for elective cesarean section and induced with 20 mg dexamethasone IM on day 1. At birth, lambs were assigned a vigor score (1 - 10) based on heart rate, respiratory rate, muscle tone, irritability reflex, and mucous membrane color. A total of 19 live and 3 dead lambs were delivered. Serum total protein (TP) was measured in lambs from 24 to 36 hours after birth. Statistical analyses were performed using R. Data on days prepartum and lamb TP were analyzed using ANOVA. Pearson's correlation analysis was used to determine associations. There was moderate correlation in days prepartum with both udder development ($r = 0.53$, $p < 0.001$) and milk pH ($r = 0.46$; $p < 0.001$), but no correlation with milk protein concentrations ($r = 0.11$, $p = 0.25$). Days prepartum did not differ between udder scores of 0 (no development: 14 ± 3.8 days) and 1 (minor development: 12 ± 1.2 days; $p = 0.96$), but did change for scores of 2 (moderate development: 6.6 ± 0.5 days) and 3 (full development: 3.4 ± 0.4 days; $p \leq 0.03$). Days prepartum differed when milk pH measured > 6.5 (4.4 ± 0.3 days), 6 - 6.5 (2.1 ± 0.2 days), and < 6 (0 ± 0 days; $p < 0.001$). Lamb TP was not affected by milk protein concentrations at d 0 ($p = 0.96$), but tended to be affected by vigor score ($p < 0.06$). We concluded that udder score and milk pH may be useful for predicting parturition in ewes. Additionally, assessing neonate vigor at birth can help influence colostrum management decisions.

Keywords: Milk pH, milk protein, neonate vigor, parturition, udder development

Canine herpes virus in a breeding kennel of Wirehaired Dachshund

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A 3-year-old, Wirehaired Dachshund presented to Auburn University Veterinary Hospital 53 days postovulation for dystocia and delivery of premature stillborn puppies. Rapid slide agglutination testing (RSAT) was performed for suspicion of *Brucella canis* infection, but was negative. Seven days later, the patient's kennelmate was presented 57 days postovulation for abortion, and *Brucella* RSAT was again negative. Canine herpesvirus (CaHV-1) was then investigated as a possible causative agent of serial abortions. A vaginal swab from the second patient was subjected to direct fluorescent antibody testing. Results were positive for CaHV-1 antigen. Symptoms of CaHV-1 infection include abortion, stillbirth, and infertility in bitches, whereas CaHV-1 can also cause "fading puppy syndrome" in neonates. Client returned 2 days later with 4 additional pregnant dams for testing. Vaginal swabs from all females tested positive for CaHV-1. All bitches also possessed a low or negative titer to CaHV-1. Based on these results, future litters were considered 'at risk' for contracting CaHV-1 infection. Recommendations included increasing the temperature in the neonatal area and implementation of biosecurity. Pregnant dams were prescribed oral acyclovir (20 mg/kg), to be administered every 6 hours until puppies were 3 weeks old. Once whelped, puppies received oral acyclovir solution at 15 mg/kg until 3 weeks old. With 3 successful litters, 13 puppies were born and alive at 6 months. Owners revealed that a new stud dog had been imported from Germany approximately 1 month prior to the initial abortion. This case highlighted the importance and magnitude of CaHV-1 infection in breeding bitches and neonates. Though most adult dogs are not clinically affected by CaHV-1, the virus can rapidly result in widespread loss in a breeding kennel by compromising the health of serologically naïve dams and neonates. In the case of this kennel's CaHV-1 outbreak, a positive outcome was reached through proper biosecurity and veterinary intervention.

Keywords: Canine herpes, abortion, stud dog

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A retrospective analysis on determinants of litter size in a colony of working dogs

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Successful breeding and increased litter size are paramount to increasing working dogs' numbers for national security. We determined key factors affecting pregnancy and litter size in Canine Performance Sciences (CPS) breeding colony. Specific aims of this research were to elucidate factors that improved breeding outcomes in the colony. Data from CPS breeding population for 55 breeding cycles for 26 females over 5 years were analyzed. Factors analyzed were: age of dam at breeding, semen type (fresh, cooled-shipped, frozen), insemination method (vaginal AI, transcervical insemination [TCI], live cover), and total number of inseminations. Multiple regression model was used. Above factors influenced ($p < 0.001$) and explained 57% of the variance in litter size. Addition of 1 TCI increased ($p = 0.005$) litter size. Addition of 1 live breeding, although not different ($p = 0.07$) had clinical relevance (increased litter size). Average litter size of a 30 month old dam using fresh semen AI was 5.45 puppies and cooled-shipped semen AI was 7 ± 1 puppies. Age of dams at breeding ranged from 13 - 88 months and there was no effect of age or parity on litter size. Results indicated that maximum litter size occurred when fresh semen was utilized, with cooled-shipped semen being equivalent. Use of frozen semen was least successful, resulting in negative effects on pregnancy rate in this population. Scrutiny of insemination methods revealed that addition of 1 live cover increased litter size by 2 puppies, whereas addition of 1 TCI increased litters by 3 puppies. In summary, incorporation of 1 live breeding and 1 TCI utilizing fresh semen increased average litter size in CPS breeding colony.

Keywords: Canine, semen, transcervical, litter, breeding

Foal born using intracytoplasmic sperm injection to a Friesian mare with unexplained subfertility

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Intracytoplasmic sperm injection (ICSI) is an advanced reproductive technology wherein oocytes are retrieved from tertiary follicles and injected with a single sperm to develop into an embryo in vitro. Unlike embryo transfer, ICSI affords versatility for mares with a suboptimum uterine environment, blocked oviducts, or at postmortem where the opportunity for natural fertilization is not possible. ICSI can also be used when limited numbers of poor-quality sperm are available.[1] This technique provides the possibility to obtain foals from mares that otherwise could not propagate their genetics. A 13 year old Friesian mare presented to the UC Davis Theriogenology service in May 2013 for breeding with cooled semen after several years of unexplained subfertility. Uterine cytology was taken at multiple intervals, with no clear evidence of endometritis. On ultrasonography, anechoic fluid was present in the uterus after all breeding attempts. Treatments included uterine lavages and oxytocin to enhance uterine clearance. In 2016, after several unsuccessful breeding cycles, laparoscopic application of prostaglandin E_2 in the oviducts was performed, with the suspicion that they could be blocked.[2] After the procedure, there were 2 more years of unsuccessful breeding attempts. In 2018, it was decided that this mare was an ideal candidate for oocyte retrieval and ICSI. In May 2018, transvaginal aspiration of oocytes was performed, where 15 follicles between 10 - 30 mm were aspirated from both ovaries. Of the 15 follicles aspirated, 9 oocytes were recovered. Four of these oocytes matured to the MII stage in vitro and underwent ICSI. Two embryos matured and were shipped as blastocysts to Colorado State University where they were implanted into 2 recipient mares. Both embryos survived until fetal heartbeats were detectable, with 1 pregnancy surviving to term. The 7 year old recipient mare was transported to UC Davis where she foaled successfully and without complications.

Keywords: Equine, intracytoplasmic sperm injection, mare subfertility

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Granulosa cell tumor and prolonged estrus in a Boxer bitch

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An 11 year old, multiparous Boxer bitch was presented to the Virginia-Maryland College of Veterinary Medicine Theriogenology Service for evaluation of persistent vulvar swelling and bloody vulvar discharge of 34 days duration. On physical examination, the vulva was markedly swollen, with a small amount of blood-tinged discharge. Vaginal cytology revealed 100% superficial epithelial cells, confirming a diagnosis of estrogen influence. Thorough history revealed no known exposure to exogenous estrogens. Abdominal ultrasonography was performed to identify potential structural abnormalities of the reproductive tract and/or adrenals which could be responsible for estrogen influence. Ultrasonography revealed a markedly thickened myometrium and endometrium, with unremarkable ovaries and adrenal glands. Furthermore, serum progesterone concentrations via chemiluminescence were1.02 ng/ml. Based on the clinical findings, our differential diagnoses were microscopic disease of the ovary or exogenous estrogen exposure. Because the bitch had no history of exogenous estrogen exposure, microscopic ovarian disease, such as a granulosa cell tumor, was considered to be the most likely differential. Despite ovarian neoplasia being rather uncommon, granulosa cell tumors account for approximately half of all ovarian tumors in bitches.[1] It is not unusual for granulosa cell tumors to produce estrogen and therefore cause clinical signs consistent with a prolonged estrus. Definitive diagnosis of granulosa cell tumors relies on histopathology of ovaries. The owner elected not to pursue recommended ovariohysterectomy and instead elected humane euthanasia due to orthopedic comorbidities, with postmortem necropsy. Histopathology of a small (2 mm in diameter) round mass within an ovary revealed a well demarcated, proliferative mass made of tubules and small cysts lined with foamy basophilic cells, consistent with a granulosa cell tumor. This case demonstrated the importance of considering microscopic disease when presented with a bitch exhibiting prolonged estrus.

Keywords: Granulosa cell tumor, prolonged estrus, ovarian neoplasia, bitch

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Detection of *Tritrichomonas foetus* in a beef herd

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A herd of 150 Angus cows had transrectal palpation for pregnancy diagnosis at the end of April 2019. Eighty two percent of cows were determined to be pregnant. Eight nonpregnant cows had pyometra that was diagnosed by transrectal palpation and confirmed via transrectal ultrasonography. Pregnancies ranged from 25 to 110 days of age. Four Angus bulls were with the cows during a 120 day breeding season. Last year's weaning in the group was 77.57%. Smegma was collected from these 4 bulls via preputial scraping utilizing Pizzle Stick (Lane Manufacturing, Denver, CO). Samples were placed in Modified Diamonds Media and submitted to Bishop-Thompson-Sparks-Alabama State Diagnostic Laboratory and tested for *Tritrichomonas foetus* (*T. foetus*) DNA, via a reverse transcriptase polymerase chain reaction (RT-PCR) assay. Two of the 4 bulls tested positive for *T. foetus,* with strong positive cycle threshold (CT) values of 33.3 and 34.2. Two bulls that tested positive were sold to slaughter and 2 remaining bulls were retested via RT-PCR and were negative again. All cows with pyometra were sold for slaughter. Remainder of nonpregnant cows were treated IM 3 times with 5 ml of dinoprost tromethamine (Lutalyse) at 45 day intervals beginning in October 2019 to aid in clearing infection. These cows were then bred via AI in January 2020. The 2 positive bulls had been previously tested for *T. foetus* via RT-PCR and were negative in 2017 - 2018. Therefore, it was speculated that *T. foetus* entered the herd through replacements from another state. This case highlighted the importance of testing all bulls yearly for *T. foetus* when the herd is not a closed herd.

Keywords: Angus bulls, *Tritrichomonas foetus,* pyometra, smegma, preputial scarping, RT-PCR

Genetic causes of Golden Retriever congenital hypomyelinating polyneuropathy

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Myelin is an important component of both central nervous system (CNS) and peripheral nervous system (PNS) by surrounding nerve cells, protecting them and aiding in perpetuation of signal conduction. Myelin deficits can occur either by demyelination (myelin that initially formed properly and was then degraded) or by hypomyelination (myelin never correctly/fully formed at all). Two cases of littermate, full-sibling Golden Retriever (GR) puppies with very young onset (i.e. congenital) had neurological deficits.[1] Peripheral nerve biopsies revealed myelin sheath changes consistent with a predominantly hypomyelinating neuropathy. Uniquely, this syndrome affected only the PNS. Previously reported hypomyelination syndromes in veterinary patients had either exclusively involved the CNS, or both the CNS and the PNS together. We acquired DNA from 4 GRs presenting with clinical signs as reported;[1] nerve biopsies confirmed these dogs to have a congenital PNS hypomyelinating neuropathy. We performed whole genome sequencing on DNA from each of these 4 dogs. Three causative private variants were identified, each in an excellent functional candidate gene known to cause Charcot-Marie-Tooth disease in humans: a loss of donor splice site variant in 1 dog, a missense variant in 1 dog, and a premature stop codon shared by 2 dogs. These 3 variants were all absent from > 1,000 canine whole genome sequences of normal dogs, and from > 200 control GRs. While this condition is rare in the GR breed, breeders could use genetic tests for these variants to avoid producing affected puppies. In conclusion, theriogenology contains within its purview inherited congenital diseases. Four cases of congenital hereditary neurological disease in Golden Retrievers, wherein, using modern whole genome sequencing technology, we identified the likely causative mutation in each of them. Therefore, this is important for theriogenology service, as we can now offer each of these 3 variants as genetic tests to Golden Retriever breeders and prevent any more puppies born affected with this condition.

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Delayed uterine prolapse in a Thoroughbred mare with retained fetal membranes

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A 6 year old Thoroughbred mare was presented to Virginia-Maryland College of Veterinary Medicine's (VMCVM) Equine Field Service for evaluation of retained fetal membranes (RFM) after aborting twins the night before. Fetal membranes retention was observed by the owners, with no progression for 5 - 6 hours. Initial treatment included gentle manual traction of fetal membranes, administration of oxytocin and flunixin meglumine, and lavage of uterus. Despite expelling large amounts of fluid after lavage, there was no further progress in fetal membranes expulsion. Mare was given another oxytocin injection which caused continued myometrial contractions; however, there was no progress. Due to duration of retention, worsening of clinical signs (increased digital pulses and injected mucus membranes), and lack of improvement, mare was referred to the VMCVM Veterinary Teaching Hospital. She was resedated, and her uterus was lavaged again without dislodging fetal membranes. Mare was placed on IV fluids and broad-spectrum antimicrobials, and was under continued sedation and oxytocin treatment. At 8 hours after presentation, mare's uterus prolapsed (everted). Mare was given an epidural, uterus was rinsed with hypertonic saline and replaced. A Caslick's procedure was performed to aid in uterine retention. There was still a small amount of RFM observed after replacement. Hospitalization for treatment included twice-daily uterine lavages, IV fluids, oxytocin, antimicrobials, flunixin meglumine, and omeprazole. Mare was also treated with ice boots, vasodilators, and antiinflammatories to prevent laminitis. Mare was discharged 4 days later with no medications and did not experience any further complications. Uterine prolapse (eversion) is rare in mares, often fatal (from infection), and usually occurs immediately or within a few hours after parturition. Aggressive and prompt treatment of RFM in mares is critical. Regardless, as observed in this case, RFM may be considered a predisposing factor for delayed uterine prolapse.

Keywords: Equine, uterine prolapse, retained fetal membranes

Importance of breeding management in abnormal estrous cycles: a case study

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In the expanding small animal breeding industry, accurate estrus detection and ovulation timing are essential for breeding management. Many clinics rely on inhouse progesterone tests for convenient and efficient results. Despite widespread use of these tests, it is critical to monitor progesterone concentrations comprehensively due to potential abnormalities in the estrous cycles of individual patients. Such an anomalous cycle was diagnosed in Suzie, a 3.5 year old Fawn Chinese Pug, who was presented to Crestwood Veterinary Centre for artificial insemination. Suzie's baseline progesterone concentration on December 18, 2019 was 0.8 nmol/l (0.25 ng/ml) with concurrent clinical proestrus signs. Regular progesterone monitoring at 2 - 3 day intervals continued until December 27, 2019, where an increase to 4.0 nmol/l (1.26 ng/ml) indicated an impending luteinizing hormone (LH) surge. Despite this, on December 30, 2019, progesterone concentration decreased abruptly and was undetectable. Vaginal cytology had cornified cells confirming estrus. Suzie was bred on January 1 and 3 via artificial insemination with fresh semen; however, pregnancy was not achieved. A theriogenologist reviewed this case and suggested that Suzie's progesterone decrease after an apparent LH surge indicated she had not ovulated, and therefore should not have been bred. Her progesterone readings suggested an atypical estrous cycle, possibly a split heat or anovulatory cycle.[1] It was recommended to monitor Suzie's next cycle using serial examinations of serum progesterone and vaginal cytology, and to breed only after confirmation of ovulation via serum progesterone 2 - 3 days after the perceived LH surge. It was also suggested to induce ovulation using gonadotropin-releasing hormone or human chorionic gonadotropin in case of repeated anovulatory cycles. With advances in theriogenology, we are better able to understand dysfunctional or abnormal canine estrous cycles and to provide suitable advice to our clients in their management.

Keywords: Estrus, progesterone, canine, luteinizing hormone, artificial insemination, ovulation

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Sertoli cell tumor in an alpaca

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A 9 year old intact male alpaca was presented with a unilaterally enlarged right testis that the owner noticed after annual shearing. He had no previous breeding history, nor history of health or behavioral problems. Physical examination was normal except for visible unilateral scrotal enlargement. Scrotal palpation findings were, a large turgid right testis and a soft left testis, with normal epididymides. Palpation elicited no pain response and ruled out the presence of epididymitis, herniation, or testicular torsion. Testicular ultrasonography revealed a heterogeneous appearance of right testis parenchyma with diffuse multifocal areas of hyperechoic foci and several anechoic pockets. Left testis was comparatively homogenous in appearance with rare hyperechoic foci. At this point, differential diagnoses included neoplasia or orchitis. A bilateral elective castration was performed, since this alpaca was not intended for breeding. After castration, right testis measured 50 x 54 mm, with 2 cystic structures and a bulging cream-colored bulging structure grossly identified upon dissection. Left testis measured 37 x 32 mm with no gross abnormalities. Histopathology diagnosed a Sertoli cell tumor that completely replaced the stroma in the right testis and diffuse degeneration with no tumor infiltration in left testis. Spermatic cord was also free from tumor involvement. The alpaca recovered from castration uneventfully. Testicular neoplasia in camelids is rare. While Sertoli cell tumors have been reported in dogs, bulls, rams, cats, and horses, apparently, this is the first report in camelids. Sertoli cell tumors are characteristically hormonally active. While no symptoms were present in this case, degeneration of contralateral testis suggested that the tumor might have been secreting estrogen. Bilateral castration proved to be curative for this alpaca; however, since no metastasis was evident, unilateral castration might have preserved fertility, if there was an intention to use the animal for breeding.

Keywords: Sertoli cell tumor, testicular enlargement, testicular degeneration, alpaca

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Scrotal hydrocele in an Angus bull

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A 5 year old, Angus, bull presented with a history of left testicular swelling. Ultrasonography of scrotum revealed a normal appearing right testis and the presence of fluid containing strands of hyperechoic material surrounding left testis. No bowel was appreciated within the scrotum. Semen evaluation revealed decreased numbers of sperm with adequate motility. White blood cells were present on the semen slide and proximal droplets were seen within individual sperm. The fluid surrounding left testis indicated unilateral scrotal hydrocele. Hydrocele is an abnormal accumulation of serous or inflammatory fluid within the vaginal cavity of testis.[1] In mature bulls, hydrocele results from local inflammation or trauma.[2] Most commonly, scrotal hydroceles present with swelling in scrotum.[3] Ultrasonographic examination reveals varying amounts of anechoic fluid surrounding the epididymis and testis with normal testicular echotexture.[3,4] With fluid accumulation, testicular thermoregulation is disrupted, resulting in decreased semen quality.[3] Treatment of this condition is directed at removing the inciting cause of fluid accumulation.[3] In this case, a hemicastration of left testis was performed with the possibility of regaining reproductive potential. Cystorelin (GnRH) was administered to increase spermatogenesis in the remaining testis. At re-examination, appropriate postoperative healing was noted. A semen sample obtained via electro-ejaculation had 16% normal cells, 79% cells having primary abnormalities and 5% cells having secondary abnormalities. Other parameters of the breeding soundness examination were concluded to be within normal limits. The scrotal circumference was 29 cm. Prognosis for reproductive performance in bulls with unilateral hydrocele is good with prompt removal of affected testis.[3] Since spermatogenesis can be impaired for 2 - 6 months following testicular insult, final recommendations regarding fertility should only be made after allowing adequate time for recovery.[3,5]

Keywords: Hydrocele, bull, castration, BSE, ultrasonography

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Bilateral ovarian adenocarcinoma in a geriatric Shih Tzu

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A 15 year old female intact Shih Tzu was presented due to vaginal discharge of 4 day duration that had been recurring for over 1 year. She had a history of an open pyometra twice in the past year. Ovariohysterectomy had not been pursued, due to owner's concern for anesthetic risk. On presentation, the patient was moderately thin, had serosanguinous vaginal discharge, a swollen vulva, and a positive flagging response; physical examination was otherwise unremarkable. Vaginal cytology revealed primarily intermediate cells with few parabasal cells. Vaginal cultures revealed growth of *Enterococcus faecalis*, *Escherichia coli*, and *Staphylococcus pseudointermedius*. Progesterone concentration was 0.79 ng/ml. Transrectal ultrasonography revealed cystic structures on both ovaries. Followup examinations within next 2 weeks revealed similar findings on vaginal discharge and cytology, and progesterone concentrations rose slowly to 2.28 ng/ml. Due to the abnormal ovarian findings and potential for hyperestrogenism, ovariohysterectomy was pursued. Preoperative bloodwork revealed a mild leukocytosis characterized by a neutrophilia with a mild left shift. Histopathology of reproductive tract revealed bilateral ovarian adenocarcinoma, severe, diffuse, chronic cystic endometrial hyperplasia, and mild pyometra. Epithelial tumors, including ovarian adenomas and adenocarcinomas, were the most common primary ovarian tumor in dogs (46%), with adenocarcinomas making up 20% of canine ovarian tumors.[1] The patient recovered without incident and was discharged to the care of her owner. Followup examination 3 weeks postoperatively, including thoracic radiographs and abdominal ultrasonography, revealed no evidence of metastasis. This case presented a unique abnormal estrous cycle secondary to a neoplastic process in a geriatric bitch with an open pyometra.

Keywords: Ovarian adenocarcinoma, geriatric dog, hyperestrogenism

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Bilateral cystadenoma with a granulosa cell tumor in a Bernese mountain dog

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A 7 year old Bernese mountain dog presented for more than 5 weeks of persistent estrus and infertility. Her history consisted of multiple attempts of unsuccessful breeding, with and without progesterone timing, and she had never produced a litter. On presentation, the bitch had normal physiological parameters with the presence of a swollen vulva and vaginal bleeding. Vaginal cytology revealed 100% superficial cells, consistent with estrus. Serum progesterone was 0.671 ng/ml, also consistent with estrus. Transabdominal ultrasonography was elected to examine for the presence of follicular activity or any abnormalities. The right uterine horn presented with a small single cyst. The right ovary was larger and with a lobulated appearance and a questionable small mass. In addition, the right ovary had increased blood flow on Doppler compared to the left ovary. Due to her advanced age, history of infertility and ultrasonographic findings, an ovariohysterectomy was performed. Morphological diagnosis on histopathology was bilateral papillary cystadenoma, a granulosa cell tumor (GCT) on the right side and aggregates of small cells, indicative of an early dysplastic change over a GCT on the left side. According to an analysis of studies of canine ovarian tumors from 1960 - 2004, granulosa cell tumors accounted for ~ 25% of all ovarian tumors.[1] A case that had a history of infertility and presented with clinical signs of estrus was diagnosed to have bilateral papillary cystadenoma and a unilateral GCT.

Keywords: Bilateral cystadenoma, granulosa cell tumor, infertility

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A third-degree rectovaginal tear in a primiparous mare

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A 10 year old Quarter Horse mare was presented for rectovaginal tear and fistula. She had foaled unattended. The large foal was found dead but appeared grossly normal. A tear was present from the mare's vulva through her perineal body and extended cranially 6 inches. The mare was medically managed with flunixin meglumine and femycin and hydrotherapy to reduce irritation from fecal contamination. The owner was advised to keep her on a soft stool diet and to continue hydrotherapy for 6 weeks prior to surgical reconstruction. Two months after the initial visit, the mare was reevaluated for surgical correction of the rectovaginal tear. On rectal palpation, a 5 x 5 inch fistula was noted and the rectal sphincter was intact. Fecal contamination of the vagina was present. Surgical correction for this case was performed in 2 phases. In the first phase, the rectal sphincter was split and the fistula was converted to a grade 3 rectovaginal tear. In phase 2, the perineal body and anal sphincter were repaired. Due to the degree of fibrosis from the severity of her injury, additional foaling was not recommended. Third-degree rectovaginal tears occur predominantly in primiparous animals, with the mare being over represented, due to the explosive nature of their parturition.[1] Third-degree rectovaginal tears are a complete communication between the vestibule and the rectum. Correction is important to either restore breeding function or to provide a better cosmetic appearance.

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Cervical adenoma and excisional biopsy in a spayed Labrador retriever bitch

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A 7 year old (42 kg), nulliparous, spayed female Labrador Retriever was presented following referral to the Virginia Maryland College of Veterinary Medicine for evaluation of chronic, intermittent, bloody vaginal discharge and acute tissue herniation from the vulva. Physical examination revealed a hemorrhagic, multilobulated, irregularly shaped mass protruding from the vulva. A digital vaginal examination revealed the presence of a firm, prominent, pedunculated mass originating from a distinct stalk. An impression smear had a monomorphic population of large, undifferentiated round cells with anisocytosis, anisokaryosis, and coarse chromatin. Following sedation with intravenous dexmedetomidine (7 μg/kg) and butorphanol (0.3 mg/kg) and administration of a lumbosacral epidural (0.25 mg/kg of 0.75% preservative free bupivacaine), digital vaginal examination revealed 2 pedunculated masses. The caudal mass originated from ventral vaginal mucosa located 5 mm cranial to the urethral orifice, and the cranial mass was located dorsolaterally, 1.5 cm cranial to the first. The owner elected excisional biopsy of both masses for histopathology performed with a vessel sealing device (Ligasure) over an episiotomy with complete excision. Although the caudal mass was easily exteriorized and excised, the cranial mass was more friable and tore during attempted exteriorization, leaving a remnant within the cranial vagina. Minimal vaginal bleeding was observed postbiopsy. The dog was discharged with carprofen (2.2 mg/kg PO, every12 hours) and trazodone (2.4 mg/kg, PO, every 12 hours) awaiting histopathology. Histopathology revealed a well-differentiated simple cuboidal and columnar epithelial population of cells, typical of the cervix, with secretory mucin-rich cystic glandular lesions consistent with cervical adenoma. Vaginoscopy was repeated at 1 month postbiopsy. The remaining mass had not changed in size or appearance; however, the previous clinical signs had resolved. While benign epithelial tumors of the vaginal mucosa have been documented, this case presents the first reported benign epithelial tumor of the cervix, as well as a minimally invasive technique for treatment.

Keywords: Cervical adenoma, Ligasure, canine, vaginal bleeding

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