ABSTRACTS

OPENING SESSION

Rho kinases inhibitor effects on postthaw survival of slow-cooled bovine blastocysts

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Slow-cooling cryopreservation protocols for direct transfer of bovine blastocysts have allowed convenient thawing and transfer into recipients without additional embryo manipulations. Rho-associated coiled-coil-containing protein serine/threonine kinases (ROCK) inhibition has improved postthaw viability of vitrified bovine embryos produced in vitro; however, it has not been evaluated in slow-cooled bovine embryos. Aim was to determine, if treatment with 10 µM Y-27632, a ROCK inhibitor, for 2 hours prior to freezing and/or in the postthaw culture media affects embryo viability. We hypothesized that ROCK inhibition improves postthaw viability (reexpansion, hatching, and lack of degeneration) in slow-cooled cryopreserved embryos. In vitro produced bovine embryos (n = 448) in the early or expanded blastocyst stage were randomly assigned to 1 of 5 groups. The nonfreeze group (n = 104) was loaded into straws containing freezing media but was not cryopreserved. The control-control group (n = 97) were cryopreserved in CRYOLOGIC slow-rate freezer and represents the standard cryopreserved embryo. Remaining 3 groups were cryopreserved in the same way with ROCKi exposure prefreezing only (ROCKi-control) (n = 84), ROCKi postthaw only (control-ROCKi) (n = 79), or ROCKi both prefreezing and postthaw (ROCKi-ROCKi) (n = 84). Embryos were observed postthaw for 48 hours using time-lapse videography. Outcomes measured included: proportions of embryos that reexpanded, hatched, or degenerated. Data were analyzed using logistic regression ANOVA, and interval to reexpansion and hatching data were analyzed using Cox's proportional hazard model. All statistical analysis was performed in JMP Pro v16. Group and replicate were included in all models and significance was set at $p < 0.05$. Reexpansion rates (%) were: 99.0^{a}, 75.3^{b}, 84.5^{ab}, 92.4^{a}, and 94.0^{a}; hatching rates (%) were: 76.9^{a}, 59.8^{b}, 59.5^{b}, 73.4^{ab}, and 80.1^{a}; and degeneration rates (%) were: 8.7^{a}, 26.8^{b}, 17.9^{b}, 8.9^{a}, and 7.1^{a} for nonfreeze, control-control, ROCKi-control, control-ROCKi, and ROCKi-ROCKi groups, respectively, with different superscripts representing the respective significant differences. Risk ratios (representing how quickly embryos reached the outcomes) for reexpansion was highest for nonfreeze^{a}, followed by ROCKi-ROCKi^{b}, control-ROCKi^{c}, ROCKi-control^{cd}, then control-control^{d}. Risk ratios for hatching was highest for ROCKi-ROCKi^{a}, followed by nonfreeze^{ab}, control-ROCKi^{bc}, ROCKi-control^{cd}, then control-control^{d}. Exposure of embryos to ROCK inhibitor prefreeze and postfreeze resulted in the best postthaw outcome with reexpansion, hatching and degeneration rates similar to uncryopreserved embryos and significantly better than the control-control group without supplementation. Exposure to ROCK inhibitor postthaw appeared to have a greater benefit compared to exposure only prefreezing. These results suggested that ROCK inhibitors may be beneficial for postthaw survival; however, embryo transfer trials are required.

Keywords: Cattle, embryo, slow-rate freezing, rho kinase inhibition, cryodamage

Uterine inflammatory response after misoprostol during estrus and diestrus in commercial embryo donor mares

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Uterine tube obstruction is the leading cause of unexplained subfertility in mares. Over the years, numerous techniques were developed to unblock uterine tubes with variable success. In recent years, misoprostol, a synthetic analog of prostaglandin E1 (deep uterine horn treatment with a flexible pipette) has regained popularity in equine practice. However, there have been some concerns regarding rare anaphylactic reactions in some mares and an exacerbated postinfusion uterine inflammatory response to misoprostol; however, this has yet to be critically studied. In addition, debate exists whether mares should receive misoprostol in diestrus or estrus, with the latter offering the advantage of potentially unblocking the mare close to breeding. This study aimed to determine the uterine inflammatory response in mares after infusion with misoprostol during estrus or diestrus. We hypothesized that misoprostol infusion induces a greater inflammatory response than sham infusion; similarly, estrus results in a greater inflammatory response than diestrus. Forty-four estrous cycles of light breed mares (n = 11) were sequentially randomly assigned to receive misoprostol or sham infusion during estrus and diestrus. The misoprostol and sham infusions were performed by directing a flexible pipetted (Minitube, Germany) deep into 1 uterine horn, and then the same pipette was redirected to the opposite side uterine horn. Each uterine horn received 200 µg of misoprostol diluted in 3

ml of lactate Ringer's solution (LRS), whereas the sham inoculation consisted of 3 ml of LRS. The cycles assigned for estrus treatment received it when preovulatory follicles ranged from 28 - 32 mm, and diestrus cycles were treated 8 days postovulation. The day after each infusion, mares had uterine lavage performed with 2 liters of LRS. Transrectal ultrasonography of the reproductive tract was performed to quantify uterine edema and intrauterine fluid accumulation immediately before (0), 24, 48, and 72 hours after treatments. At similar time points, uterine cytology samples were harvested to count the number of neutrophils in a high-power field (40 x). Once a ≥ 35 mm follicle and edema were first detected, ovulation was hastened with deslorelin (1 mg intramuscular) during estrus or the subsequent estrus after diestrus infusion. Mares received 5 mg of intramuscular dinoprost 24 hours after infusion in estrus and diestrus. Statistical analyses were performed with Graph Prisma. Neutrophil counts were analyzed with ANOVA repeated measures and Tukey's as post-hoc. Edema scores and intrauterine fluid accumulation were analyzed with Kruskal-Wallis and Dunn's post-hoc. Embryo recovery rates were evaluated with Fisher's test. There were effects of time ($p = 0.04$) but no effects of group ($p = 0.96$), or stage of the estrous cycle ($p = 0.26$), or interaction between group and time for edema scores ($p = 0.97$). Similarly, there were effects of time ($p = 0.03$) but no effects of group ($p = 0.32$), stage of the estrous cycle ($p = 0.86$), or interactions between group and time or stage of the estrous cycle for neutrophil counts ($p = 0.87$). Uterine fluid scores did not change over time ($p = 0.39$), groups ($p = 0.22$), or stage of the estrous cycle ($p = 0.77$). Embryo recovery was similar for estrus (Misoprostol 46 versus sham 46%) versus diestrus (Misoprostol 67 versus sham 46%) ($p > 0.05$). In conclusion, 200 µg of bilateral misoprostol treatment did not exacerbate uterine inflammation in mares.

Keywords: Mares, uterine tube, infertility, prostaglandin E, endometritis

Effect of sexual rest on cooling and freezing ability of stallions

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Prolonged sexual rest, particularly during winter, is apparently detrimental to semen quality, yet it is common to collect and ship or freeze semen from sexually rested stallions. When a sexually rested stallion is presented in a hasty situation, practitioners harvest and evaluate the first ejaculate, and based on quality, the semen is extended (1:1) and kept at room temperature while a second collection is attempted in approximately 1 hour, if the stallion's libido and physical capabilities allow. If the second ejaculate has better quality, practitioners use it for further processing. If both ejaculates have identical marginal quality, either or both ejaculates are processed further. Although this approach is routinely applied, there is little evidence to support it. Thus, this study aimed to assess the cooling and freezing abilities of sexually rested stallions collected 1 hour apart early in the breeding season. We hypothesized that the second ejaculate of the season has superior cooling and freezing ability compared to the first ejaculate. Stallions (n =10) were collected twice, 1 hour apart, on a dummy mount with a teaser mare present. Gel-free volume, concentration, total sperm ejaculated, and percentage of normal sperm were determined after collection. Gel-free semen was extended at 50 x 10^6/ml in INRA96; part of it was immediately stored, and the remainder was cushion-centrifuged, with the pellet resuspended in INRA96 at 50 x 10^6/ml. Extended and resuspended semen were stored in Botuflex containers for further evaluations at 24 and 48 hours. After cushion-centrifugation, half of the pellets were resuspended in Botucrio at 200 x 10^6/ml, loaded in 0.5 ml straws, and frozen over liquid nitrogen. Total motility (TM) and progressive motility (PM) were evaluated with CASA. Sperm membrane integrity (SMI) and mitochondrial membrane potential (MMP) were assessed with Zombie Green and Mitotracker Deep-Red via spectral flow cytometry. Sperm parameters were assessed at 0, 24, 48 hours of cooling and before and after thawing. A thermal longevity test was conducted on postthaw semen for 4 hours, with motility assessment every 30 minutes. Data were analyzed with paired Student's *t*-test, linear mixed model, and Tukey's post-hoc. Significance was set at $p < 0.05$. Gel-free volume was not different ($p > 0.05$) between first 77.7 ± 10.8 ml and second 64.0 ± 6.7 ml ejaculates. Sperm concentration (182.4 ± 35.1 x 10^6/ml versus 93.1 ± 1 x 10^6/ml), and total sperm ejaculated (11.4 x 10^9 versus 6.0 x 10^9) decreased by half between first and second ejaculates, respectively ($p < 0.05$). Morphologically normal sperm did not vary ($p > 0.05$) between ejaculates (68.3 ± 6.9 versus 74.0 ± 6.6%). There were no effects ($p > 0.05$) of time, of ejaculates, or their interaction on TM, PM, and SMI. Processing semen through cushion-centrifugation was beneficial and increased ($p = 0.02$) TM, PM, SMI compared to noncentrifuged semen. MMP was not affected ($p > 0.05$) by any factors or interactions. TM, PM, and SMI were reduced ($p < 0.05$) postthaw and during the thermal longevity test; however, there were no differences ($p > 0.05$) between ejaculates or interactions. In conclusion, semen had adequate cooling and freezing ability despite sexual rest. Cushion-centrifugation was a suitable technique to process semen from sexually rested stallions.

Keywords: Stallion, sexual behavior, semen quality, semen cryopreservation

A novel sperm filtration system based on microfluidics

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Objective was to compare equine semen parameters before and after filtration in a novel device based on microfluidics (VetCount™ Harvester). Hypothesis was that microfluidic-based filtration with the device improves semen parameters related to sperm quality. Pilot prospective analyses were performed in stallion semen samples. Semen samples evaluated originated from commercially frozen batches from Standardbred, Arabian, and Warmblood stallions. The tested VetCount™ Harvester

device contains a filter with 10 µm micropores, and the filtration of sperm across the filter is based on fluid dynamics and active movement. The device allows 1.0 ml of frozen thawed semen sample to be filtered into 0.8 ml of skim milk-based extender. Samples (n = 55) were all filtered using the device. Concentration, percentage of progressive motile sperm (PMS), viability using propidium iodide, and percentage of morphological normal sperm were evaluated before and after filtration of the samples. Acrosome damage and DNA fragmentation analyses were performed on a smaller sample size (n = 20). Sperm concentration and viability were evaluated using Nucleocounter® SP-100. Percentage of PMS and morphologically normal sperm, acrosome damage and DNA fragmentation were evaluated using CASA AndroVision®. Wilcoxon Signed-Rank tests were performed to assess statistical differences for sperm outcomes before and after filtration. Data were presented as median (25 and 75% percentiles). Significance was set at $p < 0.05$. Median sperm concentration was 259 x 10^6/ml (176 -368) before filtration and 18.4 x 10^6/ml (13 - 29.1) after filtration. Following parameters improved significantly after filtration through the VetCount™ Harvester device: PMS from 30 (12.9 - 36.6) to 76.0% (69.7 - 83.1) ($p < 0.0001$); viability (from 38.1 (30.0 - 46.0) to 59.0% (47.0 - 71.0) ($p < 0.0001$); morphologically normal sperm from 52.0 (45.0 -59.0) to 81% (76.0 - 86.0) ($p < 0.0001$); acrosome damaged sperm from 16.6 (11.3 - 20.2) to 2.5% (1.7 - 4.2) ($p = 0.0005$); and sperm with DNA fragmentation from 6.38 (5.61 - 10.6) to 0.9% (0.6 - 1.42) ($p < 0.0001$). Recovered volume from the device was 0.7 ml. Extracted semen samples contained 14.6 % (8.7 - 22) of PMS in the native sperm samples. Total number of PMS after filtration was 9.9 x 10^6 (5.8 - 15.9 x 10^6). Percentage of PMSs, sperm viability, sperm morphology, sperm with acrosome damage and sperm with DNA fragmentation were substantially improved in a frozen-thawed semen sample using the filtration technique in the microfluidic-based device. In practice, the device does not allow filtration of dead and damaged sperm through the micropores in the filter. This provides an improved semen sample to inseminate the mare without the negative impact of dead and damaged sperm to the uterine environment. VetCount™ Harvester device is presented as a new technique that in the future may improve the fertility of frozen equine semen samples for artificial and can improve the quality of a semen sample for insemination or other assisted reproductive techniques (e.g., intracytoplasmic sperm injection).

Keywords: Stallion, semen quality, sperm, microfluidic-based filtration

A novel method for oocyte recovery from equine postmortem ovaries

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Equine postmortem gamete recovery frequently occurs in the equine industry and is considered an emergency procedure. Obtaining postmortem oocytes provides owners with a last chance to further the genetic line of their mare and obtain valuable offspring. Currently, the industry standard for oocyte recovery uses varying sizes of bone curettes to scrape the interior follicular walls to manually detach the tightly attached cumulus oocyte complex (COC). This study aimed to evaluate a new method for oocyte recovery from equine postmortem ovaries using Tenex technology, a minimally invasive procedure used to perform a percutaneous tenotomy of fasciotomy. We hypothesized that the use of Tenex technologies to ultrasonically detach the COC from the follicular wall while maintaining the structure and integrity of the oocyte is an effective method for postmortem equine oocyte recovery. Ovaries were obtained through scheduled euthanasia and ovariectomy procedures in mares. Ovaries were extracted immediately after euthanasia or obtained shortly after ovariectomy procedures and processed within 24 hours after collection. Ovaries were stored in a passive cooling device in an effort to maintain room temperature (~ 22°C) until processing occurred. A single ovary from each mare's pairs were randomly assigned to either a control or treatment group. Both ovaries were trimmed of excessive fascia prior to processing and commercial embryo flush media was utilized. Ovarian follicles on control group ovaries were processed traditionally, through manual scraping with bone curettes. Curettes were rinsed thoroughly into a 50 ml conical tube filled with embryo flush media. Treatment group ovaries were processed with Tenex technology to aspirate individual ovarian follicles using a double lumen needle with ultrasonic energy and irrigation capabilities. With built-in suction, the machine allowed for contents to be collected into a 250 ml sterile plastic bottle. The commercial flush media collected from both control and treatment groups were placed into separate petri dishes to facilitate COC identification under dissecting microscopes. Data were analyzed using a one-way ANOVA test with significance set at $p \leq .05$. The average period spent processing control and treatment ovaries was 37.50 minutes and 34.11 minutes, respectively. Treatment ovaries processing were 3.39 minutes shorter than control ovaries, although not significant ($p = 0.342$). An average of 3.25 COCs were recovered from control ovaries (45.70%) and an average of 2.17 COCs were recovered from treatment ovaries (33.10%) that was significant ($p = 0.006$). Oocyte structure and integrity were not affected by this method of oocyte recovery. This study evaluated an alternative method for equine oocyte collection from postmortem ovaries. Although the oocyte recovery rate did not currently support transitioning to this method in particular, novel methods should continue to be explored in an attempt to improve successful outcomes.

Keywords: Mae, postmortem, ovary, oocyte, gamete

Gene expression in day 13 postovulation pregnant and nonpregnant mare endometrium

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Equine embryo has maximal mobility between days 11 - 14 postovulation to deliver signals to the endometrium for pregnancy maintenance. Embryonic factor(s) signal to the uterus to turn 'on' or 'off' genes to stop luteolysis in an intricate feto-maternal interaction. Insulin-like growth factor binding protein 1 (IG-

FBP1) is a uterine receptivity marker in the cow and sheep, and its expression is increased by prostaglandin E2 and prostacyclin (PGI2). Prostaglandin I2 synthase (PTGIS) and prostaglandin I2 receptor (PTGIR) have been characterized in ruminants and pigs; however, such investigation of prostaglandin-related genes is lacking in the horse during early pregnancy. We hypothesized that the relative expression of prostaglandin-related genes differs in the endometrium of pregnant mares compared to non-pregnant mares at day 13 postovulation. Objective was to better understand the role of prostaglandins in the endometrium during early pregnancy. Light breed mares (n = 13) were examined daily via transrectal palpation and ultrasonography from estrus to the day of ovulation (D0), and randomly assigned to nonpregnant (NP; n = 7) group or pregnant (P; n = 6) group. Mares assigned to P were bred using artificial insemination with > 500 x 10^6 normal and progressively motile sperm from a single fertile stallion. On day 13 postovulation, a uterine lavage was performed to retrieve the conceptus, and an endometrial biopsy was collected, snap frozen in liquid nitrogen, and stored at –80°C until analysis. Total RNA was extracted from endometrial biopsies and evaluated for expression of following genes: *IGFBP1*, *IGFBP2*, *NR3C1*, *CREB*, *CRTC2*, *PPAR-g*, *PTGES*, *PTGS2*, *PTGER2*, *PTGER4*, *PTGFS*, *PTGFR*, *mPGES2*, *PTGIS*, and *PTGIR* using real-time PCR. Mean threshold cycle (Cq) was determined and then normalized to the reference gene (*GAPDH* and *ACTB*) (ΔCT). Statistical analyses were performed with GraphPad Prism 9 using a Student's *t*-test or Mann-Whitney test to compare differences between NP and P endometrium at $p < 0.05$. Pregnant mare endometrium had higher ($p = 0.005$) expression of *IGFBP1* compared to NP and *PTGIS* expression was slightly higher ($p = 0.09$) in P mares. NP endometrium had higher ($p = 0.03$) expression of *PTGIR* compared to P endometrium The remainder of the genes had no significant differences. IGFBP1 increases during pregnancy in the horse. This protein can increase the availability of growth factors within the endometrium and the uterine lumen, helping to support endometrial and conceptus growth in preparation for fixation of the conceptus. Contrary to our results, in pregnant pig endometrium, *PTGIR* expression increases compared to cycling animals. PGI2 has a role in cAMP signaling and angiogenesis in pig uterus, with an increase during early pregnancy, suggesting a possible regulation by embryonic factors. Further investigation on protein concentrations and functional roles of *IGFBP1* and *PTGIR* in the equine endometrium and conceptus at various points in early pregnancy is warranted that may help to clarify the differing results observed in horse and pig.

Keywords: Pregnant mare, insulin-like growth factor binding protein, prostaglandins

Genital mycoplasma prevalence in the healthy and subfertile breeding dog

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Canine reproductive failure can be the result of poor breeding timing, genetic incompatibility, inflammation, or infectious disease. Whereas bacteria (e.g., *Brucella canis*) cause reproductive failure, mollicutes (e.g., *Mycoplasma*) have a more controversial role in canine infertility. At least 17 *Mycoplasma* spp. have been isolated from dogs, with *M. canis* and *M. cynos* as the most common isolates. *M. canis* is considered an opportunistic pathogen whereas *M. cynos* is a proven respiratory pathogen. We hypothesized that a similar pattern would be noted in breeding dogs, with the presence of *M. cynos* in semen or vaginal samples decreasing fertility, whereas the presence of *M. canis* not impacting pregnancy rates. Objectives were to determine *Mycoplasma* prevalence in the population of dogs presenting for breeding management to the University of Georgia Veterinary Teaching Hospital and to determine pregnancy rates in *Mycoplasma*-positive dogs. Dogs (n = 13) presented with a history of infertility and dogs (n = 65) presented for routine breeding management were enrolled. For each dog, 100 µl of semen (n = 31), or a vaginal cytology swab (n = 47) were collected during routine breeding management. Nucleic acids were extracted using a QIAamp cador Pathogen Kit (Qiagen, Hilden, Germany) and a QIAcube automated nucleic acid extraction system (Qiagen) and all samples were analyzed for *M. cynos* and *M. canis* using a previously validated multiplex real-time PCR assay. Pregnancy rates between positive and negative dogs were compared by Student's *t*-test. Three of 13 dogs presented with a history of infertility were positive for *M. cynos*, and 3/13 were positive for *M. canis*. Of 65 dogs presented for routine breeding management, *M. canis* was detected in 11.9% (5/42) of bitches and 12% (3/25) of stud dogs. *M. cynos* was detected in bitches and 45.8% (11/25) of stud dogs presenting for routine breeding management. When bitches were bred with semen samples with a known mycoplasma status, 50% (5/10) bred with semen positive for *M. cynos* conceived, 0/2 positive for *M. canis* conceived, and 89% (8/9) conceived with known negative samples. Of *Mycoplasma*- negative bitches that were subsequently bred, 68% (17/25) conceived, whereas 0/6 *M. canis*-positive bitches conceived, and 0/1 *M. cynos* positive bitches conceived. Collectively, this suggests that both *M. cynos* and *M. canis* may contribute to bitch infertility. Contrary to our hypothesis, *M. cynos* was present in almost half of canine ejaculates, and the presence of *M. cynos* in semen samples decreased conception rates ($p = 0.035$), but did not prevent pregnancy in the bitch. Whereas no bitches positive for *Mycoplasma spp.* conceived, and no bitches bred with *M. canis* positive semen conceived, there was a low prevalence of *Mycoplasma*-positive bitches in the breeding population, and follow up studies are recommended. In conclusion, both *M. canis* and *M. cynos* were detected in genital samples of both the healthy and infertile bitch and stud. Whereas conception rates were lower when *Mycoplasma* was detected in either the bitch or the stud, presence of *M. cynos* in the semen still resulted in 50% conception in the bitch.

Keywords: Dogs, fertility, pregnancy

Next-generation sequencing correlation with cultured samples

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Culture is the gold standard for pathogen detection and identification; however, many organisms fail to be cultured. Fungal culture may be time-consuming, and overgrowth of bacteria may prevent diagnosis of fungal agents. Next-generation sequencing (NGS) is a powerful method for detecting and identifying the presence of microbial DNA in samples. Studies comparing these 2 methodologies in equine uterine samples are lacking. Objective was to determine the correlation between fungal and bacterial culture results with NGS of the same swab used for the cultures. Purpose was to test the hypothesis that NGS and cultures are strongly correlated and NGS may provide a broader picture of the uterine microbiome. Uterine swabs (n = 63) from mares submitted for fungal culture with or without bacterial culture to the Cornell Animal Health Diagnostic Center were used. The same swab used for culture was frozen at – 20 °C and submitted in a single batch for NGS of the internal transcribed spacer region for fungal and 16S ribosomal RNA gene for bacterial organisms through a commercial molecular diagnostic laboratory (MicrogenVet). For NGS, fungal and bacterial species were reported if the organism comprised at least 2% of the identified organisms. Kappa coefficient was calculated using SAS v9.4 and interpreted as excellent (> 0.75), fair to good (0.40 - 0.75) or poor (< 0.40). Fungal cultures (n = 63) were assigned to 3 groups: positive, negative, and contaminated (bacterial overgrowth prevented diagnosis of fungal agents). None of the 3 contaminated samples had fungal DNA by NGS. Of the 16 positive fungal cultures, 5 samples did not identify fungal agents on NGS, 2 samples identified other species of fungal agents, and for 8 (50%) samples, NGS identified the same organism as culture. Of the 44 negative samples, 5 samples identified fungal organisms by NGS that may be due to potential higher sensitivity of detection, whereas 39 negative samples (88.6%) had agreeing NGS results. Fungal culture and NGS had 78.3% agreement. The Kappa coefficient for fungal culture and NGS was 0.41. Bacterial culture results (n = 54) were assigned to 3 groups: positive, no growth, and no significant organisms (growth of bacteria classified as nonpathogenic). Of the 29 positive bacteria culture samples, NGS identified the same organism in 22 (75.9%) samples and NGS identified different organisms in the remaining 7 samples. NGS detected bacterial presence in 5/14 of no growth samples. Of the 11 samples with no significant organisms, 2 samples had bacterial DNA from pathogens. The agreement between bacterial culture and NGS was 77.8% with a Kappa coefficient of 0.56. In conclusion, culture and NGS had fair to good correlation, and NGS has potential as a valuable diagnostic option for equine endometritis.

Keywords: Mare, endometrium, culture, next-generation sequencing, microbiome

Antimicrobial activity of nonbiological alternative therapies against important microorganisms causing endometritis in mares

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Endometritis is the leading cause for antibiotic usage in mares, and their indiscriminate use selects microorganisms resistant to it; thus, the development of alternative therapies is needed. This study aimed to assess the in vitro antimicrobial activity of nontraditional therapies (NTT) against microorganisms isolated from mares with endometritis. We hypothesized that NTT antimicrobial activity varies with the type of agent and microorganism. The NTT evaluated included a commercial anti-septic product (Botukiller,® BK, Botupharma, Brazil); lactated Ringer's solution (LRS as controls), LRS with 10% H_20_2, ozonated LRS (O3, 60 µg/ml of ozone gas for 10 minutes), Coca-Cola® (CC), and Coca-Cola-Zero-sugar® (CZ). The percentage of inhibition (PI) and minimum inhibitory concentrations (MIC) of each NTT was performed with the microdilution broth method using 96-well flat-bottom microplates. Clinical isolates of *Streptococcus equi* (*Strep*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*Pseudo*), *Klebsiella pneumoniae* (*Kleb*), *Staphylococcus aureus* (*Staph*), and *Candida albicans* (*Candida*) cultured from mares with clinical endometritis were used in the study. Each NTT sample was run 3 times, and within each run, 3 wells were used per microorganism. The first 3 lanes (1 - 3) of each plate served as the negative controls that consisted of 100 µl NTT and 100 µl of Mueller-Hinton (MH) (1:1). Thereafter, the remaining lanes (4 - 12) contained serial dilutions of NTT as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. Row H (lanes 4 - 12) served as positive controls and contained no NTT. After that, 5 µl (5 x 10^4 colony-forming unit) of each isolate was added to lanes 4 - 12. Then plates were incubated at 37 °C for 24 hours. The optical density of the wells was measured at 570 nm using a SpectraMax M2 spectrophotometer (Molecular Devices, San Jose, CA). The PI was calculated by the difference between the mean of the triplicates in each group and the mean of the negative control and the positive control. To determine the MIC, 10 µl of resazurin solution (0.01%) was added to each well, and the plates were kept at 37 °C for 1 hour Subsequently, a visual reading was performed to determine microorganism activity; if the well became pink, microbial activity was present, whereas no microbial activity was present if the well remained blue. Data were evaluated by ANOVA and Tukey's post hoc test. Significance was set at $p < 0.05$. There was a reduction ($p < 0.05$) in PI when compared to the positive controls for the different NTT. Specifically, H_2O_2 PI's were 1/8 *E. coli and Pseudo*, 1/16 *Staph*, 1/32 *Candida*, and 1/64 *Kleb*. The PI results for BK were 1/2 *Strep*, 1/4 *Staph*, 1/8 *E.coli*, 1/16 *Pseudo*, 1/32 *Kleb*, and 1/64 *Candida*. The O3 results were 1/2 *Staph*, *E.coli*, and *Kleb*. The PI for CZ was 1/4 *E.coli*. The NTT having MIC values were BK 1/2 *Pseudo*; 1/4 *Staph; Kleb; Candida*; 1/8 *E.coli*; and H_2O_2 1/8 *E.coli; Pseudo*, 1/32 *Kleb* and *Candida* and 1/64 *Staph*. CC had no antimicrobial activity. In conclusion, of all NTT tested in the present study, BK and H_2O_2 had the highest in vitro antimicrobial activity against microorganisms causing endometritis in mares. Furthermore, the dose-dependency suggested a different efficacy against the 6 infectious agents tested here.

Keywords: Mare, endometritis, alternative antimicrobials, bacteria, fungus

Retrograde flushing followed by slicing-floating optimized epididymal sperm recovery in donkeys and horses

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Harvesting epididymal sperm often represents the last opportunity of preserving the genetic material of a valuable sire. It can be accomplished via retrieval by retrograde flushing or by slicing the tail of epididymis and floating sperm into an extender. Our clinical experience suggests that starting epididymal flushing with retrograde flushing followed by slicing-floating optimizes sperm recovery during cryopreservation; however, this has not been critically studied. This study aimed to assess the sperm parameters of epididymal sperm harvested by applying both techniques on the same epididymis of horses and donkeys. We hypothesized that the combination of 2 techniques increases the total number of sperm harvested without affecting the semen quality. Epididymides from donkeys (n = 16) and horses (n = 20) were harvested after castration or emergency euthanasia. The tails of the epididymis were dissected and weighed. Each tail was subjected to retrograde flushing with 5 - 10 ml of freezing extender (Botucrio). After the extender, 20 - 25 ml of air was used to push the remaining extender and semen into the collection tube. The epididymis was then sliced in 1 - 2 mm pieces, covered with 5 - 10 ml of freezing extender, and left in incubation for 15 - 20 minutes at room temperature. Then semen was filtered through a gauze and recovered in a conical tube. Recovered sperm from both techniques were evaluated separately for volume, concentration, and total sperm count. Thereafter, sperm concentration was adjusted to 200 × 10^6/ml, loaded in 0.5 ml straws, frozen, and then plunged into liquid nitrogen. Thereafter, the samples were thawed at 38°C for 60 seconds. Postthaw total motility (TM) and progressive motility (PM) were evaluated with CASA. Sperm membrane integrity (SMI) and mitochondrial membrane potential (MMP) were assessed with Zombie Green and Mitotracker Deep Red via spectral flow cytometry. Data analyzes were carried out via mixed model in R. Significance was set at $p < 0.05$, a tendency at $0.1 < p < 0.05$. Donkey epididymis weighed 19.8 ± 3.2 grams and the horse 16.2 ± 2.2 grams. The concentration tended to be lower ($p = 0.09$) in the horse than in the donkey (764 ±5 9 ×10^6/ml versus 549 ± 68.5 × 10^6/ml) Total sperm harvested was affected by both technique, species, and their interactions ($p < 0.05$); the slicing technique (4.5 × 10^9 versus 10.9 × 10^9) and the horse (5. 5× 10^9 versus 10.6 × 10^9) yielded a lower amount of sperm ($p < 0.05$). Use of retrograde flushing followed by slicing-floating resulted in 64 and 43% more sperm per harvest, in the donkey and horse, respectively. TM and PM before freezing were not influenced ($p > 0.05$) by the type of technique or by the species. After thawing, SMI and MMP were not affected ($p > 0.05$) by the technique or the species. TM and PM were not influenced ($p > 0.05$) by the technique or the species but by their interaction ($p = 0.005$); the slicing technique applied to the horse resulted in lower ($p < 0.05$) TM and PM. Results suggested that the combination of both techniques was superior to a single technique, and a suitable method to optimize epididymal semen harvest in donkey and horse.

Keywords: Conservation, epididymal sperm, cryopreservation

Effects of different treatment approaches on microbial populations in vaginal discharge of cows with clinical metritis

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Clinical metritis (CM) has many costs to the dairy producer, including decreased milk production, reduced reproductive performance, and treatment costs. The more commonly isolated bacterial species in CM cases are *Escherichia coli*, *Fusobacterium necrophorum*, and *Prevotella* spp. The current treatment strategy involves systemic antibiotics, typically penicillin or a third-generation cephalosporin; however, there is increasing public health concern about judicious use of antibiotics and prevention of drug-resistant pathogens. Objective was to evaluate the effects of various treatment approaches, a nonantibiotic treatment (intrauterine dextrose) versus systemic antibiotic treatment (subcutaneous ceftiofur) on the microbiome of the vaginal discharge of dairy cows diagnosed with CM at 7 ± 3 days in milk. We hypothesized that the clinical cure rates are similar between 2 treatment groups, and therefore, their microbial populations are also similar. Cows were enrolled from a dairy farm in central Pennsylvania and were screened for CM with a

Metricheck® device at 7 ± 3 days after calving. Cows presenting with reddish-brown fetid watery discharge were diagnosed with CM and eligible for enrollment. Eligible cows were blocked by parity and randomly allocated to 1 of 2 treatments: i. intrauterine dextrose (DEX): cows received 1 liter of an intrauterine 50% dextrose solution for 3 days starting on the day of diagnosis; and ii. systemic ceftiofur (CONV): cows received 2 injections of ceftiofur (6.6 mg/Kg of BW; Excede, Zoetis Inc.) 72 hours apart, starting on the day of diagnosis. Cows were evaluated for clinical cure rate at 7 and 14 days after diagnosis. Vaginal discharge samples were collected using a Metricheck® device at enrollment day (study day 0, before treatment), day 7, and day 14 for a subset of enrolled cows (DEX = 13, CONV = 14). Vaginal discharge samples were analyzed for 16S rRNA gene sequencing to evaluate changes in the microbiome between treatment groups. Clinical cure rate between the 2 treatment groups was not different (day 7, p = 0.56; day 14, p = 0.69, GLIMMIX procedure of SAS, Cary, NC).[1] Alpha diversity (richness of microbes within each sample) did not differ (Welch's t-test) between 2 treatments at any of the 3 time points or between pre and posttreatment. Beta diversity (comparison of microbial communities between cows) based on permutational multivariate ANOVA analysis differed between treatment groups at time of diagnosis (p = 0.024) and again at the second recheck (p = 0.015), but not at the first recheck (p = 0.112). When analyzing differential relative abundance through Wald's test, bacteria of the *Prevotella* and *Fusobacterium* genera were more abundant in the vaginal discharge of the CONV group compared to DEX group at the first recheck, though at the time of diagnosis there was increased relative abundance of *Prevotella* in the CONV cows and *Fusobacterium* in the DEX cows. Whereas 16S rRNA analysis did not provide information on the viability of these bacteria, these changes in relative abundance paired with the similar clinical cure rate of the DEX group compared to the CONV group provided evidence that dextrose may be a low-cost antibiotic alternative treatment for CM in dairy cows.

Keywords: Antibiotic alternatives, cattle, dextrose, metritis, microbiome, postpartum

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Canine splenic hemangiosarcoma cells express luteinizing hormone receptors in vitro

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Hemangiosarcoma is a rapidly growing, highly invasive cancer arising from the lining of blood vessels. More than half of all canine hemangiosarcoma primary tumors arise within the spleen. Compared to other breeds, German shepherds, Golden Retrievers, and Labrador Retrievers are overrepresented. In addition to breed, gonadectomy (spaying/neutering) increases the likelihood for developing hemangiosarcoma. Spayed female dogs have 2 - 10 times the risk for developing splenic hemangiosarcoma compared to intact female dogs. Following gonadectomy, luteinizing hormone (LH) concentrations are significantly and persistently elevated. Our laboratory has previously demonstrated that formalin-fixed canine splenic hemangiosarcoma tissues express LH receptors (LHR). We hypothesized that isolated canine splenic hemangiosarcoma cells also express LHR. Objective was to use immunocytochemistry to determine the percentage of cells expressing LHR in each cell line. Immortalized cell lines isolated from 4 dogs with a primary splenic hemangiosarcoma (DAL-4, DHSA, GRACE-HSA, EFS; Kerafast, Inc., Boston, MA) were used. Cells were fixed onto coverslips and incubated with either a rabbit polyclonal antihuman LHR antibody (#NLS1436, Novus Biologicals, Littleton, CO) or a universal negative control (#NC498H, BioCare Medical, Pacheco, CA). Cells were then incubated with horse antirabbit IgG conjugated to FITC (#NB 7159, Novus Biologicals). The coverslips were then inverted and mounted to slides with medium containing DAPI (#H-1500, Vectashield® HardSet™ Antifade Mounting Medium with DAPI, Burlingame, CA). Three randomly selected fields from each cell line were captured using fluorescence microscopy (Leica Microsystems, Germany) at 400 x magnification. The mean ± SD percentages of LHR positive cells for each cell line was compared by one-way ANOVA (GraphPad Prism 8.4.3) and significance was defined as $p < 0.05$. Percentages of cells positive for LHR were 6.9 ± 2.5, 8.5 ± 1.0, 11.8 ± 3.1, and 17.2 ± 4.5% in DAL-4, DHSA, GRACE-HSA, and EFS, respectively. Percentage of LHR positive cells was greater (p = 0.0142) in EFS compare DHSA and DAL-4. This is the first study to report LHR expression in isolated canine hemangiosarcoma cells. Our laboratory is currently examining the effect of LHR activation on hemangiosarcoma cell proliferation with increasing concentrations of LHR agonists. Since LHR induced splenic hemangiosarcoma cell proliferation, future clinical trials could begin using GnRH agonists to reduce LH concentrations in gonadectomized dogs to prevent the development or recurrence of hemangiosarcoma.

Keywords: Dog, cancer, gonadectomy, immunocytochemistry

Validating thyroid hormone testing using chemiluminescence in llamas and alpacas

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Hypothyroidism is an endocrine disease in llamas and alpacas resulting from insufficient concentrations of thyroid hormones. Hypothyroidism is a cause of infertility in female camelids.[1] Elevated thyroid stimulating hormone (TSH) concentrations with normal thyroxine (T_4) concentrations are early signs of hypothyroidism.[2] Unfortunately, hypothyroidism is rarely diagnosed in camelids because of a lack of published normal ranges. Objectives were to: i. validate a commonly used chemiluminescence assay for determination of camelid TSH and T_4 concentrations; and ii. establish normal ranges for TSH and T4 concentrations in male, gelded, and female camelids. It was hypothesized that the chemiluminescence assay accurately measures TSH and T_4

concentrations in healthy camelids. To validate the precision of the IMMULITE 1000 (Siemens) assay for TSH (#TK9) and T_4 (#KT4), 7 pooled samples of varying concentrations from each species were run in octet. To validate the linearity of the TSH assay, pooled samples of varying concentrations were run in triplicate and then spiked with a known concentration and run in duplicate. To validate the linearity of the T_4 assay, pooled samples of varying concentrations were run in triplicate and then spiked or diluted with known concentrations and run in quadruplet or triplicate, respectively. Data on pooled samples before and after spiking or dilution were compared using linear regression. Following validation, TSH and T_4 concentrations were measured on archived serum samples from healthy, adult llamas and alpacas (n = 125). Coefficients of variation for precision of the assay were < 9%. The correlation coefficient for linearity was high for all pools tested (R^2 ranged from 0.94 - 0.99). Normal ranges (mean ± 2 standard deviations) of TSH and T_4 concentrations were established for alpacas (TSH- males (n = 16): 21.9 - 86.8 pg/ml; geldings (n = 9): 12.3 - 95.3 pg/ml; females (n = 12): 20.4 - 99.1 pg/ml; T_4 males (n = 8): 3.59 - 10.8 µg/dl; geldings (n = 10): 3.65 - 7.88 µg/dl; females (n = 9): 3.04 - 7.71 µg/dl) and llamas (TSH- males (n = 29): 19.3 - 118.0 pg/ml; geldings (n = 43): 21.1 - 106.0 pg/ml; females (n =14): 13.7 - 158.0 pg/ml; T_4 males (n = 9): 3.75 - 7.73 µg/dl; geldings (n = 9): 5.17 - 9.94 µg/dl; females (n = 9): 4.22 - 11.2 µg/dl). This is the first study to validate a method for measuring TSH concentrations in camelids. Further research is needed to evaluate what role abnormalities in T_4 and TSH concentrations have in camelid subfertility.

Keywords: Camelid, hypothyroidism, precision, thyroid stimulating hormone, thyroxine

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Endometrial injection of mesenchymal stem cells may revert endometriosis in mares

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Endometriosis is characterized by degenerative changes of the endometrium leading to poor conception rates and higher pregnancy losses. Chemically irritant agents (e.g., kerosene) have been proposed to revert endometriosis; however, animal welfare concerns and questionable results have discouraged many practitioners from using it. Conversely, stem cell therapy has emerged as a promising tool in regenerative medicine. Therefore, this study aimed to determine the potential regenerative effects of stem cells therapy injected directly into the endometrium of mares with endometriosis. We hypothesized that mesenchymal stem cells (MSCs) improve endometrial health of mares with endometriosis. Utilizing the Kenney-Doig scale, light breed mares (n = 15) with endometrial biopsies classified as grade IIB or III were enrolled. Ten mares were treated with an endometrial injection of autologous bone marrow mesenchymal stem cells (MSCs, 12×10^6 in 6 ml of PBS); 5 mares were sham-injected (6 ml of PBS). Injections were guided with an endoscope (Series 160 Olympus). Each treatment consisted of 12 sites (0.5 ml/point) following a horizontal line from the tip of 1 uterine horn to the contralateral uterine horn. Uterine biopsies were taken during diestrus at 15 days before (Day -15) and 60 days (Day 60) after endometrial injections. Endometrial score (H&E stain), intensity of fibrosis (Masson's trichrome blue), the extension of collagen type III, progesterone (mouse monoclonal antiprogesterone, Clone PGR 636, Dako Cytomatuon, Carlinteria, CA) and estrogen alpha (mouse monoclonal anti-ER-alpha, Clone EP1, Dako) receptors (immunohistochemistry) were evaluated in endometrial samples before and after treatment. Intensity of fibrosis and extension of collagen type III were assessed in biopsy samples using the software AVSOFT Bio-view Spectra 4.0.1. Distribution of estrogen and progesterone receptors were evaluated using a semiquantitative scoring system: 0: absence of staining; 1: 1 - 25% of positive cells; 2: 26 - 50% of positive cells; 3: 51 - 75% of positive cells; and 4: > 75% of positive cells. Data were analyzed with Kruskal-Wallis and Dunn's post-hoc. Significance was set at $p < 0.05$. Endometrial score improved in 6 mares (3 mares improved to grade I and 3 mares to grade IIA) treated with MSCs, whereas no changes were observed in the remaining 4 treated mares or 5 controls. Morphometric evaluation had a reduction in endometrial fibrosis ($p = 0.02$; Day -15: 541198 ± 750341 pixel^2; Day 60: 448714 ± 241518 pixel^2) and collagen type III ($p = 0.007$; Day -15: 726869 ± 63200 pixel^2; Day 60: 130685 ± 7555 pixel^2) in mares treated with MSCs. Additionally, an increase in the distribution of progesterone receptors ($p = 0.03$; Day -15: 3; Day 60: 4), immunolabelling for estrogen receptors tended to increase after MSCs therapy ($p = 0.08$; Day -15: 1; Day 60: 3). In conclusion, endometrial injection of MSCs reverted endometriosis in 60% of mares and increased steroid hormone receptors in the endometrium. This technique has the potential to rescue fertility of mares by regenerating endometrium; however, this remains to be confirmed.

Keywords: Barren mares, endometritis, chronic degenerative endometritis, fibrosis, cell therapy

Does uterine microbial population vary due to uterine sampling techniques and estrous cycle stage in healthy postpartum dairy cows?

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Metagenomic sequencing for genital microbial profiling is increasingly used to enhance our understanding of the impact of

microbiota on physiological functions, uterine diseases, and hence dairy cows' fertility. Researchers have utilized uterine swab (US), uterine cytobrush (UC), and low-volume uterine lavage (UL) methods to sample the uterus for bacterial population using culture and culture-independent metagenomic sequencing.[1] Interestingly, variations in uterine microbial profiles that may occur due to inherent differences in sampling methods have not been studied. As these techniques differ in the sample's depth and representative portion of the uterus sampled and the hormonal milieu differs between estrus and diestrus phases, we hypothesized that uterine microbial profiles differ among uterine sampling techniques and phases of estrous cycle. Therefore, we compared uterine microbiome profiles determined using routine bacteriological culture and next-generation sequencing as follows: i. among 3 sampling techniques; and ii. estrus and diestrus phases of estrous cycle. Clinically healthy postpartum dairy cows (n = 15) from the Rayner Dairy Research and Teaching Centre (University of Saskatchewan) between 50 and 60 days postpartum were selected. Each cow was sampled using 3 uterine sampling techniques during estrus and diestrus phases induced during the presynchronization protocol and confirmed based on transrectal palpation and assessment of ovarian structures via transrectal ultrasonography. For each cow, the US and UC samples were taken from 1 uterine horn (randomly assigned), whereas the UL sample was collected from the opposite uterine horn using sterile techniques. By the end of the experiment, 87/90 expected samples were collected. One-half of each sample was subjected to bacterial culture by incubating aerobically at 37°C for 48 hours on Colombia blood agar and resulting colonies with different morphologies were selected for identification by PCR and Sanger sequencing of the 16S rRNA gene. Preliminary results from bacterial culture indicated that the proportion of countable colony-forming units (CFU; defined as 25 - 250 CFU/plate[2]) was higher ($p = 0.04$) for uterine samples collected at diestrus than estrus phase of the estrous cycle (15/45, 33% versus 8/42, 19%, respectively; Chi-square = 3.89). However, the proportion of countable CFU did not differ ($p = 0.68$) among the sampling techniques (US versus UC versus UL: 6/29, 21% versus 7/29, 24% versus 10/29, 34%, respectively; Chi-square = 0.78). In summary, the culturable bacterial load varied with the estrous cycle stage but not among uterine sampling techniques.

Keywords: Cattle, endometrial sampling, bacteriome, next-generation sequencing

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Generation of feline oviductal and endometrial organoids

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Organoids are 3D, spherical cell clusters formed in vitro that are capable of regeneration and self-organization with similar function to their tissue of origin. Organoids overcome 2 major short-comings of the classic explant and 2D monolayer cell culture approaches by: i. maintaining viability in vitro longer (months); and ii. retaining the ability to physiologically respond to various stimuli similar to their in vivo organ counterpart, respectively. Furthermore, organoids provide physiological structures that support intercellular interaction between multiple cell types and, therefore, offer a potentially superior model for exploring disease mechanisms and investigating potential therapeutics. Importantly, organoids reduce the need for whole-animal studies, making research more efficient and cost effective while improving animal welfare. To date, equine endometrial organoids are the only reproductive organoids reported in domestic animals.[1] Therefore, our objectives were to: i. establish endometrial and oviductal organoids derived from feline tissues; and ii. trial various culture media to determine optimal growth conditions. We hypothesized that medium reported for generation of mouse oviductal organoids[2] provide the highest growth and viability. Three culture media[1-3] were trialed with oviductal organoids using previously reported methods[1] with minor modifications (scraping the tissue surface to release cells rather than collecting tissue biopsies). Dissociated cells were plated at a concentration of 5,000 per well, and oviductal organoids were assessed after 2 weeks in culture for growth and viability using brightfield microscopy in conjunction with OrgaQuant python script[4] and fluorescein diacetate and propidium iodide staining, respectively. Data were analyzed using ANOVA with Tukey's post-hoc test, and significance was set at $p < 0.05$. Establishment of feline endometrial and oviductal organoids was successfully achieved for the first time. The organoids appeared as round, cystic structures of epithelial cells, similar to endometrial organoid reports in mares and women.[1,3] Utilizing OrgaQuant, the number of established organoids varied ($p < 0.005$) among 3 media trialed. Organoid medium reported for the growth of equine endometrial organoids[1] facilitated the greatest growth and cellular viability of feline oviductal organoids. Results demonstrated that feline reproductive organoids can be established utilizing existing protocols with minor modifications as a novel 3D cell culture model that may more closely recapitulate in vivo anatomy and physiology long-term that will facilitate reduced reliance on research animals and the associated cost and welfare concerns of live-animal experimentation.

Keywords: Queen, cat, cell culture, in vitro, 3D, fallopian tube

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Cystic ovarian disease in cattle: diagnosis and differentiation via color Doppler ultrasonography and hormone assay

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Most frequently cited definition of cystic ovarian disease (COD) in cattle is an abnormally persistent follicle (> 7 - 10 days) with a diameter > 20 mm that clearly interferes with normal ovarian cyclicity.[1] Discrimination between luteal and follicular ovarian cystic structures has traditionally been conducted by measuring the rim of luteal tissue. Most common practice in field diagnosis is via transrectal palpation examination with or without the use of a B-mode ultrasonography (though misclassification of ovarian structures is estimated to be 40 - 50%). Color Doppler ultrasonography utilizes blood flow area measurements within the ovarian structure that has been proposed as a potential indirect measure for plasma progesterone (P_4) concentrations and that apparently increase in luteal cysts. Objective was to compare the diagnostic accuracy when differentiating luteal and follicular ovarian cysts using measures collected via B-mode and color Doppler transrectal ultrasonography. The consensus definition was used to identify the condition and confirmed by 2 examinations 10 days apart. A 3 mm luteal rim width was used to differentiate follicular and luteal cysts during reproductive examinations. Blood flow area measurements were recorded within the rim of cystic structures and calculated using a standard video analysis protocol. Multiparous dairy cows (n = 36) were enrolled during routine herd reproductive examination visits, with 22 and 14 having follicular and luteal cysts, respectively. Cows were examined using a Mini-Exapad (Easi-Scan, IMV Imaging Ltd., Rochester, MN) ultrasound with color Doppler capabilities. Blood samples were collected from each cow to measure P_4 serum concentrations, using ≥ 1 ng/ml concentrations as threshold value reference standard for luteal cyst diagnosis. Receiver operating characteristic (ROC) analyses were generated to compare the accuracy when differentiating follicular and luteal ovarian cysts using measurements collected via B-mode (e.g., luteal rim width) and color Doppler (e.g., blood flow area) ultrasonography, using P_4 as the gold standard measure. Among cystic structure measured parameters, luteal rim width and blood flow area were selected for further analysis because they presented the best ROC curves for differentiating COD, with an area under the curve of 0.75 and 0.80, respectively. Luteal rim width measurements alone resulted in a sensitivity and specificity of 50 and 86%, but was improved by using color Doppler flow area measurements (with values of 79 and 86%, respectively). When combining the use of luteal rim and blood flow area for differentiating cystic ovarian structures, in a parallel approach resulted in sensitivity and specificity of 93 and 73%, and in series approach resulted in sensitivity and specificity of 35 and 100%. In conclusion, discriminating between luteal and follicular ovarian cysts in dairy cattle via Doppler ultrasonography had higher diagnostic accuracy compared to B-mode ultrasonography alone; an opportunity for veterinary practitioners to enhance diagnostic ability.

Keywords: Cyst, luteal, follicular, endocrine, progesterone

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Homozygous deletion on chromosome 29 leading to disorder of sexual development

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Disorders of sexual development (DSD) are not uncommon in the horse, affecting 5.6% of the population. DSDs affect sexual differentiation, development and function of the gonads, and may be characterized by atypical chromosomal, phenotypic, or gonadal sex. Homozygous deletions in chromosome 29 have been a risk factor for DSDs. This report describes the homozygous deletion of 200,000 base pairs in chromone 29 with abnormal genitalia in a 12-month Appaloosa filly. Horse was presented for an abnormally swollen vulva. Upon parting the vulvar lips, a band of pigmented tissue of ~ 1 cm thick in the vestibule, extending 10 cm cranially to the vestibulovaginal fold. The tissue extended from the dorsal to ventral commissure of the vulva. An abnormal urethral opening and enlarged clitoris were observed protruding from the ventral aspect of this septum. Filly was sedated and a speculum examination revealed a normal vagina and cervix. Due to the abnormal appearance and suspicion of a DSD, a heparinized blood sample was submitted for a chromosomal and PCR analysis. Cytogenetic analysis (e.g., karyotyping analysis) performed at Texas A&M revealed that the filly had 64 chromosomes with a normal morphology, 2 X chromosomes; however, it was homozygous for a large deletion in chromosome 29. The PCR test for the Y-linked SRY gene was negative and the PCR test for the X-linked androgen receptor gene was normal. The fertility of this filly is unknown at this time. It has been noted that 8.1% of reproductively or developmentally abnormal horses had a homozygous deletion; however, 79% of horses with a homozygous deletion of chromosome 29 were reproductive or developmentally abnormal.[1] The deletion includes the aldo-keto reductase family 1 C (AKR1C) gene which is a ketosteroid reductase in steroid hormone development. AKR1C is involved in androgen and estrogen metabolism, and all pathways to testosterone and dihydrotestosterone production. These mutations have also been associated with human DSD. Altered AKR1C has been associated with prostate and breast cancer, endometriosis, and XY DSDs in humans.

Keywords: Disorder of sexual development, chromosome, gene deletion

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Effect of the SexedULTRA™ Genesis III technology on the quality and 24 hours longevity of sex-sorted stallion sperm

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Use of flow cytometry techniques for the sex-sorting of stallion sperm is lower than that reported for food-producing animals. Stallion-related factors and sperm susceptibility to the sex-sorting process resulted in reduced sperm quality and longevity over time that accounted for the limited use of this technology in horses. Consequently, sex-sorted stallion sperm has been used for insemination of mares closely located to the sorting facility, that limits the commercial applicability of this technology. Recently, a proprietary technology (SexedULTRA™ Genesis III, ST Genetics, Navasota, TX) has become available for sex-sorting of bull sperm. This technology reduced the sorting time and increased the sorting efficiency, quality, and fertility of sex-sorted sperm. A study was designed to determine the effects of this process on stallion sperm. Three ejaculates from 4 stallions (n = 12), were diluted with INRA-96® and processed by colloid centrifugation. The resulting pellet containing at least 2.5 x 10^9 sperm was resuspended in a proprietary chemically defined medium (ST Extender) and stored for ~ 60 minutes at (~ 20°C) before starting the sex-sorting process. Sperm quality parameters in fresh semen immediately after colloid centrifugation before staining (FRESH), immediately after sex-sorting (X-SORTED FRESH) and following 24 hours of cooled storage at 6°C (X-SORTED COOLED) were recorded and analyzed. Parameters included: total sperm motility (TMOT; %), progressive motility (PMOT; %) determined by CASA; viability/acrosomal intactness (VAI; %), lipid peroxidation in viable sperm (VLPP; %), and sperm DNA damage ($COMP_{\alpha\text{-}t}$), determined by flow cytometry. Data were rank-transformed before analysis using the General Linear Model (JMP Pro 16). Statistical significance was set at p

< 0.05. Mean TMOT was higher in FRESH than in X-SORTED FRESH or X-SORTED COOLED semen (88 versus 80 versus 67%; p < 0.05). Mean PMOT was similar in FRESH and X-SORTED FRESH semen, while higher than in X-SORTED COOLED (52 versus 59 versus 34%; p < 0.05). Mean VAI was similar in FRESH and X-SORTED FRESH semen, while higher than in X-SORTED COOLED semen (84 versus 85 versus 78%, respectively; p < 0.05). Mean VLPP was lower in FRESH than in X-SORTED FRESH semen, and higher in X-SORTED FRESH than in X-SORTED COOLED semen (8 versus 22 versus 27%, respectively; p < 0.05). Mean $COMP_{\alpha\text{-}t}$ was similar in X-SORTED FRESH and X-SORTED COOLED semen, whereas lower than in FRESH semen (3 versus 4 versus 7%; p < 0.05). Results indicated that sex-sorting and subsequent cooled storage induced a reduction in stallion sperm motility and increased sperm lipid peroxidation. Other sperm quality parameters (e.g., viability and DNA integrity) during the fertilization process were maintained after sex-sorting and 24 hours of cooled storage. Sex-sorted stallion sperm processed using the SexedULTRA™ technology can still maintain acceptable quality for breeding purposes either as FRESH SORTED or COOLED SORTED. Further studies will determine if fresh and cooled, sex-sorted semen processed with this novel technology will retain its fertilizing potential and could be used for commercial breeding purposes.

Keywords: Stallion sperm, sex-sorting, SexedULTRA™, cooled storage

Extending teasing time increases bacterial load and decreases semen longevity in normal libido normospermic stallions

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Normal libido stallions are inadvertently or purposely over teased to increase semen collection. However, the intensity and duration of teasing before semen collection increase the seminal plasma in the ejaculate that is thought to be detrimental to semen quality. This study aimed to determine whether extending the teasing time of normal libido stallions affected semen quality. We hypothesized that extending teasing time reduces semen cooling and freezing abilities and increases bacterial contamination. Forty semen collections of 10 stallions were performed at 48 hours intervals. For each stallion, 2 collections were performed using standard teasing (i.e., a successful ejaculate was procured < 5 minutes after penile washing), and 2 collections included extended teasing (i.e., after penile washing, stallions were handheld for 10 minutes before being allowed to mount the dummy mount). Immediately after collection, gel-free semen and gel volumes were weighed. Sperm concentration was determined with a Nucleocounter. The gel-free semen was extended at 50 x 10^6/ml in INRA96 cushion-centrifuged, and half of the pellets were resuspended in INRA96 at 50 x 10^6/ml and stored in Equitainer for further evaluations at 24 and 48 hours. The second half of the pellets were resuspended in an egg yolk-based extender (Botucrio) at 100 × 10^6/ml, loaded in 0.5 ml straws, and frozen over nitrogen vapor. Total motility (TM) and progressive motility (PM) were evaluated with CASA. Sperm membrane integrity (SMI) and mitochondrial membrane potential (MMP) were assessed with Zombie Green and Mitotracker DeepRed via spectral flow cytometry. Sperm parameters were assessed at 0, 24, 48 hours of cooling before and after thawing. Raw and cooled semen were aerobically cultured (48 hours), and MALDI-TOF identified isolates. Data were analyzed with a paired Student's *t*-test, linear mixed model, Fisher's Exact test, and Tukey's post-hoc. Significance was set at p < 0.05. Gel-free and gel semen volumes increased with extended (p < 0.05) teasing time from 45.5 ± 5.0 ml to 76.4 ± 5.5 ml and from 4.7 ± 2.4 ml to 21.3 ± 4.9 ml. Sperm concentration reduced (p < 0.05) from 344 ± 56×10^6/ml to 189 ± 28 x 10^6/ml when the teasing was extended. Isolates included *Actinobacillus* spp., *Corynebacterium* spp., *Staphylococcus* spp., and *Pseudomonas* spp. The extended teasing time increased (p < 0.05) the number of bacterial isolates in the raw semen from 5 to 8. Cooled semen at 24 and 48 hours had higher percentages of positive cultures in the extended teasing but not statistically significant (24 hours, standard 25 versus extended 35%; 48 hours, standard 20 versus extended 25%). TM and PM decreased after freezing and overtime after cooling (p < 0.0008) but were not affected by the teasing time (p > 0.05). SMI was not affected by group (p > 0.05) or freezing (p = 0.7), but there was an interaction (p = 0.03) between freezing and group. During cooling, SMI was affected by group (p = 0.013) but not by time (p = 0.7) or by their interaction (p = 0.27); extended teasing resulted in lower SMI at 0, 24, and 48 hours. MMP was not affected by time (p = 0.82) or group (p = 0.2) or their interaction (p = 0.5) during cooling. MMP was affected by freezing (p < 0.001) but not by group (p = 0.4) or interaction (p = 0.3). In conclusion, extended teasing decreased the longevity of the semen upon cooling and increased the bacterial load; however, it did not affect postthaw semen quality.

Keywords: Stallion, seminal plasma, sperm viability, semen freezing

Luteal tissue area and immunoreactive concentrations of progesterone in plasma of bred and nonbred mares

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Progesterone is a pivotal hormone to maintain pregnancy in the first trimester in mares. Low progesterone concentrations (< 4 ng/ml) have been associated with early pregnancy loss. Some clinicians collect 1 blood sample from mares 2 - 10 days postovulation to determine whether the mare needs progestin supplementation. A study demonstrated that mares becoming pregnant have higher progesterone on day 5 postovulation than those failing to become pregnant; however, it remains to be determined if progesterone varies on other days. In some

species, seminal plasma has a luteotropic property. Therefore, the objective of the present study was to evaluate the immunoreactive progesterone concentrations and the luteal tissue area in non-bred, bred becoming pregnant, and nonpregnant mares. We hypothesized that pregnant mares have higher progesterone concentrations than nonpregnant mares 2 - 10 days postovulation. Light breed mares (n = 14) were monitored via ultrasonography every other day until detection of a preovulatory follicle (diameter ≥ 35 mm in the presence of endometrial edema). Then mares received deslorelin acetate (1.8 mg, intramuscularly) to induce ovulation. Twenty-four hours later, mares received an AI (~ 2 x 10^9 progressively motile sperm extended in 50 ml of INRA 96) or a sham-AI (50 ml of INRA 96). Ovulation was confirmed by ultrasonography. The number of corpora lutea and the luteal tissue area were recorded daily until 10 days postovulation. Immunoreactive progesterone concentrations were assessed daily from the day of the ovulation to 10 days postovulation. Pregnancy diagnosis was carried out at 10 and 13 days postovulation. A total of 52 estrous cycles were completed; 15 were part of the control group (sham-AI), and 37 were part of the bred group, with 17 cycles nonpregnant and 20 pregnant. Data were analyzed with a mixed model, Tukey test as post-hoc, and Pearson's coefficient of correlation. Both immunoreactive progesterone concentration and luteal tissue area varied ($p = 0.001$) with time postovulation but were not affected ($p > 0.05$) by group. The number of ovulations caused an increase ($p = 0.0001$) in the immunoreactive progesterone concentrations and luteal tissue area. There was a weak but positive association between the number of ovulations and immunoreactive progesterone concentrations ($r = 0.2$; $p = 0.0001$) and a moderate positive association between the number of ovulations and luteal tissue area ($r = 0.54$; $p = 0.0001$). The lack of change in the progesterone concentrations and luteal tissue area between bred and nonbred mares suggested that horse seminal plasma did not affect luteal function in mares. All mares had progesterone above 4 ng/ml after 5 days postovulation. Thus, it is possible that if mares with abnormal progesterone concentrations were used, the results could have been different. In conclusion, pregnancy was not associated with higher progesterone concentrations or greater luteal tissue area.

Keywords: Mare, corpus luteum, pregnancy, seminal plasma

Testicular atrophy following traumatic incident in young German shepherd dog

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A 2-year, intact male German shepherd dog, was presented for nonpainful unilateral scrotal swelling of 3 days duration. Scrotal swelling can be caused by infectious organisms, neoplasia, autoimmune disease, spermatic cord torsion, inguinal herniation, or direct trauma and can rapidly result in infertility.[1] Owner wanted to preserve breeding potential and saw no improvement after administering an antibiotic and a nonsteroidal antiinflammatory from their regular veterinarian. Left testis was difficult to palpate due to edema and the right testis was smaller than expected. Diagnostics included an in-house RSAT brucellosis test (negative), semen collection/evaluation with oligospermia and teratospermia (6.66% motility, 12% normal morphology, 11 x 10^6 total sperm), prostatic fluid cytology (many neutrophils), and prostatic fluid culture (negative). Ultrasound evaluation revealed that both testes and prostate parenchyma had normal echotexture, whereas the left testis was small (2.57 x 2.4 x 2.54 cm) compared to the right testis (2.93 x 2.38 x 3.38 cm) and was surrounded by mixed echogenicity fluid. Left testis, accompanying tunics, and 6 cm of the left spermatic cord were surgically removed in a closed castration to prevent further inflammation and subsequent degeneration of the right testis. Additional diagnostics on the removed testis included a culture (negative) and histopathology (severe testicular atrophy with steatitis) which confirmed the diagnosis of inguinal herniation. Eleven days later, the scrotum was enlarged again. Ultrasound examination revealed a homogeneously echogenic, normal right testis and left scrotal side filled with hypoechoic fluid and hyperechoic debris. Daily antibiotics were continued, and a nonsteroidal antiinflammatory was prescribed along with cold compresses. Currently, the client recalled that the patient's inguinal area got caught on a fence prior to the initial visit. At a recheck ultrasound examination 7 days later, the was limited fluid and fibrin-like tissue present in the left scrotal sac and a normal right testis. Semen collection/evaluation 124 days after the previous collection/evaluation had normospermia (85.25% motility, 74% normal morphology, 425.94 x 10^6 total sperm). It is important to conserve breeding potential in sporting and working breeds with valuable genetics which, with their activities, have an increased risk of traumatic injuries. Specifically in this case, traumatic inguinal herniation and the resultant inflammation caused an increase in scrotal temperature resulting in testicular atrophy.[2] This case highlighted how successful management of testicular trauma can preserve breeding potential.

Keywords: Dog, orchitis, hemicastration, inguinal hernia

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Diaphragmatic hernia involving the gravid uterus in a dog

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A pregnant, 4-year German Shorthair Pointer bitch, was presented for severe progressive tachypnea and depression. The day of pregnancy was unknown, the only information available was that she was bred 52 days prior to the presentation. Bitch was quiet and alert with hyperemic mucous membranes. Abdomen was soft and appeared nonpainful on manual palpation. Several fetuses were palpable in the caudal abdomen.

Ventrally located lung sounds were muffled on auscultation. Radiographs and an ultrasonographic examination revealed a diaphragmatic hernia with intrathoracic displacement of a portion of the uterus. An emergency herniorrhaphy was pursued with the owner requesting to allow the bitch to maintain the pregnancy if possible. Preoperative medications included methadone and cefazolin. General anesthesia was induced with propofol to effect. Anesthesia was maintained with sevoflurane in 100% oxygen. The bitch was maintained on mechanical ventilation at a respiratory rate of 5 breaths/minute. A ventral midline celiotomy was performed to reduce the herniated abdominal organs and repair the diaphragm. A 7 cm right-sided radial tear extending from the tendinous portion of the diaphragm into the pars sternalis was noted. Tip of the left horn of the uterus containing 2 fetuses, the spleen, and right medial and lateral lobes of the liver, pancreas, and a large portion of the greater omentum were in the thoracic cavity. Herniorrhaphy was performed using both modified Lambert and simple continuous suture patterns. A rotational flap of the internal oblique muscle was used to aid in the closure of the most ventral portion of the hernia due to high tension. Porcine submucosa was sutured over it as reinforcement. Performance of an intra-operatory transuterine ultrasonographic examination of the fetuses revealed that the placental sites were intact, fetal heartbeats were present in all puppies evaluated, however, low heart rates (80 - 100 beats/minute) were attributed to uterine circulatory compromise and the effect of anesthesia; however, the decision to allow the pregnancy to continue was made. Following surgery, the bitch received supportive medical care for 3 days until its systemic status declined. She was obtunded and had severe ventral edema and swelling of the vulva. Blood chemistry and CBC showed hypoalbuminemia, leukocytosis with a left shift neutrophilia, and increased liver enzymes. Transabdominal ultrasonography revealed evidence of fetal death with lack of fetal heart rate and increased opacity of the amniotic fluid. An emergency cesarean surgery was performed. Six of 11 pups were saved; 1 fetus was underdeveloped and had a very elongated neck, presumably from entrapment within the lumen of the diaphragmatic hernia. To our knowledge, this is the first report that describes a pregnancy with survival of several fetuses following surgery for a diaphragmatic hernia with a gravid uterus displaced to the thorax.

Keywords: Cesarean surgery, ultrasonography, pregnancy, herniorrhaphy

Survey of Golden retriever breeders' expectation of reproductive services

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The importance of client communications has long been recognized in veterinary medicine. However, to a much lesser extent has this relationship been explored regarding reproductive service provided to breeders. Previous studies reported feelings of disconnect and distrust between the 2 parties. In a recent report,[1] dog breeders commented about the apparent lack of training in theriogenology among veterinarians. Conversely, veterinarians expressed disinterest in working with breeders who seem arrogant, argumentative, or inflexible. Our goal was to identify possible areas of intervention to improve communications by way of utilizing a survey format to further define the relationship between breeders and veterinarians. A survey link was distributed to the Golden Retriever Club of America list-serv, including ~ 3,900 members, with 127 complete responses used for this study. We designed the survey to have 3 areas of emphasis for analysis: communication, furthering education specific to theriogenology, and most sought-after services to provide in a veterinary clinic. Communication techniques included active listening, nonjudgmental language, and trust to create a teamwork mentality and a successful breeder-veterinarian relationship. Areas needing increased assistance from veterinarians were breeding soundness examinations, Cesarean surgeries, and cleft palates. The main reasons for conflicts between breeders and their veterinarian were inexperience (21.2%), timing of Cesarean surgeries (19.2%), lack of availability (15.4%), disagreements on when to spay or neuter (8%), communications barrier (6%) and vaccination recommendations (6%). The primary veterinary services sought by the breeders were interpretation of progesterone concentrations/cycle management (82.7%), ultrasonographic evaluation of the pregnancy (69.3%), prepartum radiographs to determine the number of fetuses present (64.6%), and health clearances (64.6%). A concerning aspect was that 19.2 and 28.8% of the participants never or rarely have had a breeding soundness exam performed before mating their pets. Breeders can include a niche clientele of individuals passionate about the health and wellbeing of their animals and the future generations of dogs. The improvement of the continuing veterinary education and the enhancement of veterinary curriculum in veterinary schools are at the base of the betterment of the relationship between breeders and veterinarians who share the common goal of improving the welfare and health of the pets.

Keywords: Theriogenology, dogs, mating, genetic counseling, breeders

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Optimizing centrifugation for cooled canine semen processing: preliminary data

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Semen processing is critical during preparation for shipped chilled semen or for semen cryopreservation to remove the seminal plasma. There is a deficiency in the knowledge for ideal processing techniques or settings for canine semen. Purpose of this study is to provide recommendations for optimizing

semen processing to yield improved shipped chilled semen conditions in dogs. Our hypothesis was that higher centrifugation speeds and longer processing times will increase sperm recovery rates but reduce sperm viability and motility. Our study aimed to determine the optimal combination of time and g force centrifugation allowing the best outcome during cooled storage. Healthy, client-owned stud dogs (n = 9) of 2 - 5.5 years of age, > 15 kg and various breeds were collected using manual technique. Only dogs with a negative *Brucella canis* serology and an ejaculate with appropriate concentration, ≥ 70% total motility and ≥ 40% normal morphology, were included. Ejaculates were divided into 6 equal volume aliquots, each subjected to 1 of 6 treatments: centrifugation at 400, 720 or 900 g, each for 5 or 10 minutes, followed by extension of the sperm pellet to the original aliquot volume with CaniPlus Chill LT (Minitube). Samples were then cooled for 24 hours according to standard packaging technique in a Minitube canine shipping box. Semen evaluations of concentration and viability by Nucleocounter® SP-100™ and total and progressive motility (TM, PM) by CASA (SpermVision™, Minitube) were performed before centrifugation (T0), immediately post-centrifugation (T1) and after 24 hours of cooling (T24). Sperm recovery rate (RR, %) was calculated as (T1 concentration)/(T0 concentration)*100 from Nucleocounter® SP-100 values. Data were analyzed by linear mixed model with significance $p < 0.05$, and results presented as mean±SD. Overall RR was 98.1 ± 9% and similar ($p = 0.912$) among treatment groups. Sperm viability, TM and PM were not affected ($p \geq 0.779$) by centrifugation type but all variables decreased ($p < 0.001$) over time. Across all samples, a significant decrease in sperm viability from T0 (84.8 ± 6.8%) was detected by T1 (76.3 ± 6.4%) with further decrease by T24 (69.1 ± 8.4%). TM/PM decreased significantly from T0 (87.6 ± 5.9%/80.5 ± 13.4%) and T1 (84.9 ± 7%/81.8 ± 7.3%) to T24 (80.7 ± 13.3%/73.7 ± 15.9%). In conclusion, our preliminary results suggested that sperm viability was affected by both processing and cooling, whereas significant changes in TM and PM were not observed immediately postcentrifugation. At this time, the specific centrifugation speed or duration used in this study did not significantly affect RR and sperm viability or motility. This study is still ongoing, and we anticipate completion of this project by May 2022.

Keywords: Dog, chilled semen, centrifugation, computer assisted sperm analysis

Hydrops fetalis diagnosed via transabdominal ultrasonography

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A 3-year, Golden Retriever bitch, was presented to Philadelphia Animal Hospital 59 days after breeding with edema in the hind limbs and a decreased appetite. Hydrops was suspected after decreased detail was noted on a single lateral abdominal radiograph performed by the referring veterinarian 1 day prior to presentation. Hydrops fetalis and hydrops amnion are congenital disease processes that result in a failure of fetal fluid homeostasis, seen primarily in brachycephalic breeds and golden retrievers.[1] More importantly, hydrops fetalis and hydrops amnion increase the risk of fetal and maternal mortality. A transabdominal ultrasonography revealed 2 fetuses in the left uterine horn with heart rates over 180 beats/minute, excellent GI definition and slightly echogenic amniotic fluid suggestive of meconium contamination. Fetus in the right uterine horn had an edematous abdominal wall, pleural, pericardial, and peritoneal effusion with an abdominal diameter of 9 cm. A complete blood count and chemistry revealed mild anemia, but was otherwise unremarkable. Dexamethasone was given subcutaneously at a dose of 0.2 mg/kg to accelerate fetal lung maturation.[2] Cesarean surgery was performed the following day. An excessive amount of fluid was present in the allantois of all fetuses. Two viable pups were removed from the left uterine horn, whereas a nonviable puppy with hydrops fetalis and a significant cleft palate was removed from the right uterine horn. Biopsies of the uterus and chorioallantois were submitted to Ohio State University. There were no marked findings in the uterine biopsy, whereas the fetal membranes presented labyrinthine mineralization of varying severity. No evidence of inflammation or lesions that may have induced dystrophic mineralization were present. This patient was bred the following cycle to a different stud and delivered five normal puppies via planned Cesarean surgery. This case illustrated the use of ultrasonography to diagnose fetal abnormalities in late term pregnancy.

Keywords: Hydrops fetalis, Golden Retriever

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Melatonin improves testicular hemodynamics and sperm quality in rams subjected to mild testicular heat stress

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Melatonin is a potent free-radical scavenger with antiinflammatory, antioxidative, and antiapoptotic effects. Our objective was to determine effects of melatonin on testicular blood flow and sperm quality after mild heat stress (HS; scrotal neck insulation) in rams. Dorset rams (n = 12) with good semen quality were group-housed indoors (~18°C). Once weekly for 2 weeks, Doppler indices (resistance index; RI and pulsatility index; RI) were measured in the supratesticular artery and semen was collected (electroejaculation). Then, rams were randomly allocated into 2 equal groups: Melatonin group (MEL), was given subcutaneously 36 mg melatonin in 1 ml corn oil under the ear or a Control group with only corn oil (CONT). On day 15 after treatment, rams were subjected to mild HS for 96 hours with blood flow measurements and semen collection performed once weekly for the next 6 weeks. Sperm motility was assessed with CASA (Sperm Vision®) and morphology using eosin-nigrosin. Data were analyzed using repeated measures, with a Bonferroni test for post hoc analysis. For total and progressive motility, there were group, week, and group*week interaction effects ($p < 0.005$) for total and progressive motility and total abnormalities, plus group and week effects for RI and PI ($p < 0.005$), with no significant difference before treatment. Changes in total and progressive motility and sperm abnormalities were evident at week 1 postHS, but MEL mitigated ($p < 0.05$) these effects from weeks 2 to 6. Furthermore, both PI and RI were reduced (i.e., better flow, $p < 0.05$) in MEL versus CONT rams on weeks 1, 3, 4, 5, and 6 after HS. In MEL rams, sperm motility and total abnormalities had recovered at weeks 5 and 6, respectively, whereas CONT rams had not completely recovered by week 6. In conclusion, melatonin treatment before HS heat stress significantly improved testicular blood flow and protected sperm motility and morphology, with potential for mitigating effects of HS under field conditions.

Keywords: Rams, heat stress, melatonin, sperm quality, Doppler indices, supratesticular artery

Safety of intrauterine infusion of the honey/propolis in normal postpartum dairy cows: a pilot study

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Endometritis affects dairy cows during the postpartum period (PP) leading to prolongation of voluntary waiting period, infertility, and financial loss. The advocated treatment of the condition is intrauterine infusion of antibiotics. However, in the context of rising antibiotic resistance worldwide, legislative restrictions for antibiotic uses, and increasing numbers of organic farms, there is an urgent need for alternative therapeutic approaches. As an alternative treatment for endometritis, some products of honeybees such as propolis and honey (Pro-honey) can substitute conventional treatments. We hypothesized that Pro-honey (organic buckwheat honey with 5% propolis) is a good alternative to treat endometritis in dairy cows. However, the safety of the product needs to be assessed first. Objective was to determine the acute clinical response of the endometrium to the intrauterine infusion of Pro-honey in normal postpartum dairy cows. Healthy cows (n = 31) with intact uterus and cycling normally formed 6 groups: group 1 (n = 6), 2 (n = 5), and 3 (n = 5) received an intrauterine infusion of 30 ml of Pro-honey, honey alone, and Cephapirin (positive control) respectively within 30 - 50 days-in-milk (DIM). In group 4 (n = 3, negative control), cows were not infused. In groups 5 (n = 8) and 6 (n = 4), Pro-honey and honey alone were infused into uterus of cows with more than 50 DIM, respectively. Transrectal ultrasonography, vaginal examination, cytological, and bacteriological evaluations of the uterus via cytobrush sampling at 0 (before infusion), 48, 96, and 192 hours were performed to assess acute response of the uterus. Percentage of PMNs (polymorphonuclear cells) in the uterus reached the utmost level rapidly at 48 hours before returning to the initial level of 96 hours in all groups with the exception of group 4 ($p < 0.001$) where no increase was measured. At 48 hours, number of PMNs in cows of group 1 (53.8 ± 30) was higher ($p < 0.05$) compared to groups 2, 3, 4, 5, and 6 with 12.6 ± 7, 16.8 ± 25, 0.3 ± 0.6, 15.9 ± 9, and 10.1 ± 8 PMNs respectively. At 48 hours, Pro-honey triggered a stronger innate immune response of the uterus in cows in early PP (30 - 50 DIM) than those more advanced in PP. Bacterial culture were negative and no changes were observed with ultrasonography. In conclusion, Pro-honey triggered a prompt innate immune response of the uterus in normal postpartum cows before returning rapidly to a quiescent status. Results demonstrated that intrauterine infusion of the Pro-honey in normal postpartum dairy cows is safe and stimulates an innate im-

mune response that could be potentially advantageous in cases of endometritis.

Keywords: Dairy cows, pro-honey, honey, intrauterine infusion, endometritis

Nonsurgical approach to sterilizing avian species using a domestic chicken model

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Overpopulation of certain avian species can have detrimental effects on the natural and human environment. Zoonotic diseases, nitrogenous pollution, and damage to human property and natural ecosystems are some concerns being raised by wildlife control agencies. Current population control efforts include hunting, poisoning, and predator introduction, all of which have substantial impacts on animal welfare of the target species. Companion bird species are subject to various diseases related to reproductive dysfunction. These diseases range from behavior manifestations of aggression and feather picking to difficulties in egg production, neoplasia and cloacal prolapse. Current therapies can be cost limiting, or in surgical cases, life threatening, creating a need for non-invasive sterilization. Effects of injecting domestic chickens with high-dose antibody-guided lipid nanocomplex (LNP) carrying the cytotoxic agent saporin were studied. Antibodies for anti-Müllerian hormone II receptors were used to target theca and granulosa cells to stop hormone production and ultimately egg production. A previous study's use of 100 nmol solution yielded egg laying cessation for 16 days whereas this project increased this dose to 1000 nmol.[1] We hypothesized that increasing the dose would extend the period of egg laying cessation. Rhode Island Red laying hens (n = 6) formed a control group and test group of 3 hens each. Test group was given LNP intramuscular injections whereas the control group was given an intramuscular injection of saline equal to the LNP volume (1.4 ml). Egg laying was monitored for 30 days. Hens were euthanized, and necropsied. Oviduct and ovaries were removed weighed and photographed. Reproductive tracts were placed in formalin and histological sectioning of ovaries were made and stained with Hematoxylin and Eosin. Follicles of each slide were evaluated for theca and granulosa cell layers. Follicles between 1 - 2 mm were counted on each slide and rated on a scale from 1 to 5 based on granulosa cell architecture and organization (1 = healthy, uniform layer; 5 = complete lack of granulosa layer present). Hens injected with LNP stopped egg laying for a minimum of 23 days; 1 control hen stopped laying for 26 days. On histology, all chickens had follicles present with a viable granulosa layer. Follicle count between 1 - 2 mm in size varied between 8 - 17 follicles per slide. All control chickens had at least 2 follicles within this size range with a granulosa rating of 1/5 indicating intact and viable granulosa layer. Test group had no follicles within this size range with a viable granulosa layer (1/5 rating). Average follicle rating for the control group ranged between 2.5/5 to 2.9/5 (SD 1.28 - 2.89), and average follicle rating for the test group was 4/5 (SD 0.57 - 0.72). Lack of statistical power was attributed to small sample size. These results reflect that increased LNP dose could potentially lead to long cessation of egg laying. Future studies with larger test groups are needed to determine if granulosa cellular architectural changes are significantly different after LNP treatment. Chicken monoclonal antibodies should be investigated for increased efficacy over the commercial mammalian sourced antibodies.

Keywords: Gonadal suppression, fertility, avian egg laying, granulosa cell, nanocomplex, saporin-6

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Anti-Müllerian hormone and inhibin B concentrations in peripubertal beef bulls before and after castration

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Anti-Müllerian hormone (AMH) and inhibin B have roles in the prenatal testicular differentiation and postnatal testicular development leading to puberty. Also, the presence of inhibin B has yet to be confirmed in the peripheral blood of bulls. In numerous species, AMH has been used as a Sertoli cell biomarker to evaluate testicular function without the need of a stimulation test. The present study aimed to assess reproductive hormones of peripubertal beef bulls before and after castration and to determine the relation of them with semen quality parameters. We hypothesized that both AMH's and inhibin B's peak coincide with puberty and that their level positively correlates with testicular function and semen quality. Spring-born Angus × Simmental bulls (n = 10) were enrolled starting their first fall. Bulls had semen and blood collected once a month for 8 times and then they were surgically castrated from 3 days after the last collection. Serum was harvested and stored until analyses. Semen was collected via electroejaculation; volume, concentration, total sperm, total and progressive motility were assessed to determine the onset of puberty. Scrotal circumference was determined. Puberty was defined when at least 50 x 10^6 sperm with at least 10% motility was present in the ejaculate. Bulls had AMH and inhibin B measured at each timepoint with immunoassays. In addition, inhibin B and AMH were measured right before and after castration. Data were normalized by the onset of puberty and analyzed with R. A Mixed model, Pearson's correlation, and

Tukey's as post hoc were used. Significance was set at $p < 0.05$. A strong correlation was defined by $r > 0.7$, a moderate by $0.3 < r < 0.7$, and a weak one by $r < 0.3$. Bulls reached puberty at 11 ± 0.3 months of age. AMH and inhibin B concentrations were significantly affected by time. Prepubertal AMH concentrations were significantly higher than postpubertal concentrations at every timepoint. AMH concentrations at puberty were 902.3 ± 484.2 pg/ml. Two pre-pubertal peaks of AMH concentrations were noted at 5 (3,385 ± 733 pg/ml) and at 3 (2545 ± 603.9 pg/ml) months before puberty; conversely, the lowest AMH concentrations were at 6 months after puberty (529.3 ± 720 pg/ml). Inhibin B peaked 5 months before puberty (74.1 ± 11.9 ng/ml), and again 3 and 5 months after puberty (79.8 ± 9.7 ng/ml,78.4 ± 11.4 ng/ml). The concentrations of inhibin B decreased after castration (before 49.3 ± 6.9 ng/ml, after 6.7 ± 4.9 ng/ml) ($p < 0.05$). Concentrations of AMH were 575.1 ± 42.8 pg/ml before castration and were undetectable after castration. There was a moderate positive correlation between inhibin and total motility ($r = 0.41$) and progressive motility ($r = 0.38$). In conclusion, puberty did not coincide with peak of AMH nor inhibin B; further studies are needed to examine the value of inhibin B as marker for bull fertility.

Keywords: Endocrinology, testicular function, blood marker

Effects of ram seminal plasma on corpus luteum function and pregnancy outcomes of ewes

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In US, profitability within the sheep industry relies on improvement of reproductive technologies to allow for dissemination of superior genetics. Laparoscopic artificial insemination (LAI) with frozen semen is the most efficient way to improve flock genetics; however, there is a need to improve estrus synchronization protocols. Role of seminal plasma proteins is of interest in synchronization programs, given that nerve growth factor-beta influences ovulation dynamics in camelids and cattle. Objective was to determine if there are factors present in ram seminal plasma that can improve pregnancy outcomes in ewes bred by LAI. We hypothesized that systemic treatment of ram seminal plasma at LAI improves corpus luteum (CL) function and pregnancy outcomes. Semen was collected and pooled from 10 rams, then centrifuged to remove sperm and debris. Ampicillin was added to both seminal plasma and PBS injections to equal a final concentration of 41 mg/ewe. Crossbred ewes (n = 36) were synchronized using an intravaginal progestin device (CIDR) that was removed after 14 days. A 2 ml dose of prostaglandin (Lutalyse) was given intramuscularly at the time of CIDR removal. At 48 hours after CIDR removal, ewes were assigned to 1 of 3 treatment groups: SP (n = 12): ewes that received 2 ml intramuscular injection of seminal plasma at the time of LAI; NEG (n = 12): ewes that received a 2 ml intramuscular injection of PBS at the time of LAI; and POS (n = 12): ewes that were turned out with a ram. Ewes undergoing LAI received 0.25 ml frozen ram semen deposited laparoscopically in both uterine horns using an aspic. Blood was collected before and ~ 4 - 8 hours after LAI or ram breeding for quantification of estradiol, luteinizing hormone, and nerve growth factor-beta. Rams were removed from the POS group after 3 days. Subsequent blood collections were performed every 4 days until day 32 to assess serum progesterone concentrations (days 4 - 20) and pregnancy-specific protein B (days 24, 28, and 32). Whole blood was flash frozen in Trizol on days 16 and 20 to quantify expression of interferon-stimulated genes in peripheral blood leukocytes. Transrectal ultrasonography was performed on days 8, 12, and 16 to measure the maximum CL diameter. Transabdominal ultrasonography was performed on day 52 to confirm pregnancy status. Data were analyzed using ANOVA (CL diameter) and a Wald test (pregnancy outcome) in R. Blood hormone and gene expression data are pending. The maximum CL diameter did not differ ($p = 0.21$) amongst treatments nor were there any effects of day ($p = 0.24$) or treatment × day interactions ($p = 0.67$). Pregnancy rates did not differ ($p = 1$) between SP (33%; 4/12) and POS (33%; 4/12), but tended to be higher ($p = 0.07$) than NEG ewes (9%; 1/11). These preliminary data suggested that factors in the seminal plasma may be beneficial for improving pregnancy outcomes in ewes undergoing LAI; however, further work is needed to determine large-scale implications.

Keywords: Ewes, breeding, ram seminal plasma, synchronization

Feeding spent hemp biomass does not affect testis weight, seminiferous tubule diameter or DAZL immunoexpression in rams

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Spent hemp biomass (SHB) is a byproduct following cannabidiol extraction of hemp. Male mice fed cannabidiol for 34 days had a 30% reduction in fertility rate and a 23% reduction in litter size.[1] Objective was to determine the effects of feeding SHB on fertility in rams. We hypothesized that feeding SHB to rams reduces testis mass and seminiferous tubule diameter. Additionally, we hypothesized that the expression of the spermatogenesis-associated protein DAZL (deleted in azoospermia-like) is reduced following feeding SHB. A feeding trial was performed with 6-month Polypay rams. Rams were randomly assigned to 5 feeding trial groups (7 per group) and fed either alfalfa (control) or SHB at 10 or 20% of their total diet for 4 weeks, with 4 weeks withdrawal from SHB, or SHB at 10 or 20% for 8 weeks. At the conclusion of the feeding trial, rams were euthanized via penetrating captive-bolt. The testes were removed, weighed, and then a 0.5 cm^3 from each testis was fixed in 10% buffered formalin and paraffin embedded. Serial sections were made from each testis and mounted onto charged slides before routine immunohistochemistry was performed using a rabbit anti-DAZL antibody (1:500; Bioss, Boston, MA) or a universal negative control antibody. An adjacent tissue section was stained with hematoxylin and eosin for histomorphometry. Images of all slides were captured using a Leica DM4000B microscope and Leica DFC295 cam-

era. Diameters were measured from 5 representative seminiferous tubule cross-sections per testis. Immunoexpression of DAZL was determined using quantitative image analysis (NIH Image J software). A one-way ANOVA was used to compare variables between groups and significance was defined as $p < 0.05$. Compared to controls, feeding SHB to rams did not significantly affect total testicular weight, seminiferous tubule diameter, or DAZL immunoexpression. Based on these results, we concluded that SHB can be used as a safe feed source for rams without negatively affecting their fertility.

Keywords: Cannabidiol, fertility, immunohistochemistry, sheep

Reference

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Measuring intracellular ice from freezing and ice growth during warming

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Survival of oocyte and embryos postthaw depends on the amount of cryodamage incurred during the freezing and thawing process. Intracellular ice formation is a critical cause of cryodamage and its growth is exponentially greater during warming than during cooling, but measuring intracellular ice during thawing is challenging. Our objective was to determine if synchrotron X-ray diffraction is able to accurately measure intracellular ice formation during freezing and if time-resolved X-ray diffraction can measure ice growth during thawing. Purpose of this proof-of-concept study is to test our hypothesis that properly vitrified oocytes and embryos have little intracellular ice from freezing but some ice growth will occur during thawing even in well-vitrified samples. Bovine oocytes and embryos were incubated in standard vitrification media containing 15% ethylene glycol, 15% dimethylsulfoxide, and 0.5 M sucrose or in diluted vitrification media, mounted on specialized cryoloops designed for synchrotron X-ray diffraction, and plunged in liquid nitrogen using the Nanuq™ cryopreservation system (MiTeGen). Vitrified samples were placed in a cryostream of liquid nitrogen vapor at T = 100 K and the amount of intracellular ice present was detected by measuring diffracted synchrotron X-rays. To measure ice growth during thawing, the nitrogen cryostream was blocked and simultaneously replaced by a flow of warm room temperature gas directed at the sample. X-ray diffraction 'movies' were acquired with 0.05 s frames starting 1 s before thawing. No ice was observed in vitrified oocytes and embryos mounted on Nanuq™ cryoloops, with minimal excess surrounding liquid when standard vitrification media was used. However, when diluted vitrification media was used, ice was first observed at 40% strength of the original concentration. Time-resolved X-ray diffraction data showed ice nucleation and growth during warming even when using standard vitrification media, with warming rates of approximately 500 K/s. The maximum ice diffraction intensity and maximum ice grain size, which are likely correlated with degree of cellular damage, increase with decreasing vitrification media concentration and increasing amount of excess liquid around the sample. The Nanuq™ cryopreservation system used with cryoloops delivers cooling rates of ~ 600,000°C/min, approximately 20 x faster than the current fastest cooling systems. This ultra-fast cooling rate allows cryoprotectant concentration to potentially be reduced to 50% strength without ice formation on cooling, and reduces ice formation during warming. In conclusion, time-resolved X-ray diffraction is a sensitive and quantitative method for measuring ice growth during warming, when the largest intracellular ice fractions and grain sizes were detected. This method may be used to correlate maximum ice fractions with biologically relevant metrics of cryodamage.

Keywords: Cattle, vitrification, intracellular ice, synchrotron, oocyte, embryo

Manipulating cumulus-oocyte-complexes and embryos using microfluidics

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Oocytes and embryos experience very different physical conditions in vivo compared to the conventional in vitro production system of embryos. Microfluidics is a powerful tool to manipulate cells, including gametes, which involves the manipulation of fluids in micro-channels. The fluidic flow and physical environment in microfluidic devices are much more representative of in vivo conditions and may improve outcomes and allow automation of in vitro fertilization. Completing in vitro embryo production in microfluidics requires many steps which introduce challenges in controlling the fluidic flow. Objective of this proof-of-concept study is to determine if controlling outflow and inflow can manipulate oocytes and embryos including positioning of the oocytes for intracytoplasmic sperm injection in-a-chip. We hypothesized that regulating outflow channels using valves and inflow channels using syringes and fluidic pumps results in successful production of embryos in a chip. Microfluidic channel patterns were created using SU-8 negative photoresist on 100 mm silicon wafer exposed to ultraviolet light, then the unexposed portion was removed using SU-8 developer. Microfluidic devices were produced by pouring polydimethysiloxane (PDMS) on the wafer and bonding the PDMS device onto glass slides. Fluidic ports were created using coring needles. Bovine cumulus-oocyte-complexes (COCs) were inserted into the device and the insertion port sealed using pipette tips sealed with PDMS. The COCs were moved to a maturation chamber of the device where maturation media was continuously flowed at 20 µl/minute for 22 hours. The COCs were denuded by using holding media with hyaluronidase passed through narrowing

channels with side channels to hold the cumulus cells. This process was inefficient and most oocytes were not completely denuded. The denuded oocytes were held in a holding chamber for intracytoplasmic sperm injection (ICSI) which was backloaded and the sperm immobilized using XYRCOS laser before injection. Injection was very inefficient with a steep learning curve. After injection, the putative zygote was moved to a culture chamber with continuous flow of embryo culture media (20 µl/minute). Embryos were observed daily for development and proper running of the microfluidic device. Embryo development rate was very low with a single blastocyst formation after 200 injections. Manipulation of COCs and embryos within the microfluidic device was challenging and there was significant risk of bubble formation and contamination during the prolonged culture. Additionally, ICSI was challenging due to the oocytes not being held by holding pipettes but rather nested in an injection cradle. Future modifications are required to increase optimization of the device and process.

Keywords: Microfluidics, intracytoplasmic-sperm injection, oocyte, embryo

Identification of candidate proteins associated with impaired acrosomal exocytosis in Thoroughbred stallions using data-independent acquisition mass spectrometry

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Thoroughbred (TB) stallions that carry the linked susceptibility genotypes A/A-A/A in exon 5 of the *FKBP6* gene (ECA13; EquCab 3.0) are uniquely subfertile because they exhibit normal-to-excellent conventional sperm quality parameters. However, when exposed to known inducers of acrosomal exocytosis (AE), sperm from affected stallions undergo AE at a lower rate than sperm from fertile stallions. In affected stallions, a higher cholesterol-to-phospholipid ratio in sperm membranes has been reported compared to sperm membranes from fertile stallions. A clear causation of the *FKBP6* exon5 genotypes and impaired acrosomal exocytosis (IAE) is still unknown. Sperm proteome in frozen/thawed semen from 3 fertile TB stallions (*FKBP6* genotypes = G/G-A/A, A/G-A/A, and G/G-A/C, respectively; n = 3) and 3 subfertile TB stallions (*FKBP6* genotype = A/A-A/A; n = 3) were studied using mass spectrometry. Sperm were incubated for up to 6 hours under conditions (reported before) to induce spontaneous AE in viable sperm (AE-Viable). At hours 0, 2, 4, and 6, sperm aliquots were analyzed for AE using flow cytometry (FITC-PSA and fixable live/dead red stain), and the sperm proteome was analyzed via data-independent acquisition mass spectrometry (DIA-MS). Student's *t*-tests, two-way ANOVA with Benjamini-Hochberg multiple testing correction (FDR q-value 0.05) were used to determine differences between experimental groups in AE-Viable and protein relative abundance. At hours 4 and 6 of incubation, the mean AE-Viable was higher ($p < 0.05$) in fertile than subfertile stallions (hour 4 = 41 versus 14%; hour 6 = 44 versus 16%, respectively;). A total of 2,220 proteins was identified by DIA-MS. Using strict selection criteria (FDR 1.0%, q-value < 0.05, and log2fold > 0.584 or < -0.584 [e.g., fold change in protein abundance between fertile and subfertile stallions > 1.5]), 140 proteins were differentially abundant in sperm from subfertile stallions compared to fertile stallions (83 less and 57 more abundant). Principal component analysis indicated that these proteins explained 65% of the difference in the sperm proteome between stallion groups. Analysis using the Reactome database and *Homo sapiens* orthologs revealed that the proteins of lower abundance in sperm from the subfertile stallions were mostly overrepresented in the metabolism (32 proteins) and the 'metabolism of lipids' (18 proteins) pathways. Of interest, 2 proteins were common between these pathways, ARSF (arylsulfatase F; fold change 4.02; $p < 0.05$) and ZPBP (Zona pellucida-binding protein; fold change 3.36; $p < 0.05$), are acrosomal enzymes with fundamental roles during sperm-oocyte binding by interacting with proteins in the zona pellucida, including ZP3. Surprisingly, none of the proteins was differentially abundant between fertile and subfertile stallions appeared to have a relationship with the protein encoded by the *FKBP6* gene. These results indicated that DIA-MS is a powerful tool to identify candidate proteins that contribute to etiology of IAE in TB stallions. These proteins might also be useful to help understand the fertilization process in horses.

Keywords: Stallion sperm, impaired acrosomal exocytosis, proteomics, mass spectrometry, cholesterol

Relationship between routine dental care and pregnancy loss in Thoroughbred broodmares

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There is a concern among some veterinarians that routine dental care (reducing long cheek teeth) during an equine pregnancy could release bacteria into the bloodstream resulting in pregnancy loss. Data regarding dental procedures, in women, has mixed findings; no effect and to beneficial effect on pregnancy outcomes. Objective was to determine if a relationship existed between routine dental care (either prior to or during pregnancy) and pregnancy loss in Thoroughbred broodmares. Records from an Equine Hospital and 3 well-managed Thoroughbred breeding farms located in Central Kentucky between January 1, 2013 and January 1, 2021 were used. Data set contained 253 mares representing 414 routine dental procedures (reduction of long cheek teeth) and pregnancies. Information collected from clinical and farm records consisted of mare identification, mare age at dental procedure, farm, date of the dental procedure, date of last breeding, mare's abortion status, abortion date, and foaling date. Dental proce-

dure time was categorized as having occurred either when the mares were nonpregnant, during 1st trimester (0 - 114 days), 2nd trimester (115 - 228 days) or 3rd trimester (> 229 days) of pregnancy. SAS (9.4) FREQ procedure was used to analyze the frequency of time of dental care, abortions by trimester, and by farm. Abortion rate was modeled using SAS GLIMMIX procedure using a binary distribution with the time of dental procedure occurring, farm, year of dental procedure and age in the model. A random statement was used with mare as the subject to account for repeated observations of mares within the data set. Significance was set at $p < 0.05$. Dental care was performed 58 times with nonpregnant mares, 86 times during the 1st trimester, 74 times during the 2nd trimester and 196 times during the 3rd trimester. Abortion rate based on the time dental care was performed was 8.6% for nonpregnant mares, 2.3% for during the 1st trimester, 4.1% during the 2nd trimester and 0% during the 3rd trimester. Percent abortion rate for each farm was 1.2, 1.0, and 0.2 respectively. As per regression model there was no effect of trimester ($p = 0.511$), farm ($p = 0.623$) or year ($p = 0.896$); however, there was an effect ($p = 0.027$) of age. An odds ratio for age was calculated as 1.126 (CI: 1.014 - 1.251), indicating for each 1-year increase in age, mares were 1.126 times more likely to abort. Results suggested that routine dental care to broodmares given any time or during pregnancy was a safe procedure at any time and did not increase the risk of pregnancy loss.

Keywords: Mare, pregnancy, float, dentistry, abortion

Evaluation of corpus luteum function following a single dose of a proprietary slow-release oxytocin formulation in mid-diestrus in the mare

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Need for minimally invasive, cost effective and reversible estrus suppression in the mare has a long-standing history in the equine industry. These objectives have been met utilizing oxytocin to prolong corpus luteum (CL) function. In ≥ 70% of treated mares CL function was prolonged with 60 units of intramuscular oxytocin treatment once daily on days 7 - 14 postovulation. Need for 8 daily injections and reproductive examinations during estrus are drawbacks to widespread clinical application of this protocol. To address these disadvantages, a proprietary slow-release oxytocin (SR-OT) formulation was used with the aim to identify a single dose at an optimum day in mid-diestrus to induce prolonged CL function. It has been established[1] that 1.0 ml (2,400 IU oxytocin) of SR-OT given intramuscularly on days 7 and 10 postovulation induced prolonged CL function in 75% of treated mares. This represents a 75% reduction in the number of oxytocin treatments required to induce prolonged CL function. Current work aimed to further reduce SR-OT to a single treatment, reduce the frequency of transrectal palpation and ultrasonographic examinations to every other day and achieve ≥ 70% prolonged CL function in treated mares. We hypothesized that a single 4,800 IU dose of SR-OT provides a sufficient dose and duration of delivery such that if given on day 8 or 9 postovulation prolonged CL function would be achieved in ≥ 70% of treated mares. Mares in estrus were examined via transrectal palpation and ultrasonographic examination every other day to determine the day of ovulation (defined as day 0). Jugular blood samples were collected on day 0, Monday, Wednesday, and Friday through day 40 to determine serum progesterone concentrations. Mares (n = 16) were evaluated and randomly assigned to 1 of 2 groups, with 8 mares receiving no treatment (control) and 8 mares receiving a single intramuscular dose of SR-OT (4,800 IU oxytocin) on day 8 or 9 (treated). Given that mares were examined every other day, treatment day may have been day 9 for some mares. The proportion of mares in each group with prolonged CL function was compared using Fisher's Exact Test (GraphPad Software, Inc., San Diego, CA). A probability of $p < 0.05$ was considered significant. Mares were classified as having prolonged CL function if their serum progesterone concentrations remained > 1.0 ng/ml throughout the sampling period. Corpus luteum function was prolonged ($p > 0.10$) in 1/8 (12.5%) of untreated control mares and 4/8 (50%) of treated mares. In conclusion, a single intramuscular dose of 4,800 IU SR-OT on day 8/9 failed to prolong CL function in ≥ 70% of treated mares. Small sample size limited the ability to find a significant difference in the proportion of mares with prolonged CL.

Keywords: Mare, estrus suppression, oxytocin, corpus luteum, prolonged corpus luteum function

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Seminal microbiome and reproductive outcomes of Louisiana Thoroughbred stallions

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Microbiome of semen has yet to be extensively investigated in mammals. Meta-analysis of seminal microbiome studies in humans has identified associations with bacterial genera and semen quality. *Prevotella* increased in reduced sperm motility subjects whereas *Lactobacillus* was associated with normal sperm morphology. Although this analysis was not able to determine the effects of seminal microbiota on human fertility, in a bull study increases in *Lawsonella* were correlated with reduced fertility. Few reports have described the presence of a seminal microbiome in normal stallions in Europe; however, reproductive outcomes were not reported. Evidence suggested that the microbiome of reproductive body sites can vary depending on environmental and host factors. Uterine microbi-

ome composition in mares, is influenced by the geographical location and estrous cycle stage. Our purpose was to describe the seminal microbiome of healthy fertile stallions housed on a commercial stud farm in Louisiana. Semen was collected in January during a routine preseason breeding soundness examination. We hypothesized that the microbiome composition of stallion semen during their prebreeding soundness examination is correlated with stallion fertility. Ejaculates from 5 Thoroughbred stallions were collected into an aseptically prepared Missouri artificial vagina prior to the 2018 breeding season. Microbial DNA was extracted from semen and the V4 variable region of 16S rRNA gene was used for DNA amplification and identification. The selected stallions, mean age 14.4 years (range 10 - 19), were determined to be in good health and satisfactory breeding potential during an annual breeding soundness examination. No pathogenic microorganisms were identified on aerobic culture of the semen or reproductive tract. Parameters of semen quality were determined to be adequate based on total motility: mean 83.75% (range: 70 - 95), progressive motility: mean 74.38% (range: 60 - 85), viability: mean 76.23% (range: 65 - 84.3), morphology: mean 63% normal (range: 53 - 87), concentration: mean 182.63 x 10^6/ml (range: 55.6 – 451 x 10^6/ml), total sperm per ejaculate: mean: 10.55 x 10^9 (range: 5.24 - 16.9 x10^9). Stallion fertility for the 2018 breeding season was determined to be sufficient based on seasonal pregnancy rates as determined by day 14 postovulation transrectal ultrasonography: Stallion 1, 43/47 (91.49%), Stallion 2, 34/38 (89.47%), Stallion 3, 50/55 (90.90%), Stallion 4, 5/5 (100%), and Stallion 5, 5/5 (100%). The semen microbiota had a high diversity (Shannon Index) and low richness (Chao Index) and was dominated by 2 bacterial classes, namely Bacteroidia and Clostridia. In conclusion, our results suggested that equine semen dominated by Bacteroidia and Clostridia in January could reflect a healthy seminal microbiome for stallions in Louisiana. Further studies are required to elucidate environmental and host factors that may influence the microbiome of stallion semen, the implications for potential future fertility and clinical value as part of the breeding soundness examination.

Keywords: Stallion, microbiome, fertility, semen

Effect of freezing extender and cryoprotectant on blastocyst production after intracytoplasmic sperm injection

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Intracytoplasmic sperm injection (ICSI) using frozen/thawed sperm is a common procedure to obtain embryos from either fertile or subfertile mares and stallions. Stallion-associated factors that impact the efficiency of ICSI have been less studied than mare-associated factors. Semen extenders for freezing stallion sperm vary in their composition, particularly egg-yolk concentration, presence of milk products, and penetrating cryoprotectant-type. For some stallions, dimethylformamide-containing extenders, irrespective of the egg-yolk concentration or presence of milk products in the base freezing extender, are reported to result in higher postthaw motility and in vivo fertility than glycerol-containing extenders. To date, no studies have determined effects of these extender components on embryonic development after ICSI. Current experiment determined the effect of 4 commercially available semen extenders containing either glycerol (LE or MFR5) or a combination of glycerol and dimethylformamide (CMLE or CMMFR5) on the blastocyst rates resulting after ICSI. Both LE and CMLE extenders are formulated with 20% egg-yolk (v:v), whereas MFR5 and CMMFR5 extenders are formulated with milk and 4% egg-yolk (v:v). Immature oocytes were recovered via transvaginal, ultrasound-guided follicle aspiration and matured in vitro. After maturation, oocytes were injected by Piezo-driven ICSI using sperm from each 1 of 3 fertile stallions that was frozen using each 1 of 4 freezing extenders. Sperm quality parameters: % total motility (TMOT), % viability, and sperm % DNA damage ($COMP_{\alpha\text{-}t}$) were determined immediately after thawing (3 ejaculates per stallion, n = 9). After injection, the presumptive zygotes were evaluated for cleavage on day 5 and for blastocyst formation on days 7 - 10. Effects of semen freezing extender, stallion, and their interaction on postthaw semen quality and blastocyst rates after ICSI were analyzed using the General Linear Model and Chi-Square analysis (JMP Pro 16.0). Significance was set at $p < 0.05$. Mean TMOT was higher ($p < 0.05$) in CMLE and LE than in MFR5 or CMMFR5 extenders (52 and 49% versus 39 and 38%, respectively). Mean viability and $COMP_{\alpha\text{-}t}$ were not affected ($p > 0.05$) by the extender (range: 50 - 56% and 9 - 14%). An extender-by-stallion interaction for blastocyst rate was not detected ($p > 0.05$). Extender MFR5 resulted in a higher ($p < 0.05$) blastocyst rate (embryos/injected oocytes) than extenders LE, CMMFR5, or CMLE (26% [21/82] versus 10% [8/77] versus 5% [4/82] versus 5% [4/80], respectively). Extenders with higher egg-yolk content (CMLE, LE) had higher sperm quality than extenders with lower egg-yolk content and milk (CMMFR5, MFR5). However, extender MFR5 had the highest blastocyst rate after ICSI, suggesting an interaction among the freezing extender components (i.e., low egg-yolk quantity, milk proteins, and glycerol) and embryonic development after ICSI. Results also indicated that the presence of dimethylformamide in the freezing extender, irrespective of egg-yolk concentrations or the presence of milk proteins, negatively affected the in vitro development of equine embryos.

Keywords: Mare, intracytoplasmic sperm injection, frozen semen, extender, blastocyst

Antimicrobial activity of platelet-rich plasma against common microorganisms causing endometritis in mares

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Ever evolving microbial resistance and tightening regulations against the indiscriminate use of antimicrobials warrant the development of alternative therapies to treat endometritis in mares. Platelet-rich plasma (PRP) has emerged as an alternative therapy to modulate persistent breeding endometritis, due to its antiinflammatory and potential antimicrobial properties. However, limited work has been done to assess the antimicrobial properties of PRP against microorganisms causing endometritis in mares. Therefore, this study aimed to assess in vitro antimicrobial activity of PRP against microorganisms causing endometritis in mares. We hypothesized that PRP antimicrobial activity is microorganism-dependent. Percentage of inhibition (PI) and minimum inhibitory concentrations (MIC) of PRP were assessed with the microdilution broth method using 96-well flat-bottom microplates. Clinical isolates of *Streptococcus equi* (*Strep*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*Pseudo*), *Klebsiella pneumoniae* (*Kleb*), *Staphylococcus aureus* (*Staph*), and *Candida albicans* (*Candida*) cultured from mares with clinical endometritis were used. Blood samples were collected from 6 mares (3/mare) and double centrifuged to produce PRP. Each PRP sample was run 3 times, and within each run, 3 wells were used per microorganism. First 3 lanes (1 - 3) of each plate served as the negative controls that consisted of 100 µL PRP (1056 ± 198 $\times 10^3$ platelets/µl) and 100 µl of Mueller-Hinton (MH) (1:1) added to10 µl calcium chloride 10%. Thereafter, the remaining lanes (4 -12) contained serial dilutions of PRP as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128. Row H (Lanes 4 -12) served as positive controls and contained no PRP. Then, 5 µl (5×10^4 colony-forming unit) of each isolate was added to lanes 4 - 12. Then plates were incubated at 37°C for 24 hours. Optical density of the wells was measured at 570 nm using a SpectraMax M2 spectrophotometer (Molecular Devices, San Jose, CA). The PI was calculated by the difference between the mean of the triplicates in each group and the mean of the negative control and the positive control. To determine MIC, 10 µl of resazurin solution (0.01%) was added to each well, and the plates were kept at 37°C for 1 hour. Subsequently, a visual reading was performed to determine microorganism activity; if the well became pink, microbial activity was present, whereas no microbial activity was present if the well remained blue. Data were analyzed by ANOVA and Tukey's post hoc test. Significance was set at $p < 0.05$. Platelet-rich plasma had a greater inhibition ($p < 0.05$) compared to the control up to titers of 1:4 (*Candida*); 1:16 (*Staph*); 1:32 (*E. coli*); and 1:128 (*Strep*; *Pseudo; and Kleb*). All PRP samples inactivated *E. coli* in the MIC assay at 1:2, whereas *Pseudo* was inactivated in 33% of the samples at 1:2. The *Staph* was inactivated only in 16% of the samples treated with PRP. The remaining microorganisms (*Strep*, *Kleb*, and *Candida*) were not inactivated. In conclusion, we demonstrated that PRP antimicrobial activity in vitro was dose- and microorganism-dependent. The findings hint that greater platelet counts could result in superior clinical efficacy and that not all microorganisms are susceptible to PRP.

Keywords: Equine endometritis, antibiotic, platelet-rich plasma, subfertility

Pentoxifylline reduces postbreeding uterine fluid accumulation in older embryo donor mares and may enhance embryo recovery

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Most donor mares in embryo transfer programs have impaired uterine clearance due to uterine and cervical problems. Additionally, repeated breeding cycles, reproductive procedures, and embryo flushing make embryo donors prone to endometritis. Anecdotally, pentoxifylline, an antiinflammatory and rheolytic drug, has been used in mares to enhance blood flow in the reproductive tract and reduce uterine inflammation. However, this has not been critically studied. This study assessed the postbreeding inflammatory response and embryo recovery of subfertile donor mares treated with pentoxifylline. We hypothesized that pentoxifylline supplementation mitigates the postbreeding inflammatory response in embryo donor mares. Estrous cycles (n = 42) of 7 embryo donor mares (age 16 ± 3.7 years; 13 ± 3 years of donating embryos) were studied. During first 2 cycles, mares received pentoxifylline (17 mg/kg/day) in 50 ml of Broodmare-Plus® (Botupharma). The 2 subsequent cycles served as washout (no supplementation), and then in the last 2 cycles, mares received 50 ml of Broodmare®. Mares were monitored daily, and then once a preovulatory follicle was detected, ovulation was induced with intramuscular deslorelin (1 mg). All mares were bred with fresh extended semen (1×10^6) from the same stallion. Immediately before (0), 24, 48, and 72 hours postbreeding, mares had transrectal ultrasonography performed to determine ovulation, quantify uterine edema, and intrauterine fluid accumulation (IUF). At similar timepoints, uterine cytology sample was collected, stained with Diff-Quik®, and polymorphous nuclear cells were counted in 10 high power fields. Embryo collections were performed 8 days postovulation, and mares received an intramuscular injection of 5 mg of dinoprost (Lutalyse®, Zoetis, Parasipanny, NJ) to return to estrus. All mares had at least 1 ovulation confirmed before the onset of the study, and then each mare started in a new group 8 days postovulation. Data were analyzed with Graph Prisma (San Diego, CA). Neutrophil counts were analyzed with ANOVA repeated measures and Tukey's as post-hoc. Edema scores and intrauterine fluid accumulation were analyzed with Kruskal-Wallis and Dunn's post-hoc. Embryo recovery rates were evaluated with Fisher's test. There was an effect ($p = 0.032$) of time for neutrophil counts in the washout cycle but not ($p > 0.05$) in the other 2 groups. Neutrophil counts did not differ ($p > 0.05$) across groups. Edema scores had effects ($p = 0.046$) of time but no effects ($p > 0.05$) of group. Similarly, there was an effect ($p = 0.038$) of time for IUF, and mares receiving pentoxifylline had reduced ($p = 0.034$) postbreeding IUF compared to washout cycles but not different ($p > 0.05$) than the group receiving just Broodmare®. Embryo recovery was not different ($p > 0.05$) across groups (but remarkably dif-

ferent as follows: washout cycle was 29% (4/14); Broodmare® was 36% (5/14), and pentoxifylline (Broodmare-Plus®) was 57% (8/14). Mares in the study were subfertile older mares used as embryo donors for multiple years, thus, justifying the low embryo recovery for the cycles with no pentoxifylline. In conclusion, supplementation with pentoxifylline may be an alternative to improve the postbreeding uterine clearance. It may enhance embryo recovery in mares serving as donors for multiple years; however, this remains to be confirmed with a larger number of mares.

Keywords: Donor mares, endometritis, uterine fluid, inflammation, fertility

A retrospective study of the impact of transrectal palpation on teaching mares

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Transrectal palpation (TRPalp) in mares is considered a potentially life-threatening, noxious procedure, particularly when performed by inexperienced individuals. Such a risk may prompt teaching institutions to consider replacing live animal laboratories with model simulators. However, removal of live animals from the veterinary curriculum would significantly minimize the amount of real-life experiential education provided to veterinary students. Objectives were 3 fold: i. identify the incidence of mare injury associated with TRPalp in teaching mares, ii. identify the number of intravenous sedation utilized to circumvent averse behaviors (i.e., behaviors interpreted as 'dislike'), and iii. identify the duration of individual mares' careers/cited reasons for retirement. We predicted that the incidence of mare injury from TRPalp will be low; additionally, we hypothesized that averse behaviors during TRPalp are not a commonly cited reason for mare retirement. Transrectal palpation records (2019 - 2021) of 48 mares (age 4 - 26 years) were evaluated. Analyzed data were collected during years 2 and 3 laboratory courses, senior student clinical rotations, and other student teaching opportunities. Documented mare injuries from TRPalp included any amount of blood on rectal sleeves and any degree of rectal tearing. Individual mare medical records documenting date of entry into the teaching herd, date of retirement, and the reason for retirement were analyzed. Per our standing IACUC, mares may be transrectally palpated up to 15 times in a 7-day period. A total of 5,801 TRPalp events occurred between 2019 - 2021, with a mare injury incidence rate of 0.76% (44/5,801); no injury required surgical correction or resulted in death. Sedation was utilized to minimize averse behaviors 0.34% of the time (20/5,801) with 11 of 48 mares having received sedation. Individual mare careers ranged from 1 - 22 years with 10 of 48 mares retiring between 2019 - 2021; no retirement was related to averse behaviors or injury experienced during TRPalp. Based on these data, the probability of a mare retiring from the teaching herd because of averse behaviors or injury from 2019 - 2021 was 0; however, being that TRPalp can certainly result in injury to a horse, our probability of 0 does not indicate that an injury leading to the retirement or death of a mare is impossible. If TRPalp was a noxious procedure, one would expect teaching mares to progressively develop aversion behaviors and require increased use of sedation, although this was not the situation in our herd. Whereas it is prudent to perform TRPalp with caution and restraint, these data indicate that injury to teaching mares can be kept to an extremely low rate of occurrence. Therefore, our impression is that concerns for animal welfare (i.e., averse behaviors and mare injury) should not be cited as a reason to minimize live animal use within the veterinary curriculum.

Keywords: Transrectal palpation, behavior, injury, attrition rate

Analysis of pH of common intrauterine therapies in mares

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Intrauterine therapies are commonly used to treat endometritis in mares and have some advantages over systemic treatments, including being able to achieve higher local concentrations and physically dilute or disrupt biofilm in the uterus. Saline (0.9% NaCl) (SAL) and lactated Ringer's solution (LRS) are 2 commonly used fluids for intrauterine lavage and treatments. Sterile saline does not contain buffers and is more acidic compared to LRS. However, some clinicians are concerned that lactate-utilizing bacteria may benefit from lactate in LRS. Intrauterine therapies may be irritating to the mare's uterus due to the drug carrier or due to extreme pH. The pH of commonly used intrauterine therapies is not reported and may be different when combined with SAL or LRS. Objective of this descriptive study was to report the pH of commonly used intrauterine therapies in mares diluted with SAL or LRS. We hypothesized that intrauterine therapies diluted with LRS are closer to neutral compared to SAL. The pH of intrauterine therapies was assessed with a hand-held device (LAQUA Twin pH Meter, Horiba) and a benchtop pH meter (Accumet AB15+, Fisherbrand). Difference in pH by fluid used, treatment, and device were analyzed using linear regression ANOVA in JMP pro v16, with significance set at $p < 0.05$. The average pH of the following intrauterine therapies were: SAL (5.5 ± 0.03), LRS (6.3 ± 0.03); Ceragyn flush in SAL (3.2 ± 0.03) and LRS (3.2 ± 0.03); vinegar 2% in SAL (3.6 ± 0.03) and LRS (4.3 ± 0.03); vinegar 10% in SAL (3.6 ± 0.02) and LRS (3.7 ± 0.17); povidone iodine solution 1% in SAL (3.2 ± 0.03) and LRS (5.0 ± 0.09); hydrogen peroxide 1% in SAL (5.6 ± 0.15) and LRS (6.0 ± 0.02); dimethyl-sulfoxide (DMSO) 10% in SAL (5.4 ± 0.09) and LRS (6.2 ± 0.02); DMSO 30% in SAL (5.9 ± 0.02) and LRS (7.1 ± 0.09); N-Acetylcysteine 3.3% in SAL (5.8 ± 0.07) and in LRS (5.9 ± 0.06); gentamycin 2 grams in SAL (6.2 ± 0.02) and LRS (6.2 ± 0.05); ampicillin 1 gram in SAL (9.1 ± 0.05) and LRS (9.0 ± 0.08); ampicillin 2 grams in SAL (9.3 ± 0.15) and LRS (9.8 ± 0.04); and ceftiofur Na 1 gram in SAL (6.5 ± 0.08) and LRS (6.6 ± 0.00). There was no significant effect of fluid or device used but a significant effect of the

treatment type. There was a strong correlation (R^2 = 0.9563) between the handheld and benchtop pH meters. Some commonly used infusions are very acidic, including Ceragyn Infuse (pH = 3.6) that has the same pH as undiluted gentamycin that is known to be irritating to the endometrium, whereas Tricide (pH = 8.4), and DMSO (pH = 11.8) are alkaline. Knowledge on the pH of commonly used intrauterine therapies of mares is valuable to guide decision making, balancing treatment of bacteria that thrive in neutral pH and acidophiles, and treating biofilm without harming the endometrium.

Keywords: Mare, intrauterine therapy, pH, uterine infusion

Pregnancy and spontaneous partial abortion associated with *Salmonella* in a bitch

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An intact, 2-year mixed breed bitch (Bernese Mountain Dog x Standard Poodle) at 50 days postbreeding was presented for a greenish-brown vulvar discharge of 12 hours duration. Dog had a stillbirth 8 hours prior. Bitch was not fed a raw diet. Due to hyporexia, the owners provided cooked chicken and eggs. Physical examination revealed a body condition score of 3/9 (Royal Canin® scale) and nonfetid brown vulvar discharge. Mammary gland secretions were absent. On vaginal examination, neither Ferguson reflex, fetuses, nor membranes were palpable. Vaginoscopy revealed brown mucoid discharge that obstructed cervical viewing. Abdominal ultrasonography revealed premature fetuses, with normal heart rates between 222 - 300 beats/minute. Abdominal radiographs revealed 10 fetuses. Serum progesterone concentrations were 1.25 ng/ml; bitch had a mild monocytosis and was hypocalcemic. An in-house serum *Brucella canis* rapid slide agglutination test and herpesvirus titer were negative. A specimen of vaginal discharge was obtained for aerobic culture, and cytology revealed nonseptic neutrophilic inflammation. Due to infection and risk of sepsis, ovariohysterectomy was offered but declined by the owner. Overnight, the bitch was given intravenous lactated Ringer's solution, terbutaline, fenbendazole, altrenogest, amoxicillin, and clavulanate potassium (Clavamox®), and clindamycin, and was discharged with oral medications the following day due to financial constraints. During hospitalization, another stillborn pup was spontaneously delivered; gross necropsy lesions were absent. On physical examination 2 days later, the bitch was clinically stable with vulvar discharge. Abdominal ultrasonography revealed live fetuses with slightly increased fetal maturation of kidneys and gastrointestinal tract, but 1 dead fetus. The bitch was not azotemic, but urine specific gravity was isosthenuric at 1.008. Preliminary vaginal culture results grew *Salmonella sp.*; based on susceptibility, clindamycin was discontinued and therapy with ciprofloxacin initiated. Five days after initial presentation, the bitch was febrile with erythematous pinnae. A marked acute inflammatory leukogram with mild hypoglycemia and hypocalcemia was present. Final vaginal culture had *Salmonella enterica* subsp. *houtenae* serovar IV 50:z4,z23:-. Due to this organism, altrenogest and terbutaline were discontinued to allow the bitch to whelp. After the last dose of altrenogest, a stillbirth occurred at 12 hours and whelping began at 60 hours, respectively. Two live pups and 1 additional stillborn were delivered at home. Upon presentation for dystocia, fetal heart rate was 120 beats/minute for 1, indicative of severe fetal distress, and absent for another. Calcium gluconate and oxytocin treatment resulted in birth of 1 live pup. Without further contractions, Cesarean section resulted in the delivery of 1 live pup and 1 necrotic fetus. Uterine aerobic culture resulted in no growth and the bitch remained intact. Due to risk of sepsis, the 4 pups were given ceftiofur, trimethoprim sulfa, and subcutaneous 0.9% sodium chloride. One pup was meconium stained; another died at home 2 days later. Seven days postpartum, the bitch was stable, and 3 remaining pups were clinically normal. Placental histopathology revealed acute suppurative chorioplacentitis, with *Salmonella* as the presumptive etiology. This case demonstrated the significance and challenges of medical management of fetal-placental infections in the bitch.

Keywords: Dog, pregnancy, *Salmonella*, infection, abortion, dystocia

Unusual disorder of sexual development in a horse: a monorchid 64, XY, SRY-positive phenotypic mare

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Disorders of sexual development (DSD) are uncommon in horses and monorchidism conditions are very rare. This case report outlines the clinical assessment of a mare with a behavioral issue that led to the diagnosis of DSD. A 4-year Quarter Horse mare was referred with a history of stallion-like behavior and an elevated serum testosterone concentration. Differential diagnoses included granulosa theca cell tumor, pregnancy, disorder of sexual development, estrus, and anabolic steroid treatment. Oral examination revealed erupting canines and the size were consistent with that of a mare. External genitalia were normal in appearance for a mare. A normal mammary gland with 2 small bilaterally symmetric teats were present. Transrectal ultrasonography revealed a structure in the right abdomen consistent with the morphologic appearance of a testis. No gonadal structure was identified on the left side. A vaginal speculum examination revealed a short, 'blind-ended' vaginal cavity, with no cervix. Endocrine evaluation consisted of serum testosterone concentrations pre- (731 pg/ml) and post- (2 hours: 762 pg/ml;

15 hours: 773 pg/ml) human chorionic gonadotropin (hCG) treatment, anti-Müllerian hormone ([AMH] 96 ng/ml), estrone sulfate (11 ng/ml), and inhibin B (76 pg/ml). All but inhibin-B concentrations were above the upper limit of the laboratory reference ranges for a nonpregnant mare. Cytogenetic analysis revealed a 64, XY karyotype. Sex determining region of the Y chromosome (SRY) was identified by PCR analysis. A standing laparoscopic procedure was performed and a cryptorchid testis was identified on the right side of the abdomen and removed. A similar gonadal structure was absent on the left side. Histopathology of the excised right gonad revealed Sertoli cells, Leydig cells, and spermatogonia within degenerate seminiferous tubules with no evidence of spermatogenesis. Additionally, a nest or island of adrenal cortical tissue was noted histologically within the cryptorchid testis and epididymal tissue was noted adjacent to the testis. Immunohistochemistry confirmed AMH presence in Sertoli cells. Reevaluation 5 weeks after surgery revealed resolution of the stallion-like behavior and normal endocrine parameters. In summary, a phenotypic mare with stallion-like behavior was diagnosed as a monorchid, 64, XY, SRY-positive, disorder of sexual development (DSD). Monorchidism was diagnosed by a combination of laparoscopic exploration of the abdomen, identification and removal of 1 gonad, and a subsequent decrease in testosterone and AMH concentrations to that consistent with a gelding. Presence of ectopic adrenal cortical tissue within the cryptorchid testis was unexpected, but has been reported in gonads of horses and other species. Finally, diagnosis of DSD conditions was based on a combination of clinical signs, endocrine evaluation, chromosomal analysis, and histopathology.

Keywords: Mare, monorchidism, disorder of sexual development, karyotype, cryptorchid

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Five-year mare with no history of foaling and early pregnancy loss had retained endometrial cups

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A 5-year, maiden Dutch Harness Horse mare, was presented for a routine breeding soundness examination prior to undergoing breeding management that would utilize cooled, shipped semen. Transrectal ultrasonographic examination revealed normal ovaries and uterus. Cervix appeared normal (speculum examination). Mare was in early estrus with a dominant follicle on the left ovary and a Grade II edema; samples for uterine culture and cytology were obtained. Cytology was normal and the culture had no growth. Due to schedule conflicts, mare was not scanned for another 5 days. At that time, mare appeared to have a corpus luteum (CL) on the right ovary - the opposite ovary from where the dominant follicle had been documented. Mare was examined 6 days later with plans to induce estrus (prostaglandin treatment): however, at this time she had a 40 mm follicle on the left ovary with the CL still present on the right ovary. Prostaglandin was not given. Four days later, the mare was again evaluated and appeared to have a CL on the left, small follicles on the right and moderate endometrial edema. Mare continued to be closely monitored due to erratic cycling with transrectal ultrasonography performed every other day. During owner consultation, owner maintained that the mare was sold as a maiden with no reported reproductive history. Over the course of her management, the mare proceeded to form a new CL without a true estrous cycle ~ every 7 days. At this time, blood equine chorionic gonadotropin (eCG) concentrations were determined (BET Labs) and were 2.9 IU/ml (normal 0.0 in nonpregnant mares), lending to a diagnosis of retained endometrial cups. Further investigation by the owner into the mare's history revealed that the mare was reportedly bred 2 years prior but never produced a foal. The owner was given the option of endometrial cup removal via laser ablation or chemical curettage; chemical curettage with kerosene was performed. Mare became appropriately cyclic immediately after treatment and was bred. Mare conceived on the first cycle following treatment and is currently ~ 210 days in foal. Endometrial cups produce eCG and form from the chorionic girdle of the fetal membranes in the mare at ~ 35 days and regress naturally at ~ 120 -150 days pregnancy. Endometrial cups will regress on their own at 120 - 150 days as the allantochorion takes over the role of progesterone production. Retaining endometrial cups past this point is abnormal but should be considered in mares with erratic cycling, incomplete estrous cycles, or luteinization of partly developed follicles. Diagnosis is best confirmed via detection of eCG. Although chemical curettage with kerosene is typically ineffective in mares with higher eCG concentrations, in the authors' experience it can be useful in treatment of mares with lower eCG concentrations.

Keywords: Mare, retained endometrial cups, equine chorionic gonadotropin

Management of postsurgical complications in a cat diagnosed with feline mammary fibroepithelial hyperplasia

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A 1-year, intact, American Domestic Shorthair queen was presented for complications associated with feline mammary fibroepithelial hyperplasia (fMFH). On presentation, the cat had pyrexia, anorexia, dull mentation, and glandular enlargement along the entirety of both mammary chains. Mammary tissues were firm, edematous, and erythematous. Patient displayed hypersensitivity to palpation of the affected area and exhibited hunched posture. Approximately 3-cm, nonhealing ulcer was noted along midline at site of previous abdominal incision. The ulcer was black, malodorous, and diffusely necrotic. A 3-cm, healing incision was identified along the patient's right flank. Following its adoption, the patient was diagnosed as pregnant

and pregnancy termination with simultaneous ovariohysterectomy (OHE) was elected. The initial surgical approach occurred via ventral midline incision but was halted due to excess hemorrhage from hyperplastic mammary tissues. Forty-eight hours later, the patient underwent OHE via flank laparotomy. Patient was presented 1 week following flank OHE due to patient's continued mammary enlargement, nonhealing abdominal wound, and overall malaise. After examination, swab specimen of the ventral midline ulcer was submitted for bacterial culture and sensitivity. Initial intervention included fluid resuscitation to correct dehydration, pain and wound management, and prolactin antagonism treatment. Immediate pain management occurred via continuous rate infusion (CRI) of hourly ketamine (0.1 - 0.5 mg/kg/hour) and once every 12 hours oral gabapentin (25 mg). A nonsteroidal antiinflammatory oral drug was incorporated (robenacoxib, 6 mg every 24 hours) as an antipyretic to reduce inflammation associated with the patient's wound and mammary hyperplasia. A course of dopamine receptor agonist oral cabergoline (25 µg) was given every 24 hours to suppress lactation via reduction of prolactin. Given the patient's febrile status and chronicity of the mammary ulceration, oral marbofloxacin (25 mg) was given every 24 hours while awaiting culture and sensitivity results. Patient sedation followed by debridement of devitalized mammary tissue was performed. Silver sulfadiazine (SSD) was liberally applied along the length of the debrided wound to stimulate epithelialization and topically treat infection. Wound debridement followed by application of SSD was continued daily. Bacterial culture results revealed heavy growth of *Staphylococcus intermedius*, resistant to marbofloxacin. Antibiotic therapy was then adjusted to oral clindamycin (150 mg), given every 12 hours, effective per susceptibility report. Due to extensive tissue injury, hyperbaric oxygen therapy (HBOT) was incorporated in the treatment plan to facilitate delivery of oxygen-rich plasma to damaged cells. Cat received a total of 3 HBOT treatments of HBOT at 48-hour intervals. One week after admission, the patient appeared bright, appetent, afebrile, and comfortable. Mammary hyperplasia had started to resolve, evidenced by decreased mammary size, and the patient was discharged to the care of the owners. This case provided an account of fMFH, a dysplastic response of mammary tissue to elevated progesterone concentrations. Tissue-healing and patient health were compromised in this case of fMFH, often a clinically benign condition, because of complications associated with ventral midline incision. To avoid additional insult of mammary tissues, flank incision for OHE may prove a superior approach in cases of fMFH. Collaboration between theriogenology service and pain management fields, and careful wound management proved beneficial for this patient.

Keywords: Cat, mammary fibroepithelial hyperplasia, hyperbaric oxygen therapy, pain, wound

Pregnancy in a *Sry*-negative XX sex reversal pig after removal of the ovotestis

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Disorders of sexual development have been documented in over 20 species including pigs in which sex reversal of XX *Sry*-negative animals has been reported to occur in 0.1 - 0.6% of the population. Reports of XX *Sry*-negative sex reversal have also been documented in dogs, goats, and horses. In these animals, 1 or both gonads has ovarian and testicular tissue and underdeveloped female reproductive tract is often present. Owing to high testosterone concentrations from testicular tissue, pregnancy is unlikely even when normal uterine structure is present. Whether removal of testicular tissue can rescue the ability to achieve a pregnancy has not been fully explored for any species with XX *Sry*-negative sex reversal. Here, we identified a newborn gilt with a 'skyhook' vulva, a common trait associated with sex reversal in pigs. At 5 months of age, the gilt displayed signs of estrus but the behavior was characterized as aggressive and reflected masculinity. Artificial insemination was attempted several times with semen from a proven fertile boar but the insemination pipette could only be advanced 10 cm into the vagina (average vaginal length in pigs is 25 cm). Gilt never achieved a pregnancy from these breeding attempts. Subsequent attempts at estrous cycle synchronization did not result in normal signs of estrus. Abdominatl ultrasonographic examination revealed uterus and left ovary appeared normal; however, contralateral gonad was abnormally shaped with a testis-like echotexture. On exploratory laparotomy, right gonad was suspected to be an ovotestis and was removed. On gross examination, the gonad had 2 distinct tissue types with clear demarcation. The presumed testicular portion was smooth and compact, whereas the ovarian portion was smaller and presented a few small follicles and 1 corpus luteum. Left ovary appeared normal and had corpora lutea (8 -10). Histologic examination confirmed the diagnosis of ovotestis. The tissue consisted of Leydig cells and seminiferous tubules but germline was absent. Cytogenic evaluation revealed the gilt to be XX, 38; *Sry*-negative. Four months after the removal of the ovotestis, the animal presented a normal estrus with complete immobility reflex and was artificially inseminated yielding a pregnancy that produced 6 healthy piglets. This novel case exemplified surgical removal as a treatment to aid in return to fertility in *Sry*-negative XX sex reversal gilts with a remaining fully developed female reproductive tract.

Keywords: Gilt, Sex reversal, hermaphroditism, pregnancy

Small colon rupture in a postfoaling mare

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A 15-year, American Quarter Horse mare, was presented to a referral hospital for further evaluation and treatment of postpartum colic. Night before presentation, the mare experienced a dystocia (anterior presentation, dorsosacral position, right forelimb flexion at the elbow); the mare delivered a foal before farm personnel could correct the foal's posture. Fetal membranes passed normally; shortly thereafter the mare appeared acutely uncomfortable/agitated but remained quiet with sedation and analgesia. Following morning, the referring veterinarian diagnosed the mare with a large colon impaction prior to referral. At presentation, the mare was quiet, alert, and re-

sponsive with a mild tachycardia (56 beats/minute); mucous membranes (mm) were pink and moist with a capillary refill time of < 2 seconds. Other vital parameters were within normal limits (WNL). Borborygmi were present in all quadrants and bilateral plaques of ventral edema, considered normal for late pregnancy, were present. Passage of a nasogastric tube yielded 9 liters of net enterogastric reflux. Postpartum hemorrhage (PPH) was suspected via transabdominal ultrasonography with moderate amount of free, hypoechoic fluid. To minimize the risk of introducing bacteria into a hemoabdomen, and taking into account the plaques of ventral edema, an abdominocentesis was not performed. Amid concern of PPH, a complete transrectal palpation was not performed. A vaginal examination with a Polanski speculum revealed mild vaginal bruising and a Grade I perineal laceration at the dorsal commissure of the vulva. Additionally, unusual gas distention of an empty rectum was appreciated. A presumptive diagnosis of PPH and resulting hemoabdomen with a large colon impaction was made. Initial medical treatment included intravenous fluid therapy and observation; enteral fluid therapy for treatment of colon impaction was not pursued due to reflux. A serum chemistry panel and complete blood count eventually revealed hemoconcentration, severe neutropenia with a degenerative left shift, and marked toxic changes; broad spectrum antibiotics were initiated due to updated concerns of a uterine or intestinal rupture. Overnight, the mare developed tachycardia (80 beats/minute) with continued reflux (~ 2- 3 liters every 2 hours), but appeared stable and mm remained WNL. Worsening tachycardia was noted the following morning (120 beats/minute) warranting further diagnostics which revealed an increasing packed cell volume (66%) and additional abdominal fluid accumulation. Abdominocentesis revealed severe septic inflammation with mixed bacteria and mm acutely appeared dusky. Exploratory enterotomy was declined due to sudden, rapid deterioration and the mare was euthanized. A 15.0 cm tear/rupture was identified in the small colon during necropsy examination, located 60 cm cranial to the anus. The affected segment was dark red to purple. The peritoneal cavity contained ~ 3 liters of yellow to red-tinged fluid with flecks of fibrin, blood clots, and fecal material. The serosal surface of the small and large intestine and peritoneum were diffusely dark red. No intestinal impaction, uterine or bladder rupture was identified. The gross findings confirmed a small colon tear/rupture resulting from segmental ischemic necrosis. Foaling in the face of malposture was considered as a potential catalyst for the small colon's segmental ischemic necrosis and rupture.

Keywords: Mare, dystocia, colic, postfoaling complication

Hamartoma in the reproductive tract of a Thoroughbred mare

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Vascular hamartomas are rare, even more so in equine patients. Hamartomas are benign, noncancerous tumor-like malformations that are made up of an abnormal mixture of cells that are native to the anatomic location. They grow at the same rate as the original tissue and are mostly due to a developmental error. Hamartomas have been reported in equine fetal tissues including lung and liver, subcutaneous tissue of a pelvic limb, spinal cord, and an ovary. More specifically vascular hamartomas have been reported in the tongue, dorsal carpal region, and an ovary of adult equine patients. A 6-year, maiden mare, recently retired from racing, was first evaluated in late January to evaluate the reproductive tract prior to breeding. At that time the referring veterinarian noted cervical bruising; the bruising did not resolve after 60 days of observation and the mare was then referred for further examination. Transrectal palpation and ultrasonographic examination revealed the mare to be in diestrus (corpus luteum and medium follicles) with both uterine horns having thickened endometrial folds with no edema but multifocal small echogenic flecks within the endometrium were noted. These numerous, multifocal, small hyperechoic regions between 0.5 - 2 cm were noted in the endometrial wall of the uterine body. Cervix appeared edematous and had multifocal to diffuse, small (0.5 cm) hyperechoic flecks. Hysteroscopy revealed severe discoloration with purple to black, hemorrhagic tissue in the vagina, throughout the cervix, and entire uterine body. Strands of fibrous adhesions were present throughout the endometrium coalescing on pale crater-like lesions between 2 - 3 cm in diameter distributed throughout the endometrial surface. Vaginoscopy revealed purplish-brown discoloration extending from a linear ventral streak on the vaginal floor to encompass the entire cervix. The appearance was consistent with a bruise or trauma. Histologically, there were numerous dilated vascular structures lined by bland endothelial cells admixed with normal endometrial structures. Neoplastic features were not identified, consistent with a vascular hamartoma. A complete blood cell count and chemistry, and coagulation profile were submitted to due concerns for clotting disorders and other systemic diseases and consequences. The bloodwork revealed a decreased platelet count, with all other blood parameters within normal limits. A poor prognosis was given for the mare's future breeding potential due to a diffusely abnormal cervix and uterine body. An abnormal presentation of uterine benign tumor that was diagnosed as a uterine hamartoma is described.

Keywords: Hamartoma, equine, uterine hamartoma

Acute testicular degeneration in a 6-year Paso Fino stallion

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Azoospermia refers to absence of sperm in an ejaculate. Etiology ranges from reversible/transient (i.e., complete blockage of the ampullae) to end-stage testicular degeneration. A 6-year Paso Fino stallion was presented to Rood & Riddle Equine Hospital (RREH) in Wellington in October 2021 for a breeding soundness evaluation following recent transfer of ownership and importation into the US just a few months prior. Prior to purchase, 2 semen evaluations were performed in Columbia (December 2020 and April 2021) and were reported to contain motile, morphologically normal sperm. However, 2 months

prior to presentation to RREH, the stallion was diagnosed with azoospermia. Stallion's history included previous production of live foals and treatment provided for piroplasmosis in early 2021. A total of 7 ejaculates were collected on 3 separate days and no sperm were observed in any ejaculate even following semen centrifugation and cytologic evaluation. Stallion had signs consistent with a normal ejaculation, including tail flagging and 5 - 6 ejaculatory jets. Alkaline phosphatase concentrations were measured on 5 ejaculates and concentrations ranged from 1158 - 5770 U/L (normal 6,913 -22,180 U/L). To rule out retrograde ejaculation, the bladder was catheterized after a collection attempt, the urine centrifuged and examined microscopically, but no sperm were identified. Ultrasonographic and manual examination of the external (testes/epididymides) and internal genitalia (ampullae) were normal. The total testicular volume was very small, 86 cc (average – 240 cc), suggesting a primary testicular problem. Two testis biopsies, 1 from each testis, were collected using a TruCut biopsy punch and submitted for histopathologic interpretation. Biopsy results indicated complete absence of spermatids and spermatocytes and only a small number of spermatogonia and Sertoli cells within the seminiferous tubules, indicating severe testicular degeneration. It is not clear what caused the apparent acute testicular degeneration. Due to the severity of the testicular degeneration, the likelihood of regeneration of the seminiferous tubules was extremely low, and retirement from breeding was recommended. One should consider in this case that the testicular degeneration may be associated with piroplasmosis and/or subsequent treatment. Equine piroplasmosis is a disease endemic to South America that is caused by infection with intracellular parasites, *Theileria equi* and *Babesia caballi*. The disease is characterized by fever, lethargy, anorexia, peripheral edema, petechiation of mucous membranes, anemia, and thrombocytopenia. Treatment consisted of intramuscular injections with imidocarb dipropionate every 72 hours for 2 or 3 treatments. Although studies demonstrating direct causation between piroplasmosis and poor semen quality are lacking, infertility has been reported in stallions. Any disease process that causes systemic illness and an increase in body temperature may have a negative effect on spermatogenesis.

Keywords: Stallion, testicular degeneration, azoospermia, piroplasmosis

Bulls voluntarily access shade during hot weather, reducing scrotal subcutaneous temperatures and improving sperm quality

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Our objectives were to establish relationships among bull location (shaded versus nonshaded), intrascrotal and ambient temperatures, and sperm quality. We hypothesized that in hot ambient temperatures, bulls seek shade, reducing intrascrotal temperatures and improving sperm quality in comparison to bulls without shade. Adult Angus bulls (n = 6) were randomly and equally allocated into 2 groups in adjacent, outdoor paddocks, with 1 containing artificial shade (metal structure ~ 3.5 x 6 meters and 2.5 meters high with a roof and 3 walls) to be accessed as desired (shaded group) and monitored with a motion camera. Temperature data loggers surgically implanted in scrotal subcutaneous tissues recorded temperature once each hour. Semen was collected by electroejaculation once weekly for 9 weeks. During the 10 hottest days, the percentage of time that a bull accessed shade increased with ambient temperature, ranging from 7.6 to 86.7% for ambient temperatures < 25°C and > 33°C, respectively. Over the 10 hottest days, scrotal subcutaneous temperature in the no-shade group was associated with ambient temperature, with the highest scrotal temperature (36.5°C) recorded when the ambient temperature was ~33°C. Conversely, bulls with access to shade had lower ($p < 0.001$) scrotal subcutaneous temperatures during high ambient temperatures, particularly when they accessed shade. During the 4 hottest days, bulls that could access shade did so most of the time from 12.00 to 17.00, coinciding with peak ambient temperatures. For total and progressive forward motility, there were group effects ($p < 0.001$ and $p = 0.023$, respectively; better motility in the shade group). Furthermore, for total abnormalities and acrosome integrity, there were group effects ($p > 0.001$ for each), and a time effect ($p = 0.009$) for acrosome integrity. For sperm kinematics (VAP, VCL, VSL, STR, and LIN) there were group effects ($p < 0.005$, $p = 0.011$, $p < 0.010$, $p = 0.020$, and $p = 0.046$, respectively), whereas ALH had a time effect ($p < 0.05$). In summary, during hot weather, bulls voluntarily accessed shade, lowering scrotal subcutaneous temperatures and improving sperm quality.

Keywords: Bulls, heat stress, shade, intrascrotal temperature

Effect of recovery rate on in vitro embryo production

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With the advent of transvaginal aspiration (TVA) of oocytes becoming a clinically relevant procedure in mares for the purposes of in vitro embryo production, a symbiotic relationship between primary veterinarians managing patient cycles and the referral facility offering outpatient TVA services has developed. In such circumstances it is common that aspirated oocytes are shipped to yet another location where laboratory personnel perform intracytoplasmic sperm injection (ICSI) and embryo culture. Unfortunately, primary veterinarians and clients may be forced to rely on opinion provided by word-of-mouth when choosing facilities, if providers of TVA and ICSI services do not to provide colleagues and owners with in-house results and statistics. Additionally, the majority of scientific reports available associated with TVA and ICSI are associated with benchtop results and typically do not continue beyond blastocyst development to report heartbeat positive pregnancy rates, likely due to a lack of access to the embryo recipient records. As little scientific research is available to provide practitioners and clients with the information necessary to make informed choices when choosing facilities, the objective was to determine the most important step for producing in vitro embryos and successful live foal rates. We hypothesized that a critical component in successful in vitro embryo production is the number of oocytes recovered from any given mare, as compared to other steps in the production process. A retrospective examination of the latest complete annual records (2020) involving our facility in collaboration with an exclusively equine reproduction veterinary practice, focused on 275 TVA sessions completed over the course of 12 months. A range of 2 - 22 oocytes were collected from a total of 46 mares. Per TVA session, an average of 11 oocytes were recovered (1,381 follicles punctured and 1,111 oocytes collected, 80% recovery rate) and an average of 7 oocytes matured and underwent ICSI per TVA session (748 oocytes injected, an oocyte maturation rate of 25%). An average of 2 embryos per TVA session were obtained. The average pregnancy rate of recipient mares receiving embryos derived from our collaboration was 87% with a live foal rate of 57%. At our institution, follicles as small as ≤ 5 mm are punctured and included when calculating recovery rates. Although aspiration of very

small and large follicles may have a negative impact on recovery rate, we included every follicle-like structure aspirated in our calculations. For our data set we concluded that the highest embryo maturation and live foal rates were achieved when a higher number of oocytes are recovered, leading to higher numbers for potential embryo development and pregnancy. To be most beneficial, a streamlined process should exist between the selection of mares with higher numbers of appropriately sized follicles and coordination between the equine practitioner, TVA specialty practice, ICSI lab, and the recipient management. As collaborators in this working partnership, working as a team to offer such services has enhanced the working relationship between practitioners and specialists and provided our clients with a greater degree of success.

Keywords: Mare, transvaginal oocyte aspiration, oocyte recovery

Deoxynivalenol and deepoxy-deoxynivalenol-induced alterations in theca cell function as a major cause of infertility in dairy cows

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Tricothecene mycotoxins such as Deoxynivalenol (DON) and its metabolite deepoxy-DON (DOM-1), can alter major intracellular signaling pathways within theca cells that can perturb normal folliculogenesis in the ovary resulting in infertility in dairy cows. They function through the activation of a specific tyrosine kinase receptor that transduces the signal by activating several intracellular signaling pathways. In our experimental study, bovine ovarian theca cells were collected from adult cows during the follicular phase of estrous cycle and were cultured at a density of 500 000 viable cells for 5 days. Cells were treated on day 5 of the culture with 1 ng/ml DON and DOM-1 for 30 minutes and a mass spectrometry approach was used to identify changes in the proteome profile of the cells. We identified approximately 93 peptides that were phosphorylated and 254 peptides that were dephosphorylated in response to DON and DOM-1 compared to non-treated control cells. Gene ontology analysis indicated that the abundance of proteins associated with cell proliferation such as MAPK3/1, MAPK14, GNGT1, EDN1 and YWHAB were up-regulated in the DON and DOM-1 compared to the control group. This study reports for the first time that DON and DOM-1 at sub-toxic level can activate major mitogen-induced proliferative molecules within theca cells that can stimulate tumorigenesis in the ovary.

Keywords: Bovine ovary, deoxynivalenol, deepoxy-deoxynivalenol, proteome, theca cell

A case of hemosemen in a Merino ram

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Aim was to investigate a case of hemosemen in a ram. A 2½ year, Merino ram was examined as part of a routine flock breeding soundness examination. Ram was from a Brucellosis free flock, and had been on the property for at least 12 months with no previous history suggestive of any health nor reproductive abnormalities. Ram was bright, alert and responsive, with a body condition score of 3.5/5, a scrotal circumference of 35 cm, and no obvious abnormalities palpable within the scrotum on 22 Feb 2021. Semen sample collected via electro-ejaculation was red in colour, suggestive of hemosemen (hemospermia). A DiffQuick stained smear made from a sample of the ejaculate had red blood cells without leukocytes. Morphological assessment (1,000 x magnification) with differential interference contrast microscopy on a 10% buffered formol saline preserved sample resulted in a spermiogram with 93% morphologically normal sperm. An assessment on 07 Apr 2021 resulted in a hemosemen sample of similar appearance, but with a more pink colouration on gross evaluation. Scrotal circumference was 32 cm, and 90% sperm were morphologically normal. There were fewer erythrocytes detected on a DiffQuick stained smear from an ejaculate sample, but there were obvious (~ 3 per high power field (HPF) x 1000 magnification) neutrophils visible microscopically. A pink coloured ejaculate was collected at a third examination on 07 Jun 2021. Abnormal ejaculate was confirmed to be from within the urethral process, illustrating the blood cells were not from a superficial lesion. There were 68% morphologically normal sperm, with most (29%) being distal mid-piece reflex abnormalities. A DiffQuick stained smear revealed fewer erythrocytes than in the original sample, and more than 3 neutrophils per HPF. Occasional macrophages were visualised. Transrectal ultrasonography of the pelvis revealed an enlarged vesicular gland, ~ 35 mm in diameter, compared to 15-20 mm in a control ram. Ram was treated with long acting oxytetracycline every second day for 3 treatments. Ejaculate was less pink in color when re-examined on 13 Jan 2022. There were more than 5 neutrophils visible per HPF on microscopic examination of a stained smear, and erythrocytes were scant. Mild blood contamination with moderate numbers of degenerate and nondegenerate cells were reported from a laboratory submitted microbiological and cytological assessment. Coccobacilli and cocci were visualised on cytospin examination, and the presence of crystals. Bacteriological culture revealed moderate mixed growth of *Staphylococcus aureus* and *Histophilus somni*. *Histophilus ovis* and *Actinobacillus seminis* are reported as causes of inflammation in the reproductive tract of rams. Hemosemen is rarely reported in ruminants, and to our knowledge there are no reports of hemosemen in the ram. This is the first report of hemosemen in a ram with identification of *Histophilus somni* in the ejaculate. This case illustrated a case of hemosemen that is likely to have been caused by *Histophilus somni* infection of the vesicular glands. A

6-week out of season, breeding with this ram resulted in 32% pregnancy rate compared to another ram (63%).

Keywords: Hemosemen, hemospermia, ram, ejaculate, *Histophilus somni*

Seasonal effects of temperature in semen quality: heat-sensitive and heat-tolerant bulls

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Breeding soundness examination (BSE) in bulls is a risk assessment on the likely infertility of a bull. Recommendation is for this assessment to be done at least 2 months prior to a producer's mating start date, reflecting the time taken for spermatogenesis and epididymal maturation time. Adverse effects of heat on the production of viable sperm are well documented. If male thermoregulating capacity is affected, the production of viable sperm can be compromised. Many cattle breeders in Australia aim for spring (August/September) calving, which means breeding typically occurs during the summer months (November-March). As a result, BSE is recommended to be carried out from July to December (winter-early summer in Australia) to facilitate timely assessment of the likelihood of infertility in bulls, and allow time for remediation, retesting or replacement of those bulls. Objective was to assess the likelihood of BSE representing the bulls' spermiogram at the time of the breeding period. Ejaculates (n = 1,271) from 79 bulls of 11 breeds that were submitted for BSE, were assessed using standard in vitro fertility parameters where poor quality was determined to be fewer than 30% progressively motile sperm and/or fewer than 50% morphologically normal sperm. Importantly, each bull was assessed using several ejaculates from throughout the year. From the results, there appeared to be at least 2 bull classifications based on spermatozoa production effects. These were correlated to climatic conditions prevailing at, or prior to, the time of sperm collection. Bulls that consistently produced good quality sperm throughout the year were classified as 'heat tolerant' bulls. Those that produced varying quality ejaculates during the year were classified as 'heat sensitive' bulls. For the heat sensitive bulls, an analysis of the correlation between sperm quality and maximum temperature (lagged from 0 to 40 days) showed that the highest correlation ($r = 0.42$; 95% CI [– 0.5, – 0.34], $p < 0.001$) was at 17 days prior to the day sperm quality was measured. This supported the notion that there was a delayed effect of temperature on sperm quality. Based on a nominal logistic regression of spermiograms for 12 heat sensitive bulls it would be predicted that 63% of spermiograms would have poor quality 17 days after the ambient temperature reached 34˚C, i.e., decreased semen quality and a likelihood of being less fertile ($p < 0.001$). Knowledge of the inability of a bull to tolerate heat stress is unknown at the time of BSE. Based on this information, a cohort of 17 bulls were subjected to artificial heat stress of 40˚C for 12 hours during winter, and their spermiograms monitored for 12 weeks. Data indicated that 3 bulls were heat susceptible with abnormal sperm morphology between 3 and 8 weeks after heat stress, before returning to normal by 10 weeks, illustrating that the classification of heat sensitive and heat tolerant bulls is scientifically sound. Work is continuing to define more clearly heat sensitivity, determine the mechanisms of heat tolerance and sensitivity in bulls, and to locate genetic markers that can be used to predict these phenotypic traits.

Keywords: Breeding soundness examination, bull, spermiogram, heat-tolerant, heat-sensitive

Fungal endometritis in a Cavalier King Charles Spaniel bitch

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A 3-year, Cavalier King Charles spaniel bitch presented for infertility testing after multiple attempts of breeding without successful pregnancy. Dog had been bred with fresh chilled semen via transcervical insemination in 2 cycles prior without a pregnancy being identified. A 6-month interestrus interval had been observed, cycle progression normal, and semen quality and quantity deemed acceptable for a favorable outcome. On physical examination, the bitch appeared in excellent physical health. Based on recent cycle activity, the bitch's progesterone concentrations were assessed in late diestrus until progesterone was < 1.5 ng/ml. Dog was sedated, and cervix visualized using a rigid ureteroscope. A small biopsy forceps was then passed through the cervical os to acquire multiple endometrial biopsies. Several endometrial biopsies were submitted for histopathologic assessment that had fibrin mats containing septate fungal hyphae and small oblong or teardrop-shaped yeast forms within the endometrium. Mild cystic change was also observed within the endometrium. Bitch was treated with ketoconazole 25 mg/kg orally every 24 hours for 90 days. At the onset of next estrus, intrauterine bacterial culture was performed via low volume lavage using a ureteroscope and 4 French catheter that yielded growth of *Haemophilus haemoglobinophilus*. Azithromycin was given at 10 mg/kg orally every 24 hours during estrus prior to breeding. Ovulation timing followed, and surgical insemination performed with chilled semen of excellent quality and quantity. At insemination, the uterus had a few small adhesions associated with the uterine body and a few small intramural cystic lesions that were expressed during the procedure. Bitch presented at 31 days postovulation that revealed no evidence of pregnancy. Bitch was culled from the breeding program after this attempt, but other approaches may have been considered to identify and speciate the fungal infiltrates, and repeat biopsy to assess if treatments had been successful. Intrauterine treatments may also be considered if oral therapy was not resolving presence of the organisms. Though these treatments have not been re-

ported in dogs, they are well described in equids and some livestock species.

Keywords: Fungal endometritis, endometritis, canine infertility

Postbreeding inflammatory response in inter- and intra-species breeding in equids

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Anecdotally, it has been suggested that subfertile mares have better fertility when bred with donkey semen (D-S) than horse semen (H-S), and this could be due to donkey seminal plasma (D-SP). Horse seminal plasma (H-SP) and D-SP have immunomodulatory properties on endometrium of females of their own species; however, it is unknown if D-SP also modulates the mare endometrium. This study aimed to: i. compare the inflammatory response of horse mares inseminated with D-S or H-S or infused with D-SP or H-SP; and ii. assess embryo recovery in mares bred with D-S and H-S. We hypothesized that: i. sperm results in a greater inflammatory response than seminal plasma regardless of species; and that ii. D-SP downregulates endometrial inflammatory response compared to H-SP. Five cycles of 22 mares were randomly assigned in a crossover design to a uterine infusion with donkey semen (D-S) and seminal plasma (D-SP) and horse semen (H-S) and seminal plasma (H-SP) or saline. Mares were examined via transrectal ultrasonography, and ovulation was induced when a dominant follicle (> 35 mm) was detected. Semen and seminal plasma were obtained from a jack and a stallion. Uterine edema, intrauterine fluid accumulation, number of neutrophils on uterine cytology were assessed at 0, 6, 24, and 48 hours postinfusion or at AI. Progesterone concentrations were assessed at 0, 6, 24, 48, 72, 96 hours postinfusion and after day 8 postovulation. Embryo flushing was performed on 8 days postovulation. R-studio was used for data analyses. Kruskal-Wallis rank-sum test was used to compare the scores given to uterine fluid and edema; a mixed linear model was used to compare the other variables. Significance was set at $p < 0.05$ and a statistical tendency a $0.05 < p < 0.1$. Although there was a transient increase in the amount of intrauterine fluid after infusion at 6 hours, there was no difference ($p < 0.05$) among groups. Embryo recovery and quality were not different ($p > 0.05$) between groups; however, it was numerically higher in D-S than H-S (59 versus 55%). There were effects of time ($p < 0.05$) and group ($p < 0.05$) but no time by group interaction ($p > 0.05$) on progesterone concentrations. After 8 days postovulation, H-S had higher ($p = 0.046$) progesterone concentrations than D-SP, tended to have higher ($p = 0.084$) concentrations than saline), and had similar ($p = 0.7$) concentrations to H-SP. Neutrophil counts changed ($p = 0.001$) over time and there was an interaction ($p < 0.001$) between group and time. At 6 hours postinfusion, the number of neutrophils on uterine cytology in D-S tended to be lower ($p = 0.074$) than H-S. Infusion of D-SP had an antiinflammatory effect ($p < 0.05$) compared to S-S and S-SP. In conclusion, D-S and H-S had similar postinfusion inflammatory responses and embryo recovery. D-SP results in a lower postinfusion inflammatory response than other combinations. Seminal plasma might be the reason that subfertile mares appear to have better fertility when bred with donkeys.

Keywords: Donkey, endometritis, horses, seminal plasma

Intrauterine chlorine dioxide for treatment of fungal endometritis in Marwari horses

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Two valuable mares of a rare Marwari breed of horse were presented with prolonged lengths of infertility (one for 4 years, and the other for 2 years). Mares had been treated by multiple veterinarians with various regimens of antibiotics and antiinfective agents for chronic endometritis. Mares were checked for a basic breeding soundness examination and the findings were the following. Mare 1 (barren for 4 years): ovaries had multiple follicles and a corpus luteum. Cervix was closed with pendulous well-toned uterus containing severe amounts of echogenic fluid accumulation in the body. Mare 2 (barren for 2 years): One ovary had 45 mm follicle and the other ovary had multiple small follicles. Cervix was relaxed and 30% dilated, and the uterus contained a small amount of echogenic fluid in the right horn and body with a fair to poor uterine tone. Mare also had mucopurulent whitish vulvar discharge that was also observed on the medial aspect of one hock. Endometrial cytology performed on efflux from small volume uterine lavage from both mares revealed fungal and yeast elements along with severe inflammation. Both cytologic smears contained neutrophils, mononuclear inflammatory cells, and plasma cells. Cellular debris also contained several coccobacilli. Due to the chronicity and refractory nature of the endometritis, an experimental trial with chlorine dioxide was suggested. Three days of intrauterine lavage was performed followed by infusion of 1 liter saline with 375 ppm, chlorine dioxide. Production of chlorine dioxide was carried out as per the patented formulation (Sureshot-EQ, India), to accomplish a final concentration of 375 ppm. No adverse reaction or pain response was noted and the efflux on subsequent uterine lavage improved. After 3 consecutive days of treatment the mares had a Caslick's performed and were sent home. Mares were examined via transrectal palpation and ultrasonography 4 weeks after treatment. Mares had active ovaries, appropriate uterine edema and negligible intrauterine fluid. Mares were bred and the referring veterinarian was advised to perform a postbreeding lavage with warm saline. Mare 1 was in foal at day 45. Mare 2 mare was lost to follow-up. Chlorine dioxide has been used historically as an active nonantibiotic biocide. We used a patented technology to produce low volume consistent release of chlorine dioxide at the rate of 375 ppm lasting for 4 hours. In this patented system, the end result of the reactions are sodium chloride and water. Additionally, chlorine dioxide has been effective in disrupting biofilm. Further research is needed to determine the effectiveness of chlorine dioxide for the treatment of endometritis.

Keywords: Mare, fungal endometritis, chlorine dioxide, Marwari

Lactate-induced spontaneous acrosomal exocytosis in stallion sperm

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An in vitro, physiologic model of acrosomal exocytosis (AE) is needed to study sperm function and fertility potential in stallions. Currently, the calcium ionophore A23187 is used to measure the ability of the acrosome to react but is recognized as a potent nonphysiologic inducer of AE. As such, A23187 may not diagnose more subtle forms of acrosome dysfunction. Incubation of stallion sperm under capacitating-like conditions by using a medium that contains only lactate as an energy source (Lac-MW) resulted in a high rate of spontaneous AE in viable sperm (% AE/Viable). Here, we determined if the Lac-MW model could be used as a more physiologic method to study acrosomal function in stallion sperm, taking into consideration other factors such as semen storage methods for the transport of equine sperm. In Experiment 1, fresh semen (n = 12 ejaculates) was incubated with A23187, Lac-MW, or Control (INRA-96®) for up to 6 hours and analyzed for AE/Viable using flow cytometry (FITC-PSA and Fixable Live/Dead Red). In Experiment 2, Fresh semen (n = 21 ejaculates) was either incubated in Lac-MW (as in Experiment 1), cool-stored for 24 hours (Cooled), or frozen in liquid nitrogen (Frozen). After cooled storage or freezing/thawing, sperm were incubated in Lac-MW for up to 6 hours and analyzed for AE/Viable. In Experiment 3, semen from 5 stallions (Stallions A, B, C, D, or E; n = 15 ejaculates) were analyzed after 24 hours of cooled storage using A23187 or Lac-MW. Also, cool-stored semen from these stallions was used to breed 143 mares (range 24 - 35 mares/stallion). For all experiments, AE/Viable was determined after 6 hours of incubation. In Experiment 3, the Total AE (only for A23187), and the per-cycle pregnancy rate (PC/PR) per stallion were also calculated. For all experiments, rank-transformed data was analyzed using the Mixed Linear Model (SAS 9.4). Statistical significance was set at $p < 0.05$. In Experiment 1, the mean AE/Viable was higher in Lac-MW than A23187, but similar in A23187 and control (42, 8 and 8%, respectively; $p < 0.05$). In Experiment 2, mean AE/Viable was similar in Fresh, Cooled, or Frozen semen (37, 38, and 46%, respectively; $p > 0.05$). In Experiment 3, whereas differences in Total AE (using A23187) were not observed among all stallions (range: 92 - 94%; $p > 0.05$), there were differences in mean AE/Viable (using Lac-MW) (A - E: 56, 45, 42, 33, and 24%, respectively; $p < 0.05$). For these stallions, the PC/PR were A: 72%, B: 65%, C: 60%, D: 54%, and E: 45%. We concluded that the use of Lac-MW to induce spontaneous AE can be considered as a more physiologic in vitro alternative than A23187 to study acrosomal function in stallion sperm, primarily because sperm viability is preserved during AE. This approach appeared to be not affected by the stallion sperm storage method. We did not observe a faster occurrence of spontaneous AE in frozen/thawed semen, suggesting that freezing/thawing did not induce capacitation-like changes in stallion sperm.

Keywords: Stallion sperm, acrosomal exocytosis, acrosome function, lactate, storage, fertility

Severe cleft palates affected an entire English Bulldog litter

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A 5-year, female English Bulldog, was presented on day 4 of estrus for a reproductive examination and determination of serum progesterone concentrations. Bitch had 4 previous breedings and 3 previous pregnancies. One pup in a previous litter was euthanized due to a severe cleft palate. Bitch had multiple reabsorptions in the previous litter but delivered 4 viable pups. On examination, all parameters were within normal limits. A vaginal cytology revealed predominantly superficial cells with a few bacteria. Vaginal mucosa appeared edematous on vaginoscopy. Patient's initial serum progesterone concentrations were 18.13 ng/ml on an in-house minividas machine, corresponding with 4 days post LH surge. Due to the previous reabsorptions and history of cleft palates in pups, the owner was giving enrofloxacin, pentoxifylline, and folic acid prescribed by another veterinarian. Enrofloxacin treatment was discontinued 4 days postbreeding. The following day, the patient was bred with fresh semen via transcervical insemination with 1.07×10^9 progressively motile sperm. Patient was bred again the following day via artificial insemination. Transabdominal ultrasonography was performed 24 days postbreeding and revealed 5 viable pups and 2 reabsorptions. One pup in the left uterine horn had a thickened placenta and areas of placental separation. Patient was given pentoxifylline at a dose of 10 mg/kg and augmentin at a dose of 10 mg/kg. A recheck ultrasonography was recommended in 5 - 7 days but declined. Bitch was presented for a cesarean surgery on her calculated due date. A complete blood count and chemistry profile were within normal limits. All 6 pups were viable; however, had severe cleft palates, and the owner elected euthanasia. Patient's female offspring has been bred to the same stud dog and is due in March of 2022. After further questioning, it was apparent that the stud had sired 3 previous litters with 1 litter of entirely nonviable anasarca pups. University of California, Davis has been contacted to determine if any useful DNA samples can be submitted for research purposes. This case study illustrated the importance of obtaining a full breeding history on the stud and bitch prior to breeding and demonstrated why a genetic test for cleft palates in bulldogs would be invaluable to breeders.

Keywords: Cleft palate, bulldog, anasarca

Granular vulvitis/balanoposthitis in a cattle herd and *Ureaplasma diversum* detection using quantitative real-time PCR and culture

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We aimed to characterize the granular lesions on the mucosae of the vulva/vestibule of females and prepuce/penis of males with the detection of *Ureaplasma diversum* using RT-PCR and culture (gold-standard). A beef cow-calf herd experienced abortions in pregnant heifers last year (cause undetermined). During the current year, 12 heifers returned to estrus within the first 3 weeks of the breeding period (75 days, 2 bulls, 43 heifers). A month into breeding, vaginal and preputial samples from 11 heifers (returned to estrus) and 2 bulls were submitted for *Ureaplasma* RT-PCR (PDS, Saskatoon). A month after the end of the breeding period, 43 heifers (Group 1), 3 second-parity (Group 2), and 5 multiparous (Group 3) cows were assessed for pregnancy status and granular vulvitis. Bulls (n = 5, 1 each for Group 1 - 3 and 2 others) were assessed for granular balanoposthitis. Two swabs from the vestibular region of randomly selected heifers (n = 23) and all cows; and prepuce of all bulls were submitted for RT-PCR (PDS, Saskatoon) and bacteriological culture (AHL, Guelph). All 11 heifers and 2 bulls tested positive for Ureaplasma RT-PCR on first sampling. On the second examination, 29/43 Group 1 heifers, 4/5 Group 2 cows and 3/3 Group 3 cows had granular vulvitis, whereas 3/5 bulls had balanoposthitis. Out of 23 sampled heifers, 18 had granular vulvitis. Overall, 28/36 tested animals had lesions, with 61% (17/28) being culture-positive, whereas 63% (5/8) of animals without lesions had a positive culture. All 5 bulls were positive for Ureaplasma RT-PCR as well as culture. Ureaplasma RT-PCR had an accuracy of 83% compared to culture as a gold standard with sensitivity and specificity of 95% and 64%, respectively. Currently, the calving data for different groups at this farm is being gathered. In conclusion, Ureaplasma can be cultured in cattle with and without granular lesions. RT-PCR likely overestimated Ureaplasma presence.

Keywords: Ureaplasma, cattle, granular lesions, vulvitis, balanoposthitis

Developmental competence of immature equine oocytes after in vitro maturation

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With increasing demand for in vitro production of equine embryos over the past 10 years, the value of each oocyte recovered has increased. Intracytoplasmic sperm injection (ICSI) is performed to fertilize oocytes reaching meiosis II stage of development, determined by microscopic evidence of an extruded polar body (PB), signifying nuclear maturation. Cytoplasmic maturation, during which replication of organelles, proteins and RNA's necessary for postfertilization development occurs, is more difficult to evaluate. However, equine oocytes contain large amount of cytoplasmic lipids, and the migration and polarization of lipid granules can be microscopically visualized in mature oocytes. Furthermore, the distribution of oocyte lipids may reflect a level of cytoplasmic capacitation (Cc) and developmental competence. Although the cleavage rate of equine oocytes fertilized following maturation culture does not appear to be significantly different based on lipid polarization,[1] this study evaluated the migration and polarization of cytoplasmic lipids (granularity) following in vitro maturation to determine if blastocyst development can be predicted in the absence of PB extrusion. We hypothesized that evidence of cytoplasmic lipid polarization may predict developmental competence of oocytes failing to demonstrate nuclear maturation following maturation culture. Cumulus-oocyte-complexes (n = 411) obtained from ovarian follicles by transvaginal aspiration were stored in holding medium overnight at 20°C before undergoing incubation at 38.5°C in 50 µl drops of equilibrated maturation media.[2] After 28.5 hours, oocytes were denuded following exposure to 80 iu hyaluronidase, and evaluated for the presence of an extruded PB and lipid reorganization. Cytoplasm of immature (MI) oocytes that did not exhibit PB extrusion was graded as a percentage of dark granular appearance. Oocytes were fertilized by conventional ICSI using ICSI-fertile stallions' semen. Fertilized oocytes were cultured[2] and examined under inverted 1,000 x light microscopy. Culture had 26% (n = 106) immature (MI [without a visible polar body] and 66% (n = 273) mature (M11 [denuded with an intact membrane and an extruded polar body]) oocytes. Of the MI oocytes injected, 23% (n = 24) were deemed to have attained some level of cytoplasmic capacitation (Cc = 40 - 70% granularity). A cleavage rate of 25.5% was recorded for injected MI oocytes. Of the 24 MI oocytes with some Cc, 37% cleaved and continued to develop, with 7 of the embryos developing to the blastocyst stage. Of the 82 MI oocytes that did not demonstrate Cc, 22% cleaved; however, no blastocysts developed. In conclusion, blastocyst developmental potential was 6.7% per total MI's injected and 19% per MI oocytes with at least a 40 - 70% Cc. Injected oocytes reached a level of cytoplasmic maturation (without nuclear maturation) resulted in blastocyst development.

Keywords: Equine oocyte, maturation, ICSI, development, blastocyst

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Efficacy of intrauterine infusion of lyophilized interleukin-1 receptor antagonist protein in the prevention of persistent breeding-induced endometritis in the mare

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A transient uterine inflammatory response is an inevitable and natural consequence of breeding by either live-cover or by artificial insemination. Inflammation is due to an antigenic response to sperm. A majority of young, reproductively normal mares eliminate inflammation within 24 - 48 hours. Unfortunately, the postbreeding inflammatory response may develop into a pathologic condition referred to as persistent breeding-induced endometritis (PBIE). This occurs in ~ 15% of broodmares. Chronic inflammation is associated with decreased pregnancy rates, premature luteolysis, and a predisposition to infectious endometritis. Aim was to compare the efficacy of intrauterine infusion of a lyophilized Interleukin-1 Receptor Antagonist Protein (IRAP) product versus oral firocoxib in the prevention of PBIE. We hypothesized that intrauterine infusion of lyophilized IRAP would improve clinical outcomes associated with PBIE. Quarter Horse mares (n = 8) aged 10 - 18 years with a biopsy score of ≥ IIa were randomly assigned to 1 of 3 treatment groups in a cross-over study design. Treatment groups consisted of i. intrauterine infusion of 20 ml of sterile PBS (control); ii. intrauterine infusion of allogenic lyophilized IRAP reconstituted with 20 ml sterile PBS; and iii. oral administration of 0.3 mg/kg firocoxib. Treatments were given 4 hours prior to insemination with 1 x 10^9 dead sperm. Mares received only1 treatment per estrous cycle. Transrectal ultrasound examinations and uterine sample collections were performed 4 hours prior to and again at 6, 24, 48, and 72 hours after insemination. At each time point, assessments of intraluminal uterine fluid depth and echogenicity, and endometrial edema were performed. Brush cytology samples were collected for uterine cytology and cytokine analysis. Cytokines to be evaluated included IL1β, IL6, CXCL8 (formerly known as IL8), TNFα, IL10, and IL-1RA. Additionally, an endometrial biopsy was collected 72 hours postinsemination for histopathologic evaluation of inflammation. Regardless of treatment group, mares mounted an inflammatory response at 6 hours postinsemination as indicated by an increase in intrauterine fluid depth and echogenicity, an increase in endometrial edema, and an increase in inflammatory cells on cytology. A progressive decrease in all parameters was noted over the 72-hour observation period. There were no differences among treatment groups relative to intrauterine fluid depth or echogenicity, endometrial edema pattern, or number of inflammatory cells on uterine cytology over the 72-hour period. Cytokine analysis and evaluation of endometrial tissue inflammation are pending. In conclusion, neither intrauterine infusion of allogenic lyophilized IRAP nor oral treatment of firocoxib 4 hours prior to insemination mitigated the uterine inflammatory response to sperm within confines of this endometritis model.

Keywords: Mare, endometritis, breeding, IRAP, firocoxib

Effect of nerve growth factor-β given after artificial insemination

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Objectives were to determine the effects of nerve growth factor-β (NGF), purified from bull's seminal plasma, given at artificial insemination (AI) on progesterone postAI, interferon-stimulated genes (ISG), and pregnancy per AI (P/AI) for lactating Holstein dairy cows enrolled in a timed AI protocol. We hypothesized that NGF treatment at AI would increase plasma progesterone postAI concentrations, upregulate relative abundance of ISG, and improve P/AI in lactating dairy cows. Holstein cows (n = 557) from a single commercial dairy farm were blocked by parity and randomly assigned to receive an injection containing 296 µg of bovine purified NGF at AI diluted in 2 ml of phosphate-buffered saline (NGF, n = 275), or 2 ml of phosphate-buffered saline (Control, n = 282). Plasma progesterone concentrations and corpus luteum (CL) size were assessed in a subset of cows (NGF, n = 32; Control, n = 36) at days 7, 14, and 19 postAI. Relative mRNA abundance of ISG (ISG15, MX1, MX2, RTP4) was assessed in peripheral blood leukocytes on day 19 postAI. Pregnancy diagnosis was performed at 37- and 65-days postAI. There was an interaction effect between treatment and parity for plasma progesterone, with NGF resulting in greater plasma progesterone postAI in primiparous cows, but there were no effects in multiparous cows. However, plasma progesterone and ISG did not differ between treatments. There were no effects of NGF for P/AI at 37 days postAI (NGF 40.0 versus Control 41.6%), 65 days postAI (NGF 36.0 versus Control 38.1%), or for pregnancy loss (NGF 8.4 versus Control 7.7%). Current study revealed that effects to NGF in lactating Holstein cows were minor and contingent with parity for progesterone concentrations, with no improvement in ISG relative abundance or P/AI.

Keywords: Cattle, NGF, progesterone, interferon stimulated gene

Power doppler as a tool to perform early indirect pregnancy diagnosis in donkeys

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Horse embryo induces a differential blood flow in the uterus of pregnant mares; however, in donkeys this is unknown. Doppler ultrasonography is becoming increasingly available in portable ultrasound machines. A sensitive, early indirect

detection of pregnancy could facilitate breeding decisions and improve reproductive efficiency. This study aimed to assess the uterine blood flow of jennies as an early indirect pregnancy diagnostic test. We hypothesized that uterine vascular hemodynamics increase in pregnant jennies, thus, serving as early indirect pregnancy diagnostic tests. Multiparous jennies (n = 44) had daily transrectal ultrasonography until a preovulatory follicle of 30 mm was detected. Jennies received deslorelin to hasten ovulation. By 24 hours postdeslorelin, jennies were bred with fresh semen (> 250 x 10^6 sperm) from 1 jack. Ovulation was confirmed by ultrasonography (Well. D, medical electronics Co, Shenzhen, China) performed every other day. Transrectal ultrasonography was performed from days 7 - 10 postovulation to assess the blood flow of each uterine horn with Power doppler. Vascularization of the endometrium was analyzed using spot meter techniques that measured mean pixel intensity. Serum progesterone concentrations were analyzed by radioimmunoassay 7 - 10 days postovulation. Pregnancy diagnosis with B-mode ultrasonography was performed on days 7, 8, 9, 10, and 15 postovulation. Data assessed for normality with Shapiro-Wilk's test and mixed models. Sensitivity, specificity, positive predictive value (PPP), and negative predictive value (NPV) for blood flow on days 7 - 10 postovulation were calculated to identify pregnant animals confirmed 15 days postovulation. Significance was set as $p \leq 0.05$ and tendency as $0.05 < p < 0.1$. Out of 44 jennies, 23 (53%) were confirmed pregnant on day 15 postovulation. B-mode ultrasonography identified 17% (4/23) of the embryonic vesicles on day 9 and 39% (9/23) on day 10; no embryonic vesicles were identified on days 7 and 8. Pregnant jennies had greater ($p = 0.007$ uterine vascular blood flow in comparison with nonpregnant jennies (144.8 ± 1.5 versus 139.2 ± 1.3 pixels/unit). There was no effect of time ($p = 0.48$) or interaction between group and time ($p = 0.70$) for the uterine blood flow. Progesterone concentrations did not change ($p = 0.81$) within 7 - 10 days postovulation and there was no interaction ($p = 0.49$) between group and time. Progesterone concentrations tended to be greater ($p = 0.07$ in pregnant than nonpregnant jennies. There was no effect of time ($p = 0.79$), group ($p = 0.77$), or interaction between time and group ($p = 0.92$) for uterine horns. Sensitivity on days 7 to 10 were 76, 42, 60, and 62%, respectively. Whereas specificity from days 7 to 10 were 70, 50, 50, and 75%, respectively. The PPV and NPV for days 7 - 10 were 71 and 15%; 75 and 20%; 75 and 33%; and 80 and 27%, respectively. In conclusion, power-doppler ultrasonography had high sensitivity and specificity for indirect detection of pregnancy in donkeys, particularly, 7 and 10 days postovulation, whereas standard B-mode ultrasonography first detected a small percentage of pregnancies on days 9 and 10. Blood flow was not associated with progesterone concentrations. Pregnant jennies tended to have higher progesterone than nonpregnant jennies.

Keywords: Donkey, B-mode, power doppler, blood flow, pregnancy outcome

Impending parturition is characterized by a reduction in progestogens and estradiol concentrations with no correlations with mammary gland secretions in donkeys

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Ability to predict parturition allows timely assistance for the dam and neonate; however, limited information is known in the donkey, including the endocrine events upon imminent parturition. This study aimed to describe progestogens and estradiol concentrations in periparturient donkeys and associations with mammary gland secretions. Multiparous jennies (n = 37) were monitored daily from 350 - 355 days of pregnancy to delivery. The pH of mammary gland secretions was assessed daily with a hand-held device (LAQUA Twin, Horiba, Irvine, CA). Aliquots of mammary secretions were frozen daily and then assessed retrospectively for electrolyte concentrations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) (UniCel 800, Beckman Coulter, Switzerland) from 5 days before foaling. Serum samples were collected daily from each jenny and frozen at – 20°C until analysed. Progestogens and estradiol concentrations were analyzed by radioimmunoassay 5 days prepartum. Statistical analyses were performed with Graph Prisma (Version 9.1, San Diego, CA). Progestogens and estradiol concentrations were analyzed with a Mixed model and Tukey test. Associations between steroid concentrations, pregnancy length, mammary gland secretions, and the progestogens:estradiol ratio (P/E) were determined with Pearson's coefficient of correlation. Statistical significance was set as $p < 0.05$. Sensitivity, specificity, negative predictive value (NPV), and positive predictive values (PPV) were calculated using a cut-off value for P/E ratio of < 10 (1:10) for parturition in 24 hours. The mean gestation length was 374 ± 8.7 days and ranged from 357 to 390 days. There were effects of time for progestogens and estradiol concentrations ($p < 0.0001$) and interaction for the concentrations of these hormones and time ($p = 0.01$). Concentrations of progestogens reduced from 18.8 ± 2.7 to 8.8 ± 0.87 ng/ml from 96 to 24 hours before parturition ($p = 0.008$). Estradiol concentrations declined from 96 to 24 hours prior to parturition (140.6 ± 8.3 -115.5 ± 8.4 pg/ml) ($p = 0.01$). The P/E ratio had 70% sensitivity for parturition in 24 hours, whereas the specificity was 35%. The PPV and NPV values were 54% and 52%, respectively. There was a weak ($r = – 0.02$) and negative correlation between progestogens concentration and gestational length, and a weak ($r = 0.17$) and positive correlation between estradiol concentrations and pregnancy length. There was a weak correlation ($r = 0.17$) between pH of the mammary gland secretions and steroid hormones concentration ($r = 0.17$ progestogens; $r = 0.01$ estradiol), and a weak and negative correlation with the P/E ratio ($r = – 0.07$). Progestogens presented a weak and negative correlation for Ca ($r = – 0.22$) and Na ($r = – 0.07$) and weak and positive correlations for K ($r = 0.19$) and Mg ($r = 0.03$). Estradiol was moderately, negatively correlated with Ca ($r = – 0.39$) and K ($r = – 0.18$) and positively correlated with Na ($r = 0.31$) and Mg ($r = 0.40$). In conclusion, the P/E ratio had moderate sensitivity but low specificity for parturition in 24 hours. There were weak correlations between the P/E ratio, mammary gland pH, and

electrolytes. This species demonstrated a reduction in these hormones like horses, but no distinct peak in estradiol as observed in ruminants.

Keywords: Donkey, steroid concentrations, progestogens, estradiol ratio, predict foaling

Donkeys have a higher incidence of hemorrhagic anovulatory follicles and multiple ovulations in spring

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In horses, hemorrhagic anovulatory follicle (HAF) is a type of ovulation failure associated with fall and spring transitions, equine metabolic syndrome, cloprostenol treatment, retained endometrial cups, aging (> 16 years), and use of nonsteroidal antiinflammatory drugs. To date, HAF has not been well described in donkeys. Thus, this study aimed to assess the associations between season, body condition scores (BCS) of mares with HAF, and multiple ovulations. We hypothesized that season and BCS are associated with HAF and multiple ovulations in jennies. Estrous cycles (n = 190) of 103 jennies kept in a humid subtropical climate were analyzed. Jennies had transrectal ultrasonography every other day and when a preovulatory follicle (≥ 30 mm) was detected, they were bred with fresh semen at 48-hour intervals (> 250 x 10^6 sperm) until ovulation was confirmed via ultrasonography. Jennies were bred in the spring, fall, and summer. Day of ovulation and first day of detection of HAF (i.e., excessive specks floating in the follicle antrum) were designated as day 0. Follicle size and BCS (1 - 5) were recorded during each ultrasonography. Statistical analyses were performed with Mixed models followed by Sidak for parametric data and Kruskal-Wallis followed by Dunn's test for nonparametric data. Significance was set as $p \leq 0.05$ and tendency as $0.05 < p < 0.1$. Overall incidence of HAF was 9.4% (18/190 cycles). The same jennies displayed 27.7% (5/18) of HAF (4 in the spring and 1in summer). There was an effect (p = 0.0009) of the season on HAF incidence. Spring cycles (19.6%; 12/61) had a greater (p = 0.0009) incidence of HAF than summer (6.6%; 3/45), and fall (4.7%; 3/45) cycles with no differences (p > 0.99) between the latter 2 seasons. Season did not affect follicular size for regular cycles (spring 38.2 ± 4.17 mm, summer 38.1 ± 4.78 mm, and fall 37.8 ± 2.88 mm) or HAF cycles (spring 36.6 ± 3.6 mm, summer 39.1 ± 5.7 mm and fall 35.3± 1.63 mm), and there was no interaction (p > 0.05) between season and follicular size, consistent with a nonseasonal species. Follicular sizes at last ultrasonography before day 0 were similar (p = 0.53) between HAF (37.1 ± 3.9 mm) and regular cycle (37.6 ± 4.1 mm). There were no effects (p = 0.59) of season or interaction with BCS. There was an interaction (p < 0.0001) between follicle size before day 0 and there was no interaction (p = 0.86) with follicles from regular cycles. Higher BCS (5) presented larger (p < 0.05) HAF sizes before day 0 (41.5 ± 2.1 mm) in comparison to BCSs 2 (36 ± 3.6 mm), 3 (35.5 ± 3.5 mm), and 4 (37 ± 4.3 mm). There was an effect (p = 0.0004) of the season on the incidence of multiple ovulations. Spring cycles (21.3%; 13/61) had a greater incidence (p = 0.61) of multiple ovulations than fall (3.6%, 3/84; P=0.003); however, not different than summer (13.3%; 6/45), and the latter was not different (p > 0.99) from each other. There was no apparent association between HAF and multiple ovulations. In conclusion, jennies had a higher incidence of HAF and multiple ovulations in spring than in summer and fall. Size of the preovulatory follicle of cycles representing normal ovulation did not differ from those with HAF. Season did not alter HAF size.

Keywords: Jennies, hemorrhagic follicle, HAF, season

Field study on effects of postbreeding uterine therapy on fertility in donkeys

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Postbreeding therapies (e.g., uterine lavage, ecbolic, and antiinflammatory drugs) are routinely applied to promote uterine clearance and enhance fertility in horses. However, the literature is scant in donkeys. Additionally, it has been suggested that donkeys are prone to postbreeding inflammation. Thus, aim of the study was to assess the effects of postbreeding therapies on pregnancy rates and losses in donkeys. We hypothesized that jennies treated 6 hours postbreeding have higher fertility than untreated jennies. Estrous cycles (n = 250) of 132 jennies were included in the study. Jennies were examined every other day until a preovulatory follicle (30 mm) was detected via transrectal ultrasonography. Then jennies were bred every other day with fresh semen (> 250 x 10^6 sperm) until ovulation was confirmed. Ovulation was not induced. Six hours after the first-AI, cycles were randomly allocated into 4 groups: a. uterine lavage (1 liter of lactated Ringer's solution (LRS) with 100 ml of DMSO added (LRS-DMSO; n = 67 cycles); b. uterine lavage, (1 liter of LRS + 20 IU intravenous oxytocin) (LRS-Oxy; n = 65 cycles); c. 20 IU single intravenous oxytocin treatment (Oxy; n = 43 cycles); and d. no treatment (Control; n = 75 cycles). Number of AI/cycle was recorded and compared among groups. Pregnancy diagnosis was performed on day 15 postovulation and confirmed on day 45 postovulation; losses between the first and second pregnancy diagnosis were accounted for as pregnancy loss. Data were analyzed with Graph Prisma (Version 9.1, San Diego, CA). Pregnancy rates and losses were analyzed with multiple logistic regression. Significance was set at $p \leq 0.05$ and tendency as $0.05 < p < 0.10$. Pregnancy rates were not different (p = 0.54): LRS-DMSO 47.7% (32/67-cycles); LRS-Oxy 32.3% (21/65-cycles); Oxy 32.3% (43/75-cycles); and Control 37.3% The pregnancy losses were not different (p = 0.93): LRS-DMSO 6.25% (2/32-pregnancies); LRS-Oxy 4.7% (1/21-pregnancies); Oxy, 2.3% (1/43-pregnancies); and Control 1.3% (1/75-pregnancies). There were no differences (p = 0.11) in AI/cycle number among groups: LRS-DMSO (1.3 ± 0.4), LRS-Oxy (1.1 ± 0.3), Oxy (1.3 ± 0.4), and Control (1.4 ± 0.5). In conclusion, uterine therapy did not improve pregnancy rates compared to control.

Keywords: Jennies, endometritis, DMSO, oxytocin, pregnancy rate

Response to deslorelin and fertility according to follicle size in donkeys

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Follicle size is the primary criterion used to determine when mares or jennies need to be bred. In horses, endometrial edema is also included in the criteria. However, donkeys are notorious for not having as reliable endometrial edema patterns as horses; thus, follicle size is even more relevant. One recent study reported ovulatory response to deslorelin in small donkeys, but not fertility. This study aimed to determine the ovulatory response and fertility according to follicle size at deslorelin administration in large donkeys. We hypothesized that follicle size at induction affects donkeys' ovulatory response and fertility. Estrous cycles (n = 100) of 64 jennies received deslorelin to induce ovulation, and 83 cycles of 51 jennies did not receive deslorelin and were considered controls. The cycles were arbitrarily arranged based on the follicle sizes at induction/control as follows: Size-1 (30 - 33mm [n = 27 induced and n = 26 control]); Size-2 (34 -37 mm [n = 40 induced and n = 38 control]); and Size-3 (38-41 mm [n = 33 induced and n = 29 control]). Ovulation was confirmed via transrectal ultrasonography performed at 12 hour intervals. After detecting predetermined follicle size, jennies were bred every other day until ovulation with fresh semen from 1 jack (> 250 x 10^6 sperm). Pregnancy diagnosis was performed on days 15 and 45 postovulation. Data were analyzed with Mixed models followed by Sidak for parametric data and Kruskal-Wallis, followed by Dunn's test for nonparametric data. There was an effect of group (Sizes 1-3) ($p < 0.0001$) and induction ($p = 0.004$) on the ovulatory follicle size. Induction of ovulation decreased ovulatory sizes among groups ($p < 0.05$), Size-1 30.6 ± 0.89 mm versus 32 ± 1.17 mm; Size-2 35.9 ± 0.84 mm versus 36.8 ± 0.4 mm; and Size-3 39.2 ± 0.9 mm versus 39.6 ± 1.2 mm for induced and control cycles, respectively ($p > 0.05$). Percentage ovulatory response arranged by 12 hour intervals were: Size-1 (induced versus control) 12 - 24 hours (3.3 versus 0%); 24 - 36 hours (7.4 versus 2.4%); and 36 - 48 hours (15.8 versus 5.2%); Size-2 (induced versus control) 12 - 24 hours (18 versus 9.5%); 24 - 36 hours (25.6 versus12.7%); and 36 - 48 hours (19.8 versus 10%); Size-3 (induced versus control) 12 - 24 hours (5 versus 5.4%); 24 - 36 hours (2.2 versus 1.2%); and 36 - 48 hours (0 versus 1%). Five jennies did not ovulate within 48 hours in the control group, and 3 in the induced group. Deslorelin resulted in 97% (97/100) of cycles ovulating in 48 hours, whereas for the control 47.4% (39/83) ovulated in the same interval ($p = 0.002$). Pregnancy rates were similar ($p = 0.37$) between induced (38%) and control (29%) cycles. Pregnancy loss was similar ($p = 0.34$) between induced 13% (5/38) and control 25% (6/24) cycles. Induced cycles had fewer ($p < 0.0001$) AI/cycle than control. Percentage of AI per cycle was (induced versus control): one-AI 74 versus 61.4%; two-AI 26 versus 25.3%; and three-AI 0% versus 13.3%. Number of AI/cycle (induced versus control were: one-AI/cycle (55 versus 17.8%); two-AI/cycle (45 versus 50%); three-AI/cycle (0 versus 17.7%). In conclusion, deslorelin was 97% efficient at concentrating ovulations in 48 hours and at reducing ovulatory follicle size and number of AI/cycle. Pregnancy rates were similar between the deslorelin and control groups.

Keywords: Jennies, deslorelin, ovulation, pregnancy rate

Effects of season on fertility and losses in donkeys

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Donkeys are deemed nonseasonal polyestrous species. They display regular cyclicity throughout the year. Other nonseasonal polyestrous species (e.g., cattle and pigs) experience seasonal variations in fertility and pregnancy losses. Season does not seem to affect fertility in donkeys kept in tropical weather; however, the seasonal variation in fertility and pregnancy losses has not been assessed in jennies kept under continental weather. This study aimed to evaluate the effects of the season on the fertility, number of AI/cycle, and pregnancy losses in donkeys kept under continental weather. We hypothesized that season affects fertility and losses in jennies. One hundred and forty-four cycles of 82 jennies were evaluated. The cycle distributions were as follows: spring (n = 53), summer (n = 46), and fall (n = 45). Transrectal ultrasonography was performed every other day until detection of a preovulatory follicle (> 30 mm). Then, jennies were bred every other day with fresh semen from 1 jack (> 250 x 10^6 sperm) until detection of ovulation. Ovulation was confirmed by ultrasonography performed at 24-hour intervals. Pregnancy diagnosis was performed on days 15 and 45 postovulation. Data were analyzed with mixed models followed by Tukey for follicle size, number of AI/cycle, and season. Chi-square test was used to assess pregnancy rates and losses. There was no difference ($p = 0.42$) in follicle size across seasons, consistent with nonseasonal species. Follicle sizes before ovulation related to the AI/cycle were: 39 ± 0.4mm, 41 ± 3.3mm in spring; 41.3 ± 4.7mm, 38.4 ± 7.9 mm in summer; and 41.5 ± 5.2mm, 39.3 ± 4mm in fall for 1 and 2 AI/cycle, respectively. Pregnancy rates were similar ($p = 0.91$ [39.6, 32.06, and 31.1% for spring, summer and fall respectively]. There was no difference ($p = 0.33$) in pregnancy loss across seasons with 4.7, 6.6, and 5.1% for spring, summer, and fall, respectively. There was no effect on AI/cycle ($p = 0.41$), season ($p = 0.95$,) or interaction between AI/cycle and season ($p = 0.21$). The fertility related to the season and number of AI/cycle were 11/30 versus 10/23; 12/35 versus 3/11; 9/29 versus 3/16 for 1 versus >1 AI/cycle in spring, summer, and fall, respectively. There was no difference ($p = 0.85$) on the overall AI/cycle and fertility; 1 AI/cycle accounted for 62/94 versus 34/50 for > 1 AI/cycle. In conclusion, season did not affect follicle size prior to ovulation, number of AI/cycle, pregnancy fertility, or pregnancy loss. The number of AI/cycle did not affect fertility.

Keywords: Jennies, follicle size, AI/cycle, fertility

Effect of different extenders used for selection of equine sperm by the swim-up technique

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Equine sperm are highly susceptible to damage under suboptimal incubation conditions. Successful assisted reproductive technologies (ART) rely on these functional aspects such as motility which may be impaired by sperm processing methods. We hypothesized that various extenders affect sperm function influencing motility parameters of the recovered samples. Frozen-thawed sperm from 7 stallions was diluted (25×10^6 sperm/ml) and evaluated after being incubated with 1 of 4 sperm media extenders including: i. modified human tubal fluid (H-HTF); ii. modified Chatot-Ziomek-Bavister (SP-CZB); iii. modified Tyrode's solution (SP-TALP); and iv. control (INRA 96). Samples were maintained at room temperature and motility was evaluated by computer assisted sperm analysis (CASA) at 4 time points (0, 2, 6, and 12 hours). Statistical analyses were performed using ANOVA for repeated measures. Type of media and culture time had a significant interaction on total motility (TM, $p = 0.0059$) and progressive motility (PM, $p = 0.0069$). The INRA extender had the highest values for TM and PM at all time points and had no significant reduction in values over time. In comparison to INRA, H-HTF did not differ in TM and PM; however, SP-CZB had lower TM ($p = < 0.0001$) and PM ($p = < 0.0001$) in comparison to control, with a significant reduction at 12 hours for TM ($p = < 0.01$) and by 6 hours for PM ($p = 0.011$). Similarly, SP-TALP had lower TM ($p = < 0.001$) and PM ($p = < 0.0001$), and a reduction at 6 hours for TM ($p = 0.0169$) and by 2 hours for PM ($p = 0.03$). Additionally, we evaluated the role of the same extenders, except INRA, on sperm selection using the swim-up method. Paired frozen-thawed sperm samples from 6 stallions were incubated under 1 ml of media (H-HTF/ SP-CZB/ SP-TALP) at 37° C at a 45-degree angle, for 2 incubation times (20 and 60 minutes). After incubation, 60% of the upper fraction of the supernatant was recovered and evaluated for motility (CASA), morphology (Hancock stain), viability (Hancock Stain), concentration (hemocytometer) and recovery rates. Statistical analysis was performed using a two-way ANOVA. Extenders and time had no effect on morphology, viability or recovery rates, although both affected TM ($p = < 0.01$; 0.027) and PM ($p = < 0.001$; 0.017). The media also had effects on path velocity (VAP, $p = < 0.001$), curvilinear velocity (VCL, $p = < 0.001$) and linear velocity (VSL, $p = < 0.001$). When comparing between groups, H-HTF media had higher values for TM ($p = < 0.001$), PM ($p = < 0.001$), VAP ($p = < 0.001$), VCL ($p = < 0.001$) and VSL ($p = < 0.0001$) in comparison to SP-CZB, and similarly, higher values for TM ($p = 0.01$), PM ($p = < 0.001$) and VSL ($p = 0.03$) in comparison to SP-TALP; the latter had higher values for VAP ($p = 0.011$) only in comparison to SP-CZB. Based on these results, it is evident that extenders used in sperm selection influence sperm functions, such as motility, and considering that no changes in recovery were detected, we concluded that the main influence of extender are changes in motility parameters of recovered sperm. This evidence affirmed the importance of culture media selection to optimize sperm recovery to improve equine ART outcomes.

Keywords: Stallion sperm, extender, motility, selection, swim-up

Effects of semen cooling extender and sperm prestaining with Hoechst 33342 on the longevity of cool-stored, sex-sorted stallion sperm

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Standardizing a protocol that allows stallion sperm to be collected in a remote location, being sent to a semen sex-sorting laboratory, and sent back to a breeding farm would permit wider dissemination of this technology in the equine breeding industry. Immediately prior to sex-sorting, sperm is stained with a DNA probe (e.g., Hoechst 33342). We studied the effect of using 2 commercial semen extenders for shipping semen and the effect of prestaining sperm before being sent to a sex-sorting laboratory, on the quality of cool-stored, sex-sorted stallion sperm. We hypothesized that there would be a significant extender effect and a significant interaction between extender and duration of exposure to the Hoechst 33324. Three ejaculates from each of 4 stallions (n = 12) were diluted at a ratio of 1: 3 with either INRA-96® (INRA) or BotuGold® (BG) semen extenders, either prestained or not with Hoechst 33342 (Yes or No) and stored for 24 hours at 6°C. After storage, the semen was processed by colloid centrifugation and sent to a sex-sorting laboratory. Sperm quality parameters were recorded immediately after sorting at 24 hours, and after cooled storage of sex-sorted sperm at 48 hours. At each time point (24 or 48 hours), sperm total and progressive motility (% TMOT and % PMOT, respectively) were analyzed using CASA, whereas sperm viability (% VIAB) and DNA damage (% $COMP_{a-t}$) were determined using a NucleoCounter SP-100™ and flow cytometry, respectively. At each time point, comparisons among treatments were made in rank-transformed data using the General Linear Model (JMP Pro 16.0). Statistical significance was set at $p < 0.05$. A stallion-by-treatment interaction was observed in 2/4 stallions ($p < 0.05$). At 24 hours, INRA-No, BG-No, and INRA-Yes resulted in similar ($p > 0.05$) mean TMOT (73 versus 75 versus 72%, respectively) whereas INRA-No and BG-No had higher ($p < 0.05$) TMOT than BG-Yes (70%). Mean PMOT was similar ($p > 0.05$) in INRA-No, BG-No, and BG-Yes (50 versus 60 versus 52%, respectively), whereas higher ($p < 0.05$) than in INRA-Yes (39%). Mean VIAB was higher in BG-No than in INRA-Yes (85 versus 82%), whereas similar to BG-Yes and INRA-No (83 and 84%, respectively; $P > 0.05$). Mean $COMP_{a-t}$ was similar ($p > 0.05$) among

all treatments (range: 4 - 5%). At 48 hours, mean TMOT and PMOT were similar ($p > 0.05$) in INRA-No and BG-No (39 and 16 versus 45 and 20%, respectively), whereas higher ($p > 0.05$) than in INRA-Yes and BG-Yes (24 and 12% versus 26 and 10%, respectively). Mean VIAB and $COMP_{a-t}$ were not affected ($p > 0.05$) by any of the treatments (range: 72 - 77% and 8 - 10%, respectively. The sperm quality parameters observed in sex-sorted stallion sperm that was prestained before submission, although slightly lower than in the nonstained sperm, were considered acceptable when compared to those observed in sex-sorted stallion sperm that was not prestained. These results indicated that stallion sperm can be processed either in INRA-96® or BotuGold® semen extenders, pre-stained with Hoechst 33342, cool-stored for 24 hours, submitted to a sex-sorting laboratory, and then cooled again after sex-sorting for additional 24 hours, opening the possibility of making this technology accessible to a wider range of breeders.

Keywords: Stallion sperm, sex-sorting, cooled storage, Hoechst 33343, INRA-96®, BotuGold®

Clinical observations of bacterial culture results after intrauterine infusion of misoprostol

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Deep horn intrauterine deposition of misoprostol has been demonstrated to be an effective method of achieving pregnancies in mares that have otherwise been subfertile.[1] As the use of this technique has increased in recent years, sporadic anecdotal reports of profound uterine inflammation have been reported. As part of standard practice, the authors routinely sample the uterus by low volume lavage for culture and cytology on the day following misoprostol infusion. To objectively assess uterine response to misoprostol infusion, the records of 36 mares infused with misoprostol during a 1-year period (August 2020 - August 2021) were evaluated. Mares were presented for this procedure for failure to have produced a 14-day pregnancy despite appropriate breeding timing and no evidence of bacterial endometritis by industry standard screening methods (endometrial swab). Two 200 µg tablets of misoprostol (Cytotec®, Pfizer) were dissolved in 3 ml sterile saline and 2 aliquots were made, 1 for each uterine horn. Aliquot was deposited at the distal end of each uterine horn by standard deep horn insemination technique using a flexible pipette and transrectal guidance. The following day, 1 liter of sterile lactated Ringer's solution (LRS) was instilled into the uterus and immediately collected back into the original bottle. This low volume lavage was submitted for culture and cytology to a single laboratory. Bacterial growth was reported as no growth, scant (< 10 colony forming units), light (growth on 1 quadrant of plate only), moderate (growth on 2 quadrants of plate), or heavy (growth on 3 quadrants of plate). Of the 36 mares infused with misoprostol, follow-up was available for 32. Of these, 7 (22%) had no bacterial growth, 5 (16%) had scant bacterial growth, 6 (19%) had light bacterial growth, 10 (31%) had moderate bacterial growth, and 4 (13%) had heavy bacterial growth. Mares were then treated for bacterial endometritis based on these results at the discretion of the attending clinician. Use of intrauterine misoprostol has expanded throughout clinical practice since publication of the technique. Whereas increased detection of previously undiagnosed bacterial endometritis was not included as a benefit of the procedure, results suggested that this may be an unintended utility. Sixty-three percent (20/32) of mares infused with misoprostol responded to the infusion with clinically significant bacterial endometritis (light, moderate, or heavy bacterial growth) that had otherwise gone undiagnosed during previous workup. This phenomenon remains unexplained, but potential hypotheses include stimulation of dormant bacteria through the inflammatory insult of intrauterine infusion of a foreign substance, physical irritation of the endometrium by the deep horn infusion technique, or acute immune system downregulation secondary to misoprostol which allows for bacterial proliferation. Despite not yet understanding the underlying mechanism by which this process occurs, results from this group of mares suggested that there is substantial clinical value in culturing mares following misoprostol infusion.

Keywords: Mare, endometritis, misoprostol, barren

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Incidence and effect of low serum progesterone concentrations at day 6 postovulation on pregnancy rates in mares

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Low serum progesterone (P_4) concentration is 1 factor that may contribute to early pregnancy failure (EPF) in the mare. The threshold value for pregnancy maintenance is 2.5 - 4 ng/ml. Mares with EPF are routinely placed on exogenous P_4. This is expensive, poses human health risks, immunosuppressive, and potentially unnecessary. Therefore, we hypothesized that measuring serum P_4 concentrations on day 6 (D6) postovulation can enhance the efficacy of P_4 supplementation on pregnancy rates. Progesterone concentrations were measured in 341 Thoroughbred mares on D6 postovulation, over 500 bred estrous cycles. The study spanned 2 consecutive breeding seasons. During estrus, daily rectal ultrasonography was performed, +/- endometrial culture and human chorionic gonadotropin or deslorelin was given 24 hours prior to mating. Ovulation was confirmed 12 - 24 hours after breeding via transrectal ultrasonography. Blood collected on D6 post ovulation (day 1 defined when the preovulatory follicle was replaced by a corpus hemorrhagicum) was cooled within 6

hours after collection and centrifuged for serum collection within 36 hours. An accurate quantitative enzyme linked immuno assay measured serum P_4. In Year 1, P_4 concentrations were determined immediately and ≤ 4 ng/ml initiated altrenogest supplementation. In Year 2, samples were stored at – 20°C and subsequently analyzed. Pregnancy was diagnosed 14/15 days postovulation via transrectal ultrasonography. Mares were assigned to 3 groups based on D6 P_4 concentrations (Group 1, ≤ 4 ng/ml; Group 2, 5 - 6 ng/ml; Group 3, > 6 ng/ml) and pregnancy rates determined. Pregnancy rates among 3 groups were compared in each season, using a Chi-squared test. Group 1 pregnancy rates were compared using a Chi-squared test between Years 1 and 2. In addition, pregnancy rates were compared between Years 1 and 2 for mares with P_4 concentrations of ≤ 2.5 and > 2.5 - 4.0 ng/ml. In Year 1, 15/149 (10.06%) and Year 2, 38/351(10.8%) mares exhibited P_4 concentrations of ≤ 4 ng/ml respectively. Pregnancy rates for 3 groups during Years 1 and 2, respectively were: Group 1, 7/15 (47%), 21/38 (55.8%); Group 2, 22/40 (55%), 67/104 (64.4%); and Group 3, 62/94 (65.9%), 130/207 (62.8%). There was no difference among groups or between Years 1 and 2 pregnancy rates with P_4 concentrations of ≤ 4 ng/ml or ≤ 2.5, > 2.5 - 4.0 or ≥ 4 ng/ml, or between ≤ 2.5, > 2.5 - 4.0, or ≥ 4 ng/ml in Year 2. Results indicated that primary luteal insufficiency occurred in 10 -11% of estrous cycles. Exogenous P_4 supplementation did not increase pregnancy rates. There was no difference in pregnancy rates of mares with concentrations of ≤ 2.5 ng/ml or > 2.5 - ≤ 4 ng/ml (61.3 versus 50%) when no exogenous P_4 was given. Although exogenous P_4 therapy did not appear beneficial, and numbers in this category were small, earlier measurement of serum P_4 concentrations on D6 may reduce overuse of exogenous P_4. Results further suggested that other factors are more likely to be the cause of EPF or poor pregnancy rates in the mare.

Keywords: Mare, progesterone, early pregnancy loss, luteal insufficiency

Ovulatory response to GnRH-agonist during early and late fall in mares

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Deslorelin acetate (deslorelin) is the most popular drug used to induce ovulation in mares, likely due to reliable response in older mares and consistent availability. Response to this GnRH-agonist during spring and summer is reported as ~ 90% with ovulation occuring ~ at 40 hours.[1] However, despite its efficacy and popularity, its ability to induce ovulation in the fall is debated. Endometrial edema score is an important clinical criterion to determine a follicle's readiness to respond to induction of ovulation; however, it is suggested that endometrial edema is lower in the fall, hence the presumed poor GnRH-response. Objective was to assess the ovulatory response of deslorelin in mares during fall by comparing success in inducing ovulation, interval to ovulation, edema scores, and parameters of the resultant corpus luteum (CL) in early and late fall. We hypothesized that deslorelin has a better ovulatory response during early fall than late fall. Mares (n = 22) kept in 40° latitude were checked via transrectal ultrasonography until a preovulatory follicle was detected then ovulation was induced with intramuscular deslorelin (1.8 mg). Ovulation was confirmed by ultrasonography performed at 24 and 36 hours postinduction and repeated at 2 hour intervals. Serum progesterone concentrations (chemiluminescence) and luteal tissue area (via ultrasound-calipers) were determined daily to assess CL function. A dose of intramuscular $PGF_{2\alpha}$ (7.5 mg) was given 8 days postovulation and each cycle was repeated up to 5 times. Associations between local weather and endpoints were analyzed. Cycles were grouped as early (n = 55) and late fall (n = 45) based on date of induction. Cycles with spontaneous ovulation < 24 hours (n = 6) or failure more than 48 hours (n = 2) from deslorelin were discarded. Parametric data was analyzed with Mixed models, ANOVA, Tukey's test, and Pearson's correlation. Nonparametric data were analyzed with Fisher's test, Kruskal-Wallis, and Dunn's test. Significance was defined as $p < 0.05$. Deslorelin effectively induced ovulation in 90% of cycles. Spontaneous, failure, and multiple ovulations were similar ($p > 0.05$) between early and late fall. The interval from induction to ovulation was identical ($p = 0.55$) in early (40.6 ± 0.4 hours) and late (41.2 ± 0.5 hours) fall. Percentage of mares ovulating between 36 - 48 hours and 38 - 44 hours postdeslorelin did not vary ($p > 0.05$), between early (91 and 62%) and late (95 and 60%) fall respectively. Follicle size at induction tended ($p = 0.07$) to be smaller in early (36.4 ± 0.4 mm) than late (37.4 ± 0.4 mm) fall. Edema scores varied ($p < 0.001$) with time relative to ovulation and were lower ($p = 0.01$) in late fall. There was a negative correlation ($r = – 0.88$, $p = 0.05$) between edema score on interval to ovulation. Progesterone concentrations varied ($p < 0.001$) with time but did not differ ($p = 0.73$) between early and late fall and correlated weakly with the luteal area ($r = 0.13$; $p = 0.031$). Lower temperature was associated with smaller follicle size at induction ($p = 0.0021$) and ovulation ($p = 0.009$), and lower relative humidity was associated with larger follicle size at ovulation ($p = 0.032$). In conclusion, the findings here demonstrated for the first time that mares respond satisfactorily to GnRH-agonists in the fall. Despite lower endometrial edema scores in late fall, the indistinguishable difference for other parameters between early and late fall suggested poor response to GnRH-agonist in the fall, independent of edema score.

Keywords: Ovulation, deslorelin acetate, progesterone, luteal function

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Sperm parameters and fertility of donkey semen cooled using skimmed milk, sodium caseinate, and egg yolk-based extenders

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In Brazil and the US, the production of mules is increasingly prized, and the high value of top mules has generated an interest in donkey reproduction. However, donkey semen does not well tolerate cooling with skim milk-based (SKM) extenders. Therefore, this study aimed to compare semen parameters and fertility of cooled donkey semen extended in 3 different extender bases in the presence and absence of seminal plasma. In Experiment 1, 18 ejaculates from 6 mature jacks were harvested and divided into 6 aliquots. Three of them were immediately diluted at 50 x 10^6 sperm/ml in 3 commercially available semen extenders: SKM containing cholesterol-loaded cyclodextrin (CLC) (BotuSpecial®); sodium caseinate-based with CLC (SC, BotuGold®); or egg yolk-based (EY, BotuCrio®) extenders. Remaining aliquots (n = 3) were also diluted at 50 x 10^6 sperm/ml with SKM (n = 1) or SC (n = 2) and then subjected to centrifugation at 600 x g/10 minutes. After centrifugation, the supernatant was discarded, and the sperm pellet of 2 out of 3 samples were resuspended with the same semen extender (SKM-C and SC-C); 1 sample previously diluted with SC was resuspended in EY (EY-C). After centrifugation, sperm were resuspended at 100 x 10^6 sperm/ml. All samples were stored in a passive cooling container at 5°C for 48 hours. Total motility (TM), progressive motility (PM), and percentage of rapid sperm (RAP) were assessed with CASA. Plasma membrane stability (PMS) and mitochondrial membrane potential (MMP) were assessed with the combination of Yo-Pro® and MitoStatusRed with flow cytometry. Semen was assessed before (0) and 24 and 48 hours after cooling. In Experiment 2, 118 estrous cycles of 15 mares were used for fertility assessment during 2 breeding seasons. Mares were randomly bred 24 hours postinduction of ovulation with semen from 2 Jacks (Jack 1, n = 90; Jack 2, n = 28) cooled for 24 hours with either of the treatments (SKM, SC, EY, SKM-C, SC-C, or EY-C). Pregnancy diagnosis was performed at day 15 postovulation; mares then received intramuscular dinoprost (5 mg) to return to estrus. Data were evaluated by ANOVA and Tukey's post hoc test. Pregnancy rates were evaluated by Fisher's Exact test. Significance was set at $p < 0.05$. There were no differences ($p > 0.05$) in TM, PM, RAP, PMS, and MMP for semen in either group at hour 0. There was a reduction ($p < 0.05$) in TM, PM, RAP, PMS, and MMP over time across groups. Groups EY, SC-C, and EY-C had superior ($p < 0.05$) TM, PM, RAP, PMS, and MMP than SKM. Semen in the SKM-C and SC groups had intermediate values ($p > 0.05$). Centrifugation positively affected ($p < 0.05$) sperm parameters in donkey semen extended in SKM (SKM versus SKM-C). Mares bred with semen extended in SC (74%, 14/19), SC-C (89%, 17/19), EY (90%, 18/20), or EY-C (79%, 15/19) had a greater ($p < 0.05$) conception rate than mares bred with SKM (30%, 6/20). Mares bred with SKM-C had intermediate conception rates (62%, 13/21). In conclusion, SC containing CLC and EY-based extender appeared more affordable to preserve the sperm characteristics and fertility in cooled donkey semen. Additionally, centrifugation positively impacted donkey semen extended in SKM-based extenders.

Keywords: Mule, jack, stallion, artificial insemination, cryopreservation

Feeding spent hemp biomass does not negatively affect ram fertility

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Industrial hemp was recently removed from the Controlled Substances Act and classified as an agricultural product. After cannabidiol (CBD) extraction, several tons of spent hemp biomass (SHB) containing a residual amount of CBD is left over. SHB is high in crude protein, omega-3 fatty acid, and omega-6 fatty acid, suggesting it could be used in livestock diets. However, use of SHB as feed in livestock diets have not been approved by the FDA yet because of the paucity of information about cannabinoid residuals and their effects on animal health, production, reproduction, and product quality. Chronic exposure to cannabinoids, including CBD, can negatively affect fertility in mice. Objective was to determine the reproductive effects of feeding SHB to rams. We hypothesized that feeding SHB to rams may adversely affect fertility. A feeding trial was performed with 6-month-old Polypay rams. Rams were randomly assigned to 5 feeding trial groups (7 per group) and fed either alfalfa (control) or SHB at 10 or 20% for 4 weeks, with 4 weeks withdrawal from SHB, or SHB at 10 or 20% for 8 weeks. Blood samples were collected from the jugular vein on days 0, 28, 56, and serum was analyzed for testosterone concentrations via chemiluminescence (Immulite 1000®, Siemens). At the conclusion of the feeding trial, rams were euthanized via penetrating captive-bolt and testes were removed. Sperm was collected from the vas deferens and evaluated for motility (total, progressive, speed), morphology, and concentration using routine methods. An analysis of variance was used to analyze the date and significance was defined as $p < 0.05$. Testosterone concentrations increased ($p < 0.01$) over time in all groups but there was no effect of animal ($p = 0.58$), treatment ($p = 0.30$), or time*treatment ($p = 0.75$). However, rams that were fed either 10 or 20% SHB for 8 weeks had higher ($p < 0.05$) total and progressive sperm motility and higher sperm motility speed compared to controls There was no difference ($p > 0.05$) in sperm concentration or the percentage of morphologically normal sperm per group Based on these results, we concluded that SHB does not have a negative reproductive effect on male endocrinology and may improve fertility with respect to sperm motility.

Keywords: Cannabidiol, hemp, motility sperm, testosterone

Effect of Caslick's surgery as a treatment for behavioral problems in mares

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Referred vaginal pain causes behavioral problems in mares (including decreased rideability), and a direct link between windsucking, pneumovagina, and subsequent chronic irritative vaginitis causing referred vaginal pain syndrome has previously been demonstrated. Aim was to retrospectively evaluate the effects of Caslick's surgery in mares with a reported history of behavioral problems. An additional aim was to describe the owner's perception of the mare's clinical presentation related to the behavioral problems. A retrospective study using an online questionnaire was performed. Participants were recruited on Danish equestrian social media platforms. Questionnaire included 19 questions and was designed in SurveyMonkey®. General variables (age, breed, use, and competition participation) and specific variables related to behavioral symptoms, estrous cycle, and age at the time of Caslick's surgery was used in statistical analyses. Total population was categorized into 3 groups based on the effect of Caslick's surgery and further divided into groups based on the frequency of occurrence of behavioral symptoms. All comparative analyses were carried out using Chi-square tests. Statistical significance was set at $p < 0.05$. In total, 406 mare owners reported that their mare had Caslick's surgery performed due to behavioral problems. 'Full effect' (disappearance of symptoms) was reported by 129 owners (31.8%), 'some effect' (behavioral problems became less apparent) in 152 mares (37.4%), and no effect was reported by 125 owners (30.8%). Symptoms were present 'constantly' in 53.2% of mares (215/404), 'in relation to estrus' in 28.7% (73/404), and with 'varying pattern' in 28.7% (116/404). The mares reported to express symptoms 'constantly' had higher prevalence of the symptoms 'increased sensitivity in the flank region' ($p = 0.013$), 'excessive tail swishing' ($p = 0.006$), and 'opposing signals from rider's legs' ($p = 0.013$) compared to mares expressing symptoms in a 'varying pattern'. Additionally, the prevalence of mares 'refusing to move forward' was higher ($p = 0.005$) within the group of mares expressing symptoms 'constantly' compared to mares only showing symptoms 'in relation to estrus'. Age ranged from 3 - 24 years (mean: 10.0 years). The 'full effect' group contained significantly more mares aged ≤ 6 years at the time of surgery compared to 'no effect' ($p = 0.033$) and 'some effect' ($p = 0.006$). More mares ($p = 0.008$) within the age interval ≤ 6 years were present in the group 'full effect' compared to 'some effect'. Mares were reported to participate in competitions by 65.7% of the owners. Higher prevalence of competing mares was in the groups 'some effect' ($p = 0.002$) and 'full effect' ($p = 0.0009$) compared to 'no effect'. Time to effect was reported to be within 3 months in 83.7% ('full effect') and 83.5% ('some effect'). Within the group 'no effect', 37.6% (47/125) of the owners reported to have another reason for the mares' problems. Caslick's surgery resolved or markedly improved behavior in almost 70% of the cases, and in most of these mares, the effect was observed within 3 months postoperatively. Despite the bias of the owners' subjective perceptions/observations, we concluded that Caslick's surgery will solve behavioral problems in mares suffering from referred vaginal pain syndrome.

Keywords: Mares, behavioral problem, Caslick's surgery

Sperm protein reactive with antisperm antibody expression in domestic and wild horse ovaries

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Sperm protein reactive with antisperm antibody (SPRASA) is a lysozyme-like protein previously identified in domestic and wild horse ovarian follicles.[1] Objective was to use image analysis software to compare SPRASA ovarian immunoexpression in all follicle stages. We hypothesized that SPRASA expression does not differ between domestic and wild mares. Routine immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections, followed by image analysis using FIJI software. Heat-induced epitope retrieval with sodium citrate (pH 6.1) was used. Anti-SPRASA polyclonal antibody (#HPA023633, Atlas Antibodies) was applied at 1:200 dilution and immunostaining specificity was verified by replacing the primary antibody with negative control rabbit serum on adjacent sections. Sections were then reacted with one-step horseradish peroxidase-conjugated polymer anti-rabbit IgG (IH-8064-custom-OrSU, ImmunoBioScience) followed by a NovaRED peroxidase substrate (#SK-4800, Vector Labs). Representative images of each follicle stage from each ovary were digitally captured using QCapturePro image capture software by a single observer at 200 x magnification (ML). Cellular expression of SPRASA was then quantified in primordial, primary, secondary, and tertiary follicles using FIJI software with RGB stack and manual thresholding to isolate areas of staining. The granulosa cells and theca cells were outlined using the freehand selection tool and mean gray value was measured. Results (mean ± SEM) were compared between domestic and wild mares using a Student's *t*-test and significance was defined as $p < 0.05$. SPRASA expression was greater in domestic compared to wild mares in granulosa cells of primordial ($p = 0.0022$), primary ($p < 0.001$), secondary ($p = 0.025$), and tertiary follicles ($p = 0.0078$), and in theca cells of tertiary follicles ($p = 0.022$) (Figure 1). Wild equid population in US has drastically exceeded the carrying capacity of the public lands where they are managed.[2] Immunization against SPRASA may prove to be a permanent, nonsurgical immunologic method for sterilizing horses, because it targets all stages of ovarian follicles.

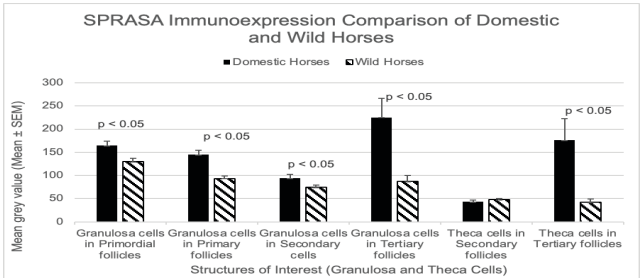

Figure 1. SPRASA expression in equine follicles

Keywords: Mare, immunocontraceptive, mare, granulosa cell, follicle

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How to work-up a mare exhibiting stallion-like behavior

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Veterinarians are occasionally asked to evaluate a mare exhibiting stallion-like behavior, which may include mounting or herding other mares, aggression toward other horses or people or other manifestations. An initial approach to the issue would be to formulate a differential diagnosis (DDX) for the abnormal behavior. Goals of the DDX are to provide a list of potential causes, guide diagnostic tests and treatment protocols, rule out life threatening or time-critical conditions and provide a pathway to a final diagnosis. Differential diagnoses for stallion-like behavior in mares includes an ovarian granulosa-theca cell tumor, pregnancy, estrus, dominant/alpha mare, disorder of sexual development, iatrogenic, idiopathic and inaccurate assessment of behavior by the mare owner or trainer. Initial diagnostic plan should include a complete and accurate history, a general physical examination (evidence of increased muscle tone, masculine physical features, 'cresty' neck, abnormal perineal region) and transrectal palpation/ultrasound (evaluation of gonads, presence/absence of a uterus, pregnancy status). Additional diagnostic tests that may be indicated pending the initial evaluation include hormone analysis (anti-Müllerian hormone, inhibin, testosterone, progesterone), observation of behavior, vaginal speculum examination (is the vaginal vault complete and is a cervix present), karyotype (chromosome analysis), behavioral response to hormone therapy (altrenogest), tissue biopsy with histopathology, and other tests. Mares in behavioral estrus may exhibit mounting behavior. Mounting behavior is uncommon in mares compared to the incidence rate in cows. Mares that exhibit mounting behavior have been reported to have higher testosterone concentrations than mares being mounted. A dominant mare may exhibit aggressive behavior toward other (subordinate) mares in the herd or harem. Dominance behavior may be more evident with introduction of a new mare into the herd. Dominant mares may have elevated testosterone concentrations of ovarian or adrenal origin. A granulosa-theca cell tumor is a benign, unilateral ovarian tumor associated with a small, inactive contralateral ovary. The presence of thecal cells is associated with elevated testosterone concentrations and subsequent expression of aggressive or stallion-like behavior. Diagnosis is based on behavioral characteristics, transrectal palpation/ultrasound and hormone analysis. Mares in mid- to late pregnancy have elevated blood testosterone, which may lead to aggressive or stallion-like behavior. The source of testosterone is the fetal-placental unit; levels peak at ~ 7 months of gestation. Mares given an anabolic steroid (e.g., boldenone undecylenate), may exhibit behavior characteristic of stallions, including aggression or fighting, flehmen response, elimination or marking behavior, and mounting and/or herding of other mares. Disorders of sexual development may be associated with stallion-like behavior in a horse with a female phenotype. One example is a 64 XY, SRY-positive horse with cryptorchid testes, elevated testosterone and variable differentiation of the perineal region. Diagnosis of this condition is chromosomal analysis of a blood or hair sample. In summary, an owner or trainer complaint of aggressive or stallion-like behavior is uncommon, but not rare. Acquisition of a complete history and an initial thorough, systematic reproductive evaluation will dictate additional diagnostic tests that will hopefully lead to a definitive diagnosis.

Keywords: Mare, stallion-like behavior, testosterone, karyotype

Induction of labor in the mare: review of principles, procedures, and pitfalls

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Induction of labor allows for an opportunity to have professional assistance available to assist with foaling and care of the newborn foal from a high-risk pregnancy. Clinical indications for a controlled induction of labor in a mare include management of high-risk pregnancies, and monitoring of labor in mares that have had a history of dystocia, stillbirth, premature separation of the placenta, or other foaling complications. Induction of labor may also be performed as an emergency procedure on a late-term pregnant mare with a life-threatening medical condition, such as severe acute laminitis, ruptured prepubic tendon, placental hydrops or other issues. Elective induction of labor in the mare for nonmedical reasons is generally not recommended. Induction of foaling in mares can be predictable and successful with strict adherence to guidelines for assessment of fetal readiness for birth. However, emergency medical situations may over-ride the standard guidelines. To be considered for induction of labor, a mare should have a gestation length of at least 330 days, have an enlarged udder and engorgement of the teats with colostrum and, if measurement is possible, a mammary fluid calcium carbonate level of greater than 200 ppm. Hormonal techniques evaluated for induction of labor in the mare have included corticosteroids, prostaglandins and oxytocin. Corticosteroids (e.g., dexamethasone) are effective in inducing parturition in ruminants, but generally unreliable in inducing labor in mares. Prostaglandins have been used successfully in mares, but interval from administration to rupture of the chorioallantois and subsequent foaling is not consistent or predictable. In author's opinion, the most reliable technique for induction of labor in mares is treatment with oxytocin. Dosage and treatment regimens described for oxytocin include treatment of small (2.5 - 20 units) oxytocin boluses, intravenously or intramuscularly, at 10 - 30 minute intervals, treatment of a larger bolus (40 - 60 units) intramuscularly, and treatment of 75 - 100 units of

oxytocin diluted in 1 liter of saline through an intravenous catheter at a rate of 1 unit/minute or until the fetus is delivered. Author prefers giving small intravenous boluses of oxytocin for induction of labor in mares. The interval from first oxytocin treatment (5 units) the first signs of labor (uterine contractions, discomfort, sweating) is 5 - 10 minutes; a second dose of oxytocin (10 units) is given 15 minutes after the initial dose. Rupture of the chorioallantois usually occurs within 5 - 10 minutes after the second dose and delivery of the fetus usually 10 - 15 minutes thereafter. In conclusion, controlled induction of labor can allow for a safe, predictable foaling when breeding farm and veterinary medical support personnel and emergency equipment are available. Conversely, induction of labor can be disastrous if guidelines for selection of mares are not followed or if an accurate breeding date is not known. Induced parturition may also be associated with dystocia, retained fetal membranes, premature placental separation, dysmaturity, and failure of passive transfer.

Keywords: Mare, induction, labor, obstetrics, oxytocin

Outcome of periparturient peritonitis secondary to uterine perforation in mares

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This study investigated signalment and presenting signs of mares with suspected uterine perforation and their association with duration of hospitalization, survival, and subsequent fertility. It was hypothesized that affected mares differ from clinical and clinicopathological reference ranges of nonaffected mares, and survivors and nonsurvivors also differ in the magnitude of these changes. Medical records of mares (n = 46) presented to a large central Kentucky referral hospital between 2011 and 2020 with uterine perforation and resultant periparturient peritonitis were reviewed. Affected mares ranged between 4 and 22 years of age with mean ± standard deviation (mean ± SD) of 10.5 ± 3.7 years. Duration of hospitalization was (mean ± SD) 7 ± 5 days, with surviving mares 3 ± 3.94 days (range 0 - 10 days) and nonsurviving mares 8 ± 5.03 days (range 4 - 22 days). Most consistent findings on initial examination were tachypnea 76%, apparent abdominal discomfort 63%, and tachycardia 56%. Clinicopathological abnormalities commonly detected were hyperlactatemia 80%, bandemia 63%, hemoconcentration 49%, and leukopenia 33%. Peritonitis was diagnosed on the basis of abdominocentesis, transabdominal ultrasonography, and exploratory celiotomy. Common peritoneal fluid abnormalities included elevated protein concentrations 100% and leukocytosis 94%. All mares suspected of uterine perforation underwent exploratory celiotomy. Location of uterine defect was evenly distributed between the uterine body or the horns. Most common postsurgical complication was peritonitis 75%. Medical treatment consisted of treatment of intravenous fluids, antibacterials, nonsteroidal antiinflammatory drugs, and peritoneal lavage (intraoperative and postoperative). Survival to discharge was 82.6% (38/46). Of nonsurvivors, 87.5% (7/8) were humanely euthanized based on prognosis 71.4% (5/7), intraoperative findings 14.3% (1/7), and postanesthetic misadventure 14.3% (1/7). Factors associated with nonsurvival (odds ratio, 95% confidence interval) included hypercreatininemia (39, 1.86 - 817.63), leukocytosis (5.16, 0.65 - 40), absence of fever (3.66, 0.35 - 38.03), increased peritoneal fluid volume (3.20, 0.33 - 31.42), and absence of apparent abdominal pain at admission (1.87, 0.22 - 15.93). Sample size limitations are evident in calculation of confidence intervals. For surviving mares not lost to follow up, 80% (24/30) produced another foal with (mean ± SD) 1.48 ± 0.66 years (range 1 - 3 years) until successful delivery. In summary, survival of mares with uterine perforation leading to peritonitis is good. Reproductive potential is favorable. Presenting signs and clinicopathological variables available at initial examination differ from healthy mares and these are also differentially associated with nonsurvival.

Keywords: Mare, peritonitis, uterine perforation, foaling, hypercreatininemia, colic

A case of paraphimosis of 7 years' duration in a Percheron stallion

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A 15-year, Percheron stallion, was presented for evaluation of paraphimosis of 7 years' duration. Available history indicated that the paraphimosis was subsequent to a 2013 kicking injury. Vital parameters were within normal limits (WNL). Penis and prepuce were markedly firm and swollen, glans penis extended to tarsus level, and chronic penile exteriorization was evident (epithelium was thickened, friable, and cool to the touch). Since the stallion had an apparent lack of sensation, as evidenced by the lack of response when the penile skin was pinched, a phallectomy and castration were recommended. Prior to induction of general anesthesia, the stallion received prophylactic antibiotics and antiinflammatory drugs; presurgical packed cell volume (PCV) was 32% with total solids (TS) of 8.7 g/dl. Following anesthetic induction, the periscrotal area and penis were aseptically prepared and a stallion urinary catheter was placed. To reduce the likelihood of urethral stricture, the Williams' technique was selected. Use of electrocautery and an Esmarch tourniquet at the base of the penis provided hemorrhage control. A urethrostomy was performed by making 3 cm long incisions, forming a triangular-shaped wedge on the ventrum of the penis. Catheter was removed and a stoma was created by securing the urethral mucosa to the epithelium with simple interrupted sutures. Penis was amputated 5 cm distal to the urethrostomy site. A simple interrupted suture pattern provided compression and closure of the corpus cavernosum around the urethral lumen. Finally, a closed castration was performed with Serra emasculators. No intraoperative hemorrhage was noted. Recovery from anesthesia was uneventful and the patient experienced mild postoperative hemorrhage from the stoma. Physical examination findings were unremarkable. Approximately 12 hours postsurgery, the gelding presented with dull mentation, pale mucous membranes, tachycardia (72 beats/minute), and tachypnea (32 breaths/minute). Gelding was given infusions

of aminocaproic acid, 0.05% formalin, and a whole blood transfusion (7 liters). By the following afternoon, PCV had increased from 18 to 22% and TS increased from 5 to 6.8 g/dl; vital parameters were WNL. Gelding was discharged 5 days postsurgery with normal physical examination findings. Medications included oral twice daily phenylbutazone (1.5 gram), trimethoprim sulfamethoxazole (30 mg/kg), and Red Cell® (30 ml). Instructions provided to owners included stall rest, daily hydrotherapy, and short hand walks twice daily to manage postoperative edema. Four days after discharge, owners returned the gelding for assessment of dull mentation; vital parameters were WNL except for mild tachycardia (56 beats/minute). Urethral stoma appeared to be healing appropriately; PCV was 16% and TS was 7.4 g/dl at presentation. Gelding remained stable and was discharged 2 days later. Instructions included continued stall rest and hydrotherapy in addition to application of Femycin ointment to penis. This case described resolution of a severely protracted paraphimosis and successful management of common, albeit concerning, postoperative complications.

Keywords: Paraphimosis, stallion, phallectomy, castration

Evaluating susceptibility of fungal biofilm to mucolytics used to treat mares

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Fungal endometritis in mares causes infertility and is difficult to treat. Fungal agents form biofilms that impede the immune system and may enhance antifungal resistance. Mucolytics are commonly used to disrupt fungal biofilm but the efficacy of these intrauterine treatments is not well known. We aimed to investigate the susceptibility of *Candida* sp. biofilm to commonly used intrauterine treatments. We hypothesized that biofilm from various *Candida* species have distinct susceptibility to mucolytics. Biofilm was produced by culturing 5 *Candida* isolates (1 *C. albicans*, 2 *C. parasilopsis*, and 2 *C. tropicalis*) obtained from clinical cases of equine endometritis in RPMI media for 24 hours in 96-well plates. Initial biofilm amount was determined by spectrophotometric absorbance at 600 nm. Biofilm was exposed to 1 of 15 treatments: Lactated Ringer's solution (LRS) as control; Ceragyn Lavage, Ceragyn Uterine infuse, dimethylsulfoxide (DMSO) at 10, 20, and 30%; hydrogen peroxide at 0.5 and 1 %; povidone iodine at 1 and 1.5%; N-acetylcysteine at 3.3 and 20%; Tricide®; and vinegar at 2 and 10%. Treatment was applied to the biofilm for 5 minutes, then washed before repeating spectrophotometric reading. Each isolate was tested at least 3 times and each treatment was tested in 4 replicates each time. Percent reduction in biofilm absorbance was calculated and used as the dependent variable. Statistical analysis was performed using linear regression ANOVA with Dunnett's post hoc test in JMP pro v16 with significance set at $p < 0.05$. Independent variables tested were: replicate, treatment, species, isolate, and interactions. There was a significant interaction of treatment by species, indicating various fungal species had varied susceptibility to the mucolytics, and the analysis was repeated stratifying for fungal species. Isolate and treatment were still significant, indicating that even within a fungal species, biofilm from various isolates have varied susceptibilities to mucolytics, and all results were reported by isolate. For *C. albicans*, treatment with DMSO at 30% and hydrogen peroxide at 0.5 and 1% had higher percent biofilm reduction compared to LRS. For *C. parasilopsis*, biofilm from 1 isolate was more susceptible to DMSO at 20 and 30%, and hydrogen peroxide at 0.5 and 1%; whereas biofilm of the other isolate was more susceptible to DMSO at 10, 20, and 30%, povidone iodine at 1 and 1.5%, N-acetylcysteine at 20%, and Tricide compared to LRS, but was not susceptible to hydrogen peroxide. For *C. tropicalis*, biofilm from 1 isolate was susceptible to DMSO at 10, 20, and 30%, hydrogen peroxide at 0.5 and 1%, and N-acetylcysteine at 20%; whereas biofilm of the other isolate was only more susceptible to DMSO at 30% compared to LRS. Replating fungal biofilm posttreatment for 24 hours for *C. albicans* resulted in no significant difference in growth compared with LRS, whereas for *C. tropicalis* and *C. parasilopsis* treatment with Ceragyn Lavage and Uterine Infuse, DMSO at 20 and 30%, Tricide®, and N-acetylcysteine resulted in higher inhibition of fungal growth. These results indicated a need for more studies to understand fungal biofilm susceptibility.

Keywords: Mare, Candida, mucolytic, biofilm

Efficacy of nano-colloidal silver treatment for postpartum endometritis in dairy cows

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Postpartum endometritis due to bacterial infection is 1 of the most common postpartum diseases affecting dairy cows, and antibiotics are widely utilized for treatment. Recently, bacterial resistance to antibiotics has become the most important problem. Aim was to detect the efficacy of nano-colloidal silver in treatment of postpartum endometritis in dairy cows. We hypothesized that intrauterine treatment of nano-colloidal silver eliminates bacterial infection causing endometritis, due to its wide spectrum of bacteriostatic and bactericidal activity against enormous range of bacterial strains at low concentration. Consequently, such recovery can be detected initially by peripheral concentrations of some chemokines and acute phase proteins and subsequently by pregnancy rate. In large private dairy farm, Holstein cows (n = 21) were diagnosed with postpartum endometritis at 35 - 40 days postpartum via clinical and ultrasonographic examinations. Cows received 40 ml of intrauterine nano-colloidal silver solution (25 ppm/ml, particle diameter of ~ 15 - 25 nm) for 5 consecutive days and 1 dose of prostaglandin $F_{2\alpha}$ analogue (cloprestenol) for cows with a corpus luteum. Blood samples were drawn just before the treatment and on day 7 after treatment. Serum concentrations of ceruloplasmin, CRP were assessed (immune-turbidmetry), in addition to serum amyloid –A (SAA), haptoglobin, (TNF-α), and (IL-6) using ELISA Kits. After treatment, uterine status was monitored via transrectal ultrasonography.

Animals were reexamined at 49 - 54 days postpartum to assess recovery rate. Cured animals were artificially inseminated on their observed estrus; pregnancy was diagnosed on day 40 after insemination. Data were analyzed by Student's *t*-test using SPSS software. Treatment with nano-colloidal silver, decreased ($p < 0.001$) serum concentrations of SAA, CRP, TNF-α, and IL-6. Haptoglobin and ceruloplasmin concentrations did not change ($p < 0.001$) after treatment. Uterine secretions decreased or disappeared, and endometrial thickness decreased on day 7 after treatment. At reexamination, 14 of 21 cows (66.6%) had recovered from endometritis and pregnancy rate was 71.4% (10/14). In conclusion, the study indicated the effectiveness of nano- colloidal silver in treatment of postpartum endometritis in dairy cows. So, it could be used instead of antibiotic treatment for antibiotic resistance problems. Further studies are needed to investigate the effect of different treatment period and solution concentrations of nano-colloidal silver on treatment of endometritis in dairy cows.

Keywords: Dairy cows, postpartum endometritis, nano-silver

Transcriptomic analysis reveals the complex regulatory networks in equine chorioallantois during spontaneous term labor

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Equine chorioallantois (CA) undergoes complex physical and biochemical changes during labor. However, the molecular mechanisms controlling these changes are still unclear. Therefore, the aim of the study was to characterize the transcriptome of equine CA during spontaneous labor and compare it to normal preterm CA. Placental samples were collected postpartum from mares with normal term labor (TL group, n = 4) and from preterm not in labor mares (330 days GA; PTNL group, n = 4). Our study identified 4,137 differentially expressed genes (DEGs) (1,820 upregulated and 2,317 downregulated) in CA during TL as compared to PTNL. TL was associated with the upregulation of several proinflammatory mediators (*MHC-I*, *MHC-II*, *NLRP3*, *CXCL8*, and *MIF*). Also, TL was associated with the upregulation of matrix metalloproteinase (*MMP1*, *MMP2*, *MMP3*, and *MMP9*) with subsequent extracellular matrix degradation and apoptosis, as reflected by upregulation of several apoptosis-related genes (*ATF3*, *ATF4*, *FAS*, *FOS*, and *BIRC3*). Additionally, TL was associated with downregulation of 21 transcripts coding for collagens. The upregulation of proteases, along with the downregulation of collagens, is believed to be implicated in separation and rupture of the CA during TL. Furthermore, TL was associated with downregulation of transcripts coding for proteins essential for progestin synthesis (*SRD5A1* and *AKR1C1*) and angiogenesis (*VEGFA* and *RTL1*), as well as upregulation of prostaglandin synthesis-related genes (*PTGS2* and *PTGES*) that could reflect the physiological switch in placental endocrinology and function during TL. In conclusion, our findings revealed the equine CA gene expression signature in spontaneous labor at term that improves our understanding of the molecular mechanisms triggering labor.

Keywords: Mare, parturition, placenta, pregnancy, chorioallantois, transcriptome

Evaluation of chemiluminescent assays for canine progesterone and its clinical implications

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Detection dogs perform a vital role in national security. Currently there is a national shortage of domestic detection dogs within US, so it is important to maximize reproductive efficiency. Working with Auburn's Canine Performance Sciences Program, the goal of this study was to evaluate the relative accuracy of ovulation timing using 2 commonly used methods to determine serum progesterone concentrations and determine whether the differing values would influence clinical decisions. We hypothesized that progesterone concentrations measured at Auburn University's Veterinary Teaching Hospital (AUVTH) and measured at East Alabama Medical Center (EAMC) do not differ enough to alter the treatment plan for the patient. Two different chemiluminescent immunoassays were used. AUVTH uses the Immulite 1000 whereas the EAMC uses the Access CLIA. The complete estrous cycle of 4 dogs were followed from the start of proestrus to diestrus was detected using vaginal cytology. Data were analyzed statistically focusing on values of 1 - 10 ng/ml since these are the most clinically relevant. A Bland-Altman analysis and Passing Bablock regression were used along with a Wilcox signed rank test to analyze the data. Although progesterone concentrations increased over the course of the estrous cycle, there were significant differences between these 2 methods. For the Wilcox signed rank test, the median difference between the 2 methods was compared to zero and a p value of 0 was determined. As the values increased, the difference between them increased. The Bland-Altman highlighted the proportional systematic bias between the 2 methods. Mean difference between 2 methods was 1.29 with a confidence interval of 0.89 - 1.7. Because 0 does not fall in the confidence interval of the mean difference, the bias can be considered significant. The line of regression for the Passing Bablock has a slope of 1.2726 and y-intercept of 0.4074, which highlights the poor agreement between the two methods. In conclusion, the Access CLIA reported consistently higher values than the Immulite 1000. There was poor agreement between the 2 methods of measurement, with the Access machine reading an average of 1.29 ng/ml higher than the Immulite 1000. However, the Access CLIA has not been validated for use in the dog and should ideally be compared to a radioimmunoassay, where reference ranges for canine progesterone concentrations were determined to compare accuracy. Further evaluation is necessary to determine if this difference was substantial enough to influence clinical decisions. Findings suggested that it is possible. Lastly, currently there is no clinical way to confirm the time of canine ovulation. With the use of vaginal cytology, cytologic diestrus can help confirm the date of ovulation in retrospect and was the most reliable tool available for this study.

Metagenetic analysis of pregnant mare's placental microbiome

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Placentitis is the leading cause of infectious abortion in the horse and contributes to roughly 19% of all abortions in US. It continues to cause episodic abortions, in addition to weak and/or growth restricted offspring. It has been reported that the equine placenta harbors a unique microbiome as collected at term delivery from healthy mares. Aim was to identify the core microbial communities in various body sites of the pregnant mare in early pregnancy to describe a core microbiome that may be perturbed in pathologic pregnancies such as in placentitis. We hypothesized that the equine placenta harbors a distinct resident microbiome in early pregnancy when characterized by metagenetics and that there is a disparity in bacterial communities from the oral, vaginal and fecal microbiome. Furthermore, we believed metagenetics reveals distinct communities and indicator taxa that characterize healthy and dysbiotic states in the equine placenta. Eight pregnant pony mares (bred to the same stallion) were used between 40 - 120 days of pregnancy. Sample swabs were collected from oral cavity, vagina, anus, and the allantoic portion of the allantochorion. The V4 region of the 16S rRNA gene was amplified for Illumina Miseq sequencing to examine core bacterial communities present in various body sites. Microbial community composition of the pregnant ponies by body site differed ($p = 0.001$, PERMANOVA with Bray-Curtis dissimilarity of 16S Amplicon Sequence Variant's relative abundance). The allantois was different from feces ($p = 0.006$), oral cavity ($p = 0.02$), and the vagina ($p = 0.016$) using a PERMANOVA of Bray-Curtis dissimilarity. Using beta dispersion, a calculation based on centroid distance between samples, the allantois grouped most closely together with the vagina. Microbial communities within the feces were also similar to some vaginal samples, but the allantois and feces were significantly different from each other. Alpha diversity measuring Shannon diversity matrix

was different (p = 0.0002) with the body sites being a compounding variable meaning there was a difference in richness and evenness in the different microbial communities. When using Tukey's multiple comparisons, the allantois was most similar to the oral cavity when using alpha diversity alone. In conclusion, metagenetics revealed distinct community differences in the oral, fecal, vaginal, and allantoic cavities of the horse. Equine placenta had some similarities in its microbial communities to the oral cavity (alpha diversity) and the vagina (beta diversity). Further research is needed to investigate how bacteria are translocated to the placenta from these other body sites and how they contribute to the development of placentitis. We intend to develop a screening method using next generation sequencing to rapidly identify microbial community dysbiosis to monitor mares that could potentially develop placentitis.

Keywords: Mare, pregnancy, placenta, microbiome

Effect of corn-based diets on beef bull semen characteristics

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In modern production operations, bull development and maintenance nutrition are often unbalanced leading to detrimental effects on semen quality. There are unpublished reports recognizing a correlation between high corn-based feedstuff consumption and poor results on breeding soundness evaluations (BSE) in bulls. We hypothesized that the consumption of high corn-based diets negatively affect sperm by increasing morphologic defects, resulting in bulls having an unsatisfactory classification on a BSE. Healthy beef bulls were presented to veterinary teaching hospitals for BSE's were enrolled with mean age 42 months and average pounds/day corn-based diet consumed being 15 pounds. All bulls in this study were given free choice trace minerals. Angus was the most commonly presented breed (47%), followed by Brahman influence (23%), and Hereford (17%). A total of 8 bulls (16%) were classified as unsatisfactory and 39 classified as satisfactory with average normal cells being 72% and most common deformity a midpiece abnormality. Preliminary data were used to create a statistical model with results of BSE (unsatisfactory/satisfactory), age, weight, amount of corn product consumed and sperm abnormalities. Computed models were used to analyze and compare characteristics of age, weight, and corn product consumed (pounds/day) between bulls with a satisfactory/unsatisfactory BSE result. Statistical significance was set at $p<0.05$. No significant associations were established at this time ($p > 0.05$). However, with each 1 month increase in age, the odds of satisfactory classification on BSE decreased by 98.74% (CI 100 - 40.05%, p =0.25); for each unit increase in pounds/day of consumption of corn product, the odds of satisfactory classification on BSE decreased by 99.42% (CI 100 - 54.45%, p = 0.27); and for each unit increase in weight, the odds of satisfactory classification on BSE decreased by 8.03% (CI 24.54 - 1.07%, p = 0.25). Bulls maintained on a grass diet seemingly had fewer morphological defects analyzed at time of BSE. In conclusion, amount of corn-based product, age of bull, and weight had a negative relationship with satisfactory characteristics for a BSE. Additional data are needed to further assess effects of corn-based diet on semen morphology to make accurate and practical recommendations for producers.

Keywords: Bull, corn, sperm morphology

A case of penile denervation in a Brangus bull

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A 3-year, Brangus bull, was presented for evaluation of a preputial injury that had been managed medically for several weeks. At the second visit, the owner reported that the patient had a 50% pregnancy rate in the previous breeding season and had been observed having difficulty in achieving intromission. Based on the history and the owner's description of the bull's courtship and breeding behavior, impotence due to an inability to copulate (*impotentia coeundi*) was suspected. In order to better define the condition, a test mating was arranged to allow evaluation of the bull's ability to breed. Bull was placed in a 12 x 15 foot stall with a heifer in estrus. Bull initiated normal courtship behavior and had interest in the female. An initial mount was not accompanied by erection (false mount) and the bull resumed courtship behavior after dismounting. At the next mounting attempt, the bull achieved full erection and extension of penis. Bull was observed making normal searching motions with glans penis but failed to locate the vulva. Bull did not achieve intromission, and the erect penis eventually extended along the left side of the heifer before the bull dismounted. On a subsequent breeding attempt, bull mounted the heifer appropriately, initiated penile searching motions, probing the area of the escutcheon with the fully erect penis, but failed to achieve intromission, ultimately placing the erect penis along the right side of the heifer prior to dismounting. Observation of active searching with the glans penis accompanied by failure to achieve intromission with the fully erect penis is diagnostic of damage to the dorsal nerve of the penis. Supplemental testing of nerve conduction may be used to confirm the diagnosis, although the equipment and expertise required is seldom available to private practitioners. In this case, the owner declined further diagnostics, and chose to salvage the bull at slaughter. An alternative method of determining dorsal penile nerve function is via a bovine artificial vagina after proper stimulation and mounting are allowed.[1] However, lack of ejaculate via artificial vagina method could also indicate inadequate stimulation from female exposure, that the artificial vagina does not match the bull's preference, or musculoskeletal pain during mounting.[1] This method may also not be definitive since the more caudal portions of the penis may still be adequately stimulated if the penis is inserted far enough into the artificial vagina. Penile denervation should not interfere with erection or production of viable semen.[2,3] Affected bulls of sufficient value can have semen collected via electroejaculation for cryopreservation.[2,3]

Keywords: Penile denervation, preputial prolapse, test mating, Brangus

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Hydroallantois as a result of a Longhorn and bison mating

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A 6-year, Longhorn domestic cow (*Bos taurus*), was presented for acutely nonambulatory and recumbent condition. Cow had extreme bilateral abdominal distension and had been recumbent for ~ 5 hours. It was determined that cow was in active labor. It was suspected that cow had been bred by a bison (*Bison bison)* bull. Delivery was assisted, and an extreme volume of serous fluid was expelled from the uterus. Other than the large amount of fluid expelled there were no complications with the delivery and a live and seemingly healthy heifer calf was delivered. A diagnosis of dystocia due to hydroallantois was determined. Hydroallantois is a condition that complicates 1:7,500 pregnancies.[1] In uncomplicated pregnancies, the volume of allantoic fluid is ~ 15 liters,[2] whereas in pregnancies complicated by hydroallantois there can be up to 175 liters of fluid in the allantoic cavity.[3] Hydorallantois occurs due to ineffective placental membranes.[4] Supportive care was given including intravenous boluses of hypertonic saline and plasmalyte, intramuscular injections of dexamethasone, and assistance in standing. After 4 days of hospitalization with no

improvement she was euthanized and a necropsy performed. Relevant necropsy findings were consistent with severe adventitious placentation and a venous thrombus occluding the caudal vena cava. Adventitious placentation is characterized by abnormal placentome development, decreased numbers of placentomes, and decreased vascularization of the placenta.[1] Pregnancies sired by a bison bull with a *Bos taurus* dam, result in abortions in 1:1.5 confirmed pregnancies.[5] Of the live births, 90% were female.[5] Abortions occurred in 7% of confirmed pregnancies sired by a *Bos taurus* bull to a bison cow, of the live births there was a 1:1 sex ratio.[5] Most common cause of abortion in bison and domestic cattle crosses has been hydramnios.[6] Hydramnios has more viscus fluid (25 liters).[7] In uncomplicated pregnancies the amnionic fluid is between 3 - 5 liters.[7] Hydramnios is caused by a lack of fetal ingestion of the amnionic fluid usually associated with fetal facial deformities.[8,9] There has been no evidence of facial deformities in historical records kept of attempted crosses between a bison bull and *Bos taurus* cow. Similar issues with adventitious placentation leading to hydroallantois, as commonly observed in abnormal offspring syndrome, have been associated with a lack of epigenetic processing of genes important to placental growth on the Y-chromosome.[10,11] Failure of hybrids are normally attributed to dissimilarities of the karyotypes of the parent species, the only structural difference between the karyotypes of the domestic cattle and bison are that of the Y-chromosome.[12] In bison, the Y-chromosome is acrocentric whereas in domestic cattle it is metacentric.[12] Therefore, it has been hypothesized that bison bulls lack a gene on the Y-chromosome critical for placentation that domestic cattle possess. It is also hypothesized that the bison cow has a gene that negates the importance of the gene that regulates placental growth on the Y-chromosome.

Keywords: Hydroallantois, adventitious placentation, Bison cross

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A case of suspected vaginal hyperplasia in a Chihuahua

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Vaginal hyperplasia is often associated with younger intact females.[1] A 10-year, recently spayed female Chihuahua, was referred for evaluation of suspected vaginal hyperplasia. Patient was suspected to have pyometra and was spayed 3 days prior to presentation. Prolapsed tissue became evident through the vulva 1 day after surgery. Initial differential diagnoses included vaginal prolapse, vaginal hyperplasia, or vaginal neoplasia. Although initially suspected by the referring veterinarian, the likelihood of vaginal hyperplasia in this patient was low based on the history. Vaginal hyperplasia is more often associated with younger intact females and under the influence of estrogen. Suspected pyometra indicated that the patient was likely in diestrus and under the influence of progesterone. Patient had a body condition score of 9/9 and a 2 cm in diameter section of tissue was protruding through the vulvar lips. On further evaluation, the tissue appeared well pedunculated and originated from the ventral vaginal wall. Prolapsed tissue appeared dry and black in color, suggesting necrosis. Due to the tissue originating from the ventral vaginal wall and the urethra identified in a normal location, it became less likely to be a vaginal prolapse or hyperplasia. Vaginal neoplasia became the most likely diagnosis. Patient was sedated with hydromorphone (0.1 mg/kg) and intravenous dexmedetomidine (375 μg/m^2). Stalk of the protruding tissue was clamped with Crile hemostatic forceps and transected near the base of the mass with electrocautery, taking care to avoid iatrogenic damage to the vaginal epithelium. Once the initial mass was removed and the patient relaxed under sedation, a second, smaller mass was identified near the same location. Second mass was also transected and both were submitted for histopathology. Dexmedetomidine was reversed with atipamezole (375 μg/m^2) and the patient recovered uneventfully. Two most common vulvovaginal neoplasms include leiomyoma and leiomyosarcoma. Ninety percent of cases are benign leiomyoma.[2,3] Histopathological evaluation of the mass revealed subepithelial fibrous stroma with a densely cellular, nonencapsulated neoplasm. Vaginal mucosa was extensively ulcerated with intralesional coccoid bacterial colonies. Pathologist confirmed a diagnosis of leiomyoma. Leiomyoma is a benign neoplasia affecting the muscle cells within the vulva. It is often a solitary lesion in spayed females, but may be diffuse and hormonally responsive in intact females.[4,5] As performed in this case, primary treatment for leiomyoma is local excision

and ovariohysterectomy. This case identified the importance of taking a complete and thorough history as the smallest piece of information (e.g., reason for spaying) can be the key to narrowing differentials and for accurate diagnosis.

Keywords: Dog, vaginal neoplasia, leiomyoma, vaginal hyperplasia

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Medical treatment of pyometra in young queens

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Two intact female cats from the same cattery were presented with vaginal discharge. A 10-month, intact Siamese female, was presented with purulent vaginal discharge without a history of breeding. Abdominal ultrasonography revealed 0.7 cm of fluid within uterus. Pyometra was diagnosed and treatment consisted of enrofloxacin (15.6 mg once daily subcutaneously [SQ] for 7 days) and dinoprost (lutalyse; 0.3 mg twice daily intramuscularly [IM] for 2 days). Lutalyse was discontinued on uterine fluid clearance. Patient remained alert, responsive, and afebrile throughout treatment. Queen recovered adequately and was placed with a male 1 month later. She delivered 7 healthy kittens. A second 1-year queen presented with vaginal discharge after she was with a male. On ultrasonography, multiple fluid filled loops of uterus with 0.5 - 1 cm flocculated fluid was noted. Moderate degenerated neutrophils were noted in vaginal cytology. General physical examination was normal. Pyometra was diagnosed and the patient was started on enrofloxacin (9.5 mg IM once daily) and lutalyse (0.19 mg SQ twice daily for 7 and 2 days). Fluid within the uterus decreased daily and completely resolved after 48 hours. Enrofloxacin was continued for 14 days. Final examination after a week revealed normal sized uterus with no discharge. Queen was placed with a male 1 month later, bred successfully, and gave birth to 4 healthy kittens. Pyometra is either acute or chronic inflammation of the uterine wall with accumulation of purulent material resulting from cystic endometrial hyperplasia (CEH).[1] In queens, incidence of pyometra is lower than in bitches because queens are induced ovulators.[2] Over time, prolonged secretion of progesterone leads to endometrial hyperplasia, and bacterial contamination leads to pyometra.[1] In some reports, female cats housed alone or without an opportunity to copulate experienced pyometra that suggested spontaneous ovulation.[1] In the first queen, spontaneous ovulation is suggested as the underlying cause of the patient's pyometra because there was no male. This queen was able to successfully breed and had 3 more litters after her first litter. Abdominal ultrasonography or radiography are indicated to determine uterine size and shape and to rule out pregnancy.[1] Bacterial culture and measurement of serum progesterone concentrations supported a diagnosis of pyometra.[1] *Escherichia coli* is the predominant bacterium present in pyometra; however, more than 1 species may be present and some cultures may be negative.[3] For reproducing females, standard treatment of pyometra involves multiple injections of prostaglandin to evacuate purulent material from the uterus, plus antibiotic therapy.[1] Prognosis for reproductive success following medical treatment depends on the severity of endometrial pathology[1] and response to treatment. Although treatment may be successful in queens, reoccurrence rate is 70% within 2 years.[4] These cases are important because pyometra is commonly observed in older animals that have had exposure to progesterone causing uterine pathology. In these young cats, pyometra was not due to uterine pathology and hence a higher success rate.

Keywords: Cat, pyometra, cystic endometrial hyperplasia, vaginal discharge

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Disorder of sexual development in a Chinese Crested dog with mixed testicular tumors

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A 10-year, intact female Chinese Crested dog, was presented for evaluation and further diagnostics due to persistent symptoms of vulvar swelling and vaginal discharge, with an 8-year

history of acyclicity. Generalized hyperpigmentation and truncal alopecia were identified, along with no aberrations of the female phenotype. Vaginal cytology at multiple previous veterinary visits confirmed the influence of estrogen. Positive assay results for anti-Müllerian hormone confirmed gonad presence. Exploratory abdominal laparotomy was performed and gonadal tissue and associated tubular structures were submitted for histopathology. Histopathologically, gonads were identified as abnormal testes containing Sertoli and interstitial (Leydig) cell tumors. Histopathologic diagnosis of testes and concurrent normal external phenotypically female genitalia in the patient led to a diagnosis of a disorder of sexual development (DSD). Karyotype evaluation was pursued, and the molecular analysis revealed a mosaic pattern of XX (80%) and XY (20%) cells among the blood lymphocytes, and PCR test was positive for the Y-linked *SRY* gene. Skin biopsy karyotype (to determine if this is a case of XX/XY leukocyte chimerism or a case of whole body XX/XY mosaicism) is pending. Hair follicles are currently examined for *SRY* status. This is the first known case report of a canine with a karyotypic diagnosis of mixed sex chromosomes with completely normal female phenotypic external genitalia. This patient was diagnosed with a DSD at the chromosomal level as a suspected blood chimera or whole body XX/XY mosaicism. The pathophysiology of this DSD is from *SRY* gene expression on the genital ridge during sexual differentiation to begin testicular differentiation. Whereas male external genitalia development is hormonally driven, the female phenotypic development is not hormonally driven.[1] In this patient, it was suspected that an aberration in the process of testicular development led to an androgen deficiency, allowing the external genitalia to develop as female. Clinical signs that led to the patient's specialty referral were due to the patient's abdominally retained testes and resultant hormonally active Sertoli cell tumors that caused a feminizing paraneoplastic syndrome.[2,3] Subsequently, a diagnosis of a DSD was also made. After recovery from surgery, the patient's clinical signs have completely resolved. This case illustrated a distinct presentation for hormonally active Sertoli cell tumorigenesis and demonstrated that surgery is curative for feminizing paraneoplastic syndrome.

Keywords: Dog, disorder of sexual development, chimerism, mosaicism, Sertoli cell tumor, Leydig cell tumor

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Cervical wedge resection treatment in a pyometra mare

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A 15-year, pony mare, with no known previous history was obtained by the current owner. During a routine pregnancy examination (exposed to a stallion), she had intrauterine fluid and purulent vulvar discharge. Further treatment was declined, and she returned to normal, nonbreeding activity. Approximately 4 months later, she was represented with excessive vaginal discharge. Distended uterus (12.5 cm) with purulent fluid in the vaginal canal and throughout the uterine body and horns were detected via transrectal palpation and ultrasonography. Digital examination of the cervix revealed excessive transluminal adhesions and lack of patency. Adhesions were broken manually and a catheter was passed through the cervix. Five gallons of purulent material was drained and a uterine lavage was performed to clear the purulent material from uterus. A Gram stain and culture were performed on a sample from the collected material. Gram stain had substantial number of Gram-positive cocci; however, aerobic culture results were negative. Uterine lavages were continued daily with dilute betadine solution (0.05%) until the fluid returned clean. Four days after presentation, the mare was placed in a set of stocks and sedated with butorphanol (3 mg) and detomadine (3 mg) and an epidural containing carbocaine (40 mg) and xylazine (50 mg) was placed. Cervix was retracted caudally using stay sutures and a triangular section of the dorsal portion of the caudal cervix (~ 2/3 of the cervix) was removed. After surgery, intramuscular ceftiofur crystalline-free acid (3,000 mg) was given. Lanolin-based ointment containing dexamethasone and oxytetracycline was applied to cervix to prevent adhesion formation. Ointment was applied every 3 - 4 days for 2 weeks along with manual breakdown of adhesions. A month after surgery, the mare's cervix had healed adequately. Digital examination of cervix revealed that the resection was still open and the cervix was patent. Minimal fluid (via transrectal ultrasonography) was present in the uterus. Surgery was considered a success and the mare has returned to her athletic function with no clinical symptoms associated with uterine distension since the cervical wedge resection. Pyometra is often caused by poor perineal conformation or cervical adhesions preventing drainage of the infected uterus and resulting in purulent fluid accumulation.[1-4] Traditional treatment of fluid accumulation caused by pyometra involves uterine lavage, intrauterine antibiotics, and continual breakdown of cervical adhesions to maintain patency.[1-4] This method is often used in mares planned for breeding. Without proper maintenance of a patent cervix, the animal is susceptible to repeat infections or failure of the uterus to adequately drain resulting in recurrent accumulation of fluid.[2] A cervical wedge resection performed on mares that did not respond to traditional therapies or are no longer desired for breeding enables the cervix to remain permanently open and any fluid to freely drain, thereby preventing subsequent fluid accumulation.[2] This method can be used as an alternative option to ovariohysterectomy as abdominal surgery on mares

with pyometra increases risk of contamination within the abdomen that may result in severe complications.[2,5]

Keywords: Mare, pyometra, cervical wedge resection, cervix

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In utero diagnosis of bilateral cataracts and hydrops in a mule pregnancy

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A 17-year, Thoroughbred mare, was bred to a donkey jack. A routine monthly transrectal ultrasonography was performed during pregnancy. At 186 days of pregnancy, the fetus was abnormally located deep in the mare's abdomen. Only a large amount of fetal fluid could be imaged transrectally. Two weeks later, at 200 days pregnancy, the head was detected transrectally via ultrasonography, having an eye with a hyperechogenic lens, suggestive of congenital cataract. Transabdominal ultrasonography revealed several fetal abnormalities, such as small biparietal diameter and small eye volume consistent with intrauterine growth retardation (IUGR), and hyperechogenic bowels. Fetal heart rate was within the normal limit but on the lower side of the range. As pregnancy advanced, IUGR became more pronounced and more abnormalities were observed with an abnormal twisted umbilical cord, hyperechogenic medulla of the right kidney, posterior presentation at 240 days of pregnancy, and an increased maximum fluid depth. Additionally, amniotic and chorioallantoic membranes were juxtaposed with a minimal amount of allantoic fluid, suggesting a hydrops amnion condition. Fetus was identified dead by the loss of heartbeat at 272 days without clinical signs of impending abortion in the mare. Amniotic membrane was punctured with a thoracic trocar, and ~ 20 liters of fluids were slowly evacuated. Fetus was manually delivered in a posterior presentation, with extensive umbilical cord twists. Fetal membrane had an enlarged avillous area at cervical star and fetus had bilateral congenital fetal cataracts and abnormal right kidney. Histologically, the right kidney had a severe diffuse lobulation/malformation, and there was lymphocytic inflammation in the upper esophagus. This is the first report of an in utero diagnosis of fetal congenital cataracts associated with hydrops, highlighting the importance of ultrasonographic assessments of pregnancy and fetal well-being.

Keywords: Mare, congenital cataracts, hydrops condition

Successful pregnancy in a dog treated for a deep surgical infection with abdominal wall abscess after surgical artificial insemination

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Dogs are bred via natural mating or artificial insemination (AI). Methods of AI include transvaginal, transcervical, and surgical. Surgical AI is frequently performed with either frozen or poor-quality semen, in cases of previous infertility, or on an elective basis.[1] As in any surgical procedure, there are potential complications after surgical AI, including postsurgical infection, unsuccessful fertilization, and complications associated with anesthesia. A 3-year, Labrador Retriever bitch, was presented to the emergency service 2 days after surgical insemination for anorexia and lethargy. Dog was pyrexic, lethargic, tachycardic, and a diffuse cellulitis with associated pain on palpation of her incisional site were noted. A complete blood count had severe leukocytosis characterized by a mature neutrophilia, eosinophilia, and monocytosis. Abdominal radiography and ultrasonography confirmed soft tissue swelling and fluid accumulation around the incisional site. Septic exudate was aspirated via ultrasonography guided technique. After initial stabilization with intravenous fluids, hydromorphone and gabapentin for analgesia, and ampicillin/sulbactam as an antimicrobial, surgical exploration was performed and confirmed a deep surgical site infection of subcutaneous tissue and an abdominal wall abscess communicating with the abdominal cavity. Surgical debridement with peritoneal lavage was performed and the abdomen was closed after placement of an active Jackson-Pratt® drain that was removed after 4 days. Skin and subcutaneous layers were left open for 3 days for a vacuum-assisted closure to allow secondary wound closure. Continued medical support consisted of gabapentin, hydromorphone, carprofen, and intravenous ampicillin/sulbactam. Microbial culture was positive for *Staphylococcus pseudintermedius* sensitive to beta-lactamase inhibitors. Clinical status improved quickly and the patient started eating well on day 2 after surgery. She was discharged 7 days after admission on oral carprofen and amoxicillin-clavulanic acid. Approximately 1 month after discharge, her incision site was healed, and she was diagnosed pregnant (medium to large litter size) via abdominal ultrasonography. At 66 days after estimated luteinizing hormone surge, she successfully whelped and subsequently raised 7 viable pups without complication.

Keywords: Dog, surgical insemination, abscess, pregnancy

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Postparturient metritis in a primiparous Belgian mare

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Metritis is a common postpartum complication in domesticated species, including horse. Although all equine breeds are affected by metritis, it appears that Thoroughbred and Belgian horses are more severely affected by this disease. If left untreated, metritis can be life-threating and result in laminitis. Retained fetal membranes and dystocia are common predisposing factors to metritis, but mares with an otherwise normal parturition and release of fetal membranes can also develop the condition. Clinical signs include fever (typically > 102.5°F); depression, tachycardia, purulent vulvar discharge, and secondary laminitis in advanced cases. Treatment consists of a combination of broad-spectrum antibiotics, nonsteroidal antiinflammatory drugs, uterine lavage, ecbolics, and in severe cases, fluid therapy. A 4-year, primiparous Belgian mare, delivered a filly uneventfully at 340 days of pregnancy. Fetal membranes were passed completely 1.5 hours postpartum, and the filly stood and nursed by 2 hours postpartum A dose of flunixin meglumine (1.1 mg/kg) was given intravenously to prevent any foaling induced pain. By 2 hours postpartum, the mare had mild signs of foal rejection (stall walking, pinning her ears at the foal, and not standing to nurse) that was mitigated following treatment with intramuscular cloprostenol (500 µg) and butorphanol (10 mg) treatment. Twenty-four hours postfoaling examination was unremarkable. By 48 hours after foaling, the mare had fever (103.7°F; 99 -101.5°F), tachycardia (60 beats/minute; 28 - 44 beats/minute), tachypnea (30 breaths/minute, 10 - 24 breaths/minute) and mucoid vulvar discharge. Uterine lavage was performed with 0.1% iodine solution and the fluid recovered had abundant strands of fibrin and debris. The mare was given intravenously potassium penicillin G (22,000 units/kg, every 6 hours), gentamicin (6.6 mg/kg, every 24 hours), and oral metronidazole (15 mg/kg, every 8 hours) for 5 consecutive days. Intravenous fluid therapy (lactated Ringers, 3 liters/hour) was initiated. A commercial multi-electrolyte solution (CMPK, containing calcium; magnesium, phosphorus and potassium was included at 50 ml/hour) and oxytocin (4 units/hour) were given via constant rate infusion for ecbolic action to aid in uterine clearance. Other treatments included flunixin intravenous meglumine (1.1 mg/kg, every 12 hours), oral omeprazole (4 mg/kg every 24 hours) and probios (60 grams loading dose and then 30 g every 24 hours). Mare developed a slightly increased digital pulse and temperature that were treated with distal limb cryotherapy for 48 hours. Uterine lavage (80 liters/session) was repeated 3 times/day in the first 72 hours, and then reduced to 2 times a day from 96 to 120 hours; and then once daily from 144 to 168 hours postpartum. Clinical signs progressively improved starting 12 to 96 hours postpartum. Mare was discharged after the last treatment with sulfadiazine oral trimethoprim (24 mg/kg, every 12 hours) for 7 days and intramuscular oxytocin (20 units, every 12 hours). Breeding the mare on foal-heat was strongly discouraged. Mare was reexamined by the referring veterinarian at the '30-day heat'. Mare was rebred, diagnosed pregnant at 15- and 25-days postovulation. Prognosis for metritis can be poor for draft mares due to secondary laminitis. In the present case, early diagnosis and aggressive intervention resulted in successful therapy for metritis.

Persistent Müllerian duct syndrome in a German Shorthair Pointer

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A 2-year, intact male, German Shorthair Pointer, was presented for a routine castration. Prepuce was located further caudal than normal, and penis was malformed and hypoplastic. Scrotum was not present, and testes were not appreciable on palpation. Cryptorchidism was suspected. A ventral midline laparotomy was performed to remove suspected retained testes. In the abdomen, there was a structure that resembled a uterus, with uterine horns and a testis at the end of each horn. This structure was removed; histopathology confirmed that the reproductive tract had bilateral testes, both with an epididymis that was attached to a hypoplastic uterus. Testes had a pampiniform plexus and were composed of seminiferous tubules that lacked spermatogonia, but had Sertoli and Leydig cells. Each testis was connected to Müllerian and Wolffian duct systems. Uterine tissue was devoid of glands. Although karyotyping was not performed to confirm the diagnosis, clinical findings and histopathology were consistent with Persistent Müllerian duct syndrome (PMDS), a form of male pseudohermaphroditism. During normal fetal development, Müllerian inhibiting substance (MIS) is secreted and causes regression of the Müllerian ducts. In male dogs with PMDS, there is a genetic defect in MIS or its receptor, MISRII, so the Müllerian ducts persist. Müllerian ducts are responsible for development of the uterus, fallopian tubes, and cranial vagina. With PMDS, these normally female structures are present in a dog that is phenotypically male.[1] Persistent Müllerian duct syndrome is a heritable, autosomal recessive trait primarily observed in homozygous male Miniature Schnauzers and is considered rare but has been reported in other breeds.[2] A gonadectomy, as performed in this case, is recommended in dogs with PMDS to prevent further reproductive disorders such as pyometra, urinary tract infection, or prostate infection.[3]

Keywords: Dog, Persistent Müllerian duct syndrome, PMDS, pseudohermaphrodite

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Anencephaly in a French Bulldog pup

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Neural tube defects (NTDs) are the second most common neonatal abnormality reported in human medicine. In veterinary medicine, 3 - 25% of neonatal mortality in livestock and companion species has been associated with developmental anomalies. It is not unusual that French Bulldogs are reported to possess NTDs in the form of vertebral column abnormalities, including spina bifida. However, to date, there have not been any reported cases of the NTD identified as anencephaly in the French Bulldog. An intact, 4-year, female French Bulldog, 60 days into pregnancy with her third litter for a prepartum management of an elective cesarean surgery. All previous litters were healthy and fully developed upon delivery. Dog's ovulation date had previously been determined to be October 30, 2021, via serum progesterone concentrations; a transcervical insemination was subsequently performed with chilled semen on day 2 after ovulation. On day 60 of pregnancy, the bitch appeared normal in physical examination and had marked mammary development. A transabdominal ultrasonographic examination of the uterus was performed; multiple fetuses were visualized with heart rates within normal limits (190 - 220 beats/minute), indicating that the fetuses were not distressed. All visualized fetuses had good cortico-medullary definition of the kidney and detailed intestinal definition, although peristalsis was only seen in 2 fetuses. Shortly thereafter, serum progesterone concentrations were 2.0 ng/ml and the patient was prepared for Cesarian surgery. One pup was diagnosed with the more common NTD of a cleft palate that extended the entirety of the hard and soft palates. However, the second pup had less common NTD in that it was missing the dorsal aspect of its skull and cerebrum, leaving the entirety of the eye globes exposed along with what little portion of brain matter existed. A diagnosis of anencephaly was made based on macroscopic observation. Both pups were subsequently euthanized, leaving 5/7 completely healthy and viable puppies. At the owner's request, ovariohysterectomy was performed concurrently with the cesarean surgery. Recovery from anesthesia was uneventful, with recently nursed pups and bitch were discharged. Neonatal abnormalities are undesirable in any litter. However, until corresponding factors have been identified, clients and practitioners need to be aware that developmental abnormalities of varying degrees can occur. Cleft palates, umbilical hernias, and atresia ani are common conditions that every veterinarian should routinely inspect neonates for prior to discharge. In humans, it is estimated that 1 in every 4,600 infants is afflicted with the NTD of anencephaly. The specific cause of anencephaly in individual cases are unknown but are thought to include chromosomal abnormalities, environmental factors, folic acid deficiency, or exposure to teratogenic medications. Recommendations for pet owners in these cases may also include avoidance of a repeat breeding between the impacted litter's dam and sire. This case illustrated that clients and practitioners should be prepared for varying degrees of neonatal abnormalities, even if previous reports may not exist.

Keywords: French Bulldog, anencephaly, neural tube defect

Iatrogenic foreign body in urinary bladder secondary to vaginal cytology procedure

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A 2-year, multiparous Golden Retriever bitch, was presented for breeding management. Dog had bloody vaginal discharge for 5 days. During vaginal smear collection the bitch unexpectedly jumped and the tip off of the wooden cotton swab was broken and it was not palpable in the caudal vagina nor was not seen on the floor. A rigid endoscope was used to perform a vaginoscopy, but the missing segment of the cotton swab was not visualized. Abdominal ultrasonography revealed a normal, small, hypoechoic bladder with no evidence of foreign material. Dog was taken outside to confirm that she was able to urinate. Dog was sent home with instructions to monitor for changes in behavior until her next scheduled appointment in 2 days. Owners returned with the bitch 2 days later and reported that she was urinating normally the day following the accident but had started to experience discomfort when urinating within 12 hours after presentation. Transabdominal ultrasonography was performed again that now revealed a linear hyperechoic structure in the lumen of the bladder. Dog was placed under general anesthesia and underwent a cystoscopy. Foreign material (broken cotton tip of the swab) located in the trigone of the bladder was removed. There was moderate urinary bladder and urethral trauma from the inadvertent catheterization of the urethra and the splintered wooden shaft of the cotton tipped applicator freely floating within the urinary bladder. Dog was sent home with carprofen 2.2 mg/kg and amoxicillin 15 mg/kg to help manage inflammation and prevent infection from the procedures. Progesterone concentrations were monitored and had a normal profile. Dog was bred via transcervical insemination with frozen semen 5 days after cystoscopy and pregnancy confirmed via transabdominal ultrasonography at 30 days of pregnancy. She then went on to have 8 healthy pups delivered via caesarean surgery. Cotton tipped applicator was probably broken off within the urethra during the vaginal cytology procedure and traveled (retrograde) to the urinary bladder. We suspect that the tip was not visualized in the initial ultrasonography performed immediately after it was broken was most likely due to the limitations of visualizing the pelvic urethra. However, the combination of an attentive owner and subsequent ultrasonography enabled prompt removal of the tip of the swab without any detrimental effects on the bitch's health and fertility.

Keywords: Dog, cystoscopy, vaginoscopy, vaginal cytology, foreign body

Cryptorchidism and laparoscopic assisted cryptorchidectomy in Nigerian dwarf goats

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Cryptorchidism is reportedly uncommon in small ruminants. Objective was to describe clinical features and laparoscopic assisted cryptorchidectomy in 18 Nigerian dwarf goats presented at the WSU Theriogenology Services. All goats were companion animals and ranged from 2 months to 1 year. There were 15 (83%) unilateral and 3 (17%) bilateral cryptorchids. Two of 3 bilateral goats were purchased as wethers. Owners were concerned when they began to display male like behavior and odor at ~ 6 months of age. The remaining bilateral goat was presumably castrated by banding at 1 week of age. Of the 15 unilateral, 11 retained right testis and 4 retained left. In this case series there were 3 full brothers (2 bilateral, 1 unilateral) and 5 half-brothers (same sire). Retained testis were located intra-abdominally near the bladder, via transcutaneous inguinal ultrasonography with curvilinear 3.5 - 5 MHz probe. Retained testis length and width were 21.8 ± 2.1 mm and 18.3 ± 1.5 mm, respectively (mean ± SD). For laparoscopic assisted cryptorchidectomy, goats were fasted for 18 hours. After sedation with butorphanol, anesthesia was induced with propofol and midazolam and maintained with isoflurane in oxygen. Animals were placed in dorsal recumbency, and the scrotum and ventral abdomen prepared aseptically. A 1 cm skin incision was made ~ 4 - 6 cm lateral to midline, contralateral to the side of the retained testis. Abdominal cavity was insufflated with medical grade CO_2 through a teat cannula prior to insertion of a 6 mm trocar and canula which served as the laparoscope portal. Patient was placed in Trendelenburg position to visualize testis. A second incision was made parallel to the inguinal ring on the side of the retained testis. Babcock forceps were introduced into the second portal and testis was grasped at the level of the gubernaculum. Incision length was extended to allow testis exteriorization. Spermatic cord was transfixed with 0 PDS suture and testis was excised with Metzenbaum scissors. Incision was sutured in 2 planes with 2-0 PDS to close the body wall with a simple continuous pattern and skin with an intradermal pattern. A single cruciate suture was used to close the laparoscope portal incision. Scrotal testis was castrated using a closed technique. For bilaterally cryptorchid cases, both testes were removed from the same paramedian instead of inguinal incision. There were no surgical or postsurgical complications. This case series suggested that cryptorchidism, may be more common and possibly hereditary in Nigerian dwarfs. Cryptorchidism may be missed by owners during castration. In companion goats, cryptorchidism is undesirable because of the male behavior and odor. Laparoscopic assisted cryptorchidectomy is minimally invasive and is highly recommended for small sized animals.

Keywords: Goat, laparoscopy, testicular descent, inguinal, ultrasonography

Uterine hemangioma in an alpaca (*Vicugna pacos*)

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Uterine hemangioma has been described in women, but occurs rarely in animals. Here we describe the clinical and pathological features of a case of uterine hemangioma in an alpaca. A 10-year, multiparous female, was presented with a complaint of postmating hemorrhage. Alpaca had undergone a term cesarean surgery, due to uterine torsion, 8 months prior to presentation. Physical examination parameters were within normal limits. On transrectal ultrasonography, the uterine wall appeared thickened and lined with several round echogenic areas 9 - 13 mm in diameter. A 4 mm cyst was visualized in the middle of the left uterine horn. Right ovary was static, whereas left ovary had multiple small follicles. Vaginoscopy did not reveal any abnormalities, and cervix was open. Alpaca was sedated with intramuscular butorphanol tartrate (0.05 mg/kg) and hysteroscopy was performed using a 9 mm pediatric gastroscope. Body of the uterus and uterine horns had several raised congested areas. Two biopsies were obtained from the lesions through the working channel of the endoscope. Examination was halted because of bleeding following biopsy. Alpaca was treated with flunixin meglumine and long acting ceftiofur, then placed under observation overnight. Blood was observed dribbling from the vulva but stopped after 4 hours. Biopsies taken by hysteroscopy were nondiagnostic. The owner declined further diagnostics and the animal was discharged with instruction not to breed her until another examination was performed. One year after presentation the animal was euthanized because of severe respiratory distress. No necropsy was performed; however, the uterus was submitted for examination. Full thickness histological preparations were made of the raised lesions and surrounding tissue. Uterine hemangioma was diagnosed based on presence of irregularly shaped cavernous vascular spaces in the endometrium and between myometrial fascicles. Clinical and pathological description of uterine hemangiomas is scarce in veterinary medicine. In humans, the condition can be congenital or acquired and is associated with menorrhagia and pregnancy complications. This case may have been acquired. Hysteroscopy should be considered to further investigate lesions observed on ultrasonography.

Keywords: Alpaca, neoplasia, infertility, uterus, hysteroscopy

Sertoli cell tumor in a cryptorchid dog

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Cryptorchidism is a relatively common condition in male dogs that can predispose the animal to neoplastic development and feminizing paraneoplastic syndrome. A 6-year, male castrated Boxer, was referred for evaluation of chronic generalized hair loss and thickening of skin on its chest and abdomen; pet owner also informed that dog had only 1 scrotal testis and was removed at 6 months. Initial evaluation (dermatology service) revealed symmetrical alopecia and seborrhea, lichenification of scrotal skin and an edematous, pendulous prepuce. Further evaluation (theriogenology service) revealed an enlarged and elongated nipple; cytology of penis/prepuce revealed cornified epithelial cells. A brief focal transabdominal ultrasonography was performed and a 4 - 5 cm globoid mass with heterogenous echogenicity was imaged on the left abdomen, ventrocranial to the urinary bladder; the prostate length and width measured 3.2 and 2.8 cm, respectively. Surgical removal of the abdominal mass was recommended, and the surgery was scheduled for 4 days atter initial diagnosis of cryptorchidism and presumptive Sertoli cell tumor. A preoperative comprehensive abdominal ultrasonography further detected the presence of medial iliac lymphadenopathy and splenic nodules. Fine needle aspirates were obtained from the enlarged medial iliac lymph node and spleen; the splenic cytology had hyperplasia and low-grade extramedullary hematopoiesis, and the lymph node cytology, though poorly cellular, had no evidence of neoplastic cells. Thoracic radiographs revealed no abnormalities. Complete blood cell count and serum chemistry had a stress leukogram and hypercholesterolemia, but it was otherwise unremarkable. During surgery, the left cryptorchid testis and a mildly enlarged medial iliac lymph node were removed. Histopathology of these tissues confirmed the mass to be a Sertoli cell tumor and the lymph node had no evidence of metastasis. Serum inhibin B concentrations decreased from 805 pg/ml prior to surgery to < 6 pg/ml 3 weeks after the surgery, and anti-Müllerian hormone (AMH) decreased from 8435 ng/ml to 56 ng/ml during that period. Patient recovered uneventfully but it was not brought in for an abdominal ultrasonography to rule out potential abdominal metastasis at 3 months after surgery. Pet owner reported the dermatoses began to subside by 2 months after surgery and only the 2 cranial thoracic nipples remained mildly enlarged. This report highlighted the practical and diagnostic value of penis/prepuce cytology, brief focal ultrasonographic examination and measurement of serum inhibin B and AMH concentrations in diagnosing cryptorchidism and a Sertoli cell tumor in dogs.

Keywords: Dog, sertoli cell tumor, cryptorchidism, inhibin B, anti-Müllerian hormone

Fetal mummification in a goat

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Fetal mummification occurs in most species; however, is not a common finding in goats. Not much is published in goats. A 5-year doe, 1 month after her expected day of kidding, was presented with a history of vaginal discharge and fetal membranes hanging from her vulva. She was bright alert and responsive, and had increased heart rate, elevated respiratory rate and effort along with decreased ruminal contractions. Transabdominal ultrasonography was nondiagnostic. Bloodwork had multiple electrolyte abnormalities with elevated lactate. Vulva appeared hypoplastic and urethra felt firm on palpation. Reddish-brown mucoid discharge was noted and fetal membranes were palpable. Transrectal ultrasonography revealed a fetus with bony structures resembling thorax with no heartbeat. Cesarean surgery was performed via a left flank approach and a mummified fetus was extracted from the left uterine horn.

Keywords: Goat, mummification, Cesarean surgery

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Umbilical torsion abortion in a multiparous quarter horse mare

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Umbilical cord torsion is the main cause of abortion in the United Kingdom; however, in US it accounts for a small fraction of abortions. A 21-year, multiparous Quarter Horse mare, was presented for evaluation postabortion with an accompanying 8-month fetus. One week prior to abortion, the mare had an apparently unremarkable transrectal ultrasonographic evaluation. The mare's past breeding history included susceptibility to persistent breeding induced endometritis and enlarged pendulous uterus. She was up to date on vaccinations, deworming, and had no history of disease. Initial physical examination was within normal limits. Udder was moderately developed, and milk was present. Transrectal palpation and ultrasonography revealed a distended uterus with decreased tone and a large cyst in the uterine body. Vaginal examination revealed moderate bruising inside the vestibule and a mild amount of straw-colored fluid in the uterine body and cranial vagina. Evaluation of aborted fetus suggested that the fetus was slightly small for its fetal age and had no hair but was otherwise developmentally normal. Fetal membranes had thin tissue with diffuse pallor of the chorion and there was

lack of focal thickening. Necrotic villi near the cervical star indicated premature separation at the caudal pole. Umbilical cord was increased in length (~ 122 cm) and had several twists and moderately thickened umbilical vessels and urachus near the carcass. Collectively, these findings were consistent with fetal death due to umbilical cord torsion. Due to the enlarged uterus, the mare was at risk to develop metritis and secondary laminitis; thus, therapy was initiated to prevent these issues. During hospitalization, large volume of uterine lavage was performed 1 or 2 times per day. An ecbolic agent (oxytocin 20 units, every 6 hours) was given intramuscularly and the mare was exercised daily to aid in uterine clearance. The mare was placed on oral trimethoprim sulfa (30 mg/kg, every 12 hours) to treat potential infection, flunixin meglumine (1.1 mg/kg, every 12 hours) was given intramuscularly to manage inflammation. Additionally, a temporary Caslick's vulvoplasty was performed using nonabsorbable suture to reduce severe pneumovagina. After 4 days of hospitalization, mare recovered without having any signs of metritis, but developed urovagina/urometra. As urovagina/urometra can be transient in postpartum/postabortion mares, a reevaluation was scheduled in 60 days. Owner was instructed to monitor mare's rectal temperature daily for 7 days and bring the mare back if any fever or depression was noted. Power walks were recommended to aid in uterine involution and reduce urine pooling, along with intramuscular oxytocin (20 units, every 12 hours) for 2 weeks. Cause of umbilical cord torsion remains unknown; certain Thoroughbred blood lines are prone to it. The condition has been rarely detected in Quarter Horses. The umbilical length was >100 cm that makes this mare 3 times more likely to abort from umbilical torsion. It remains to be determined if the mare's incredibly pendulous uterus served as a predisposing factor for umbilical cord torsion. Although reoccurrence of umbilical cord torsion appears to be rare, the owner was offered the possibility of performing embryo transfer to prevent the mare from carrying her own foals.

A case of placental edema and premature lactation in a Thoroughbred mare

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A 17-year, Thoroughbred mare, was presented at 302 days of pregnancy for intensive management of placentitis. Mare was confirmed pregnant at 40 days with no abnormalities identified. Referring veterinarian made the diagnosis of placentitis based upon a relaxed and elongated vulva, premature lactation with a milk calcium of > 2,000 ppm and an increased combined thickness of the uterus and placenta (CTUP) of 19 mm. On presentation, the mare's physical examination findings were within normal limits. Evidence of premature lactation was noted on the hind legs, but no vulvar discharge was present. On transrectal ultrasonography, CTUP measured 21 mm with the placenta appearing markedly edematous around the cervical star; fetal movement was appreciated. A vaginal speculum examination was performed, and findings were unremarkable. A fetal thorax was identified on the right side of the mare via transabdominal ultrasonography with no heartbeat; however, fetal movement was detected on subsequent transrectal palpation. Based on these findings, ascending placentitis was the most likely differential. Medications included potassium intravenous penicillin (22,000 IU/kg, 4 times daily), gentamicin (6.6 mg/kg, once daily), flunixin meglumine (1.1 mg/kg, twice daily), oral altrenogest (0.088 mg/kg, once daily) and pentoxifylline (8.4 mg/kg, 4 times daily). Transrectal and transabdominal ultrasonography were performed regularly to monitor progress. Improvement in the CTUP at the cervical star was noted so treatment was continued. On day 307 of pregnancy, transabdominal ultrasonography revealed a second fetal thorax with a heartbeat of 80 beats/minute on the mare's left side. This made complications from a twin pregnancy the top differential. It was hypothesized that recent fetal death incited an inflammatory response that resulted in premature lactation and mimicked the signs of placentitis. With this change, the treatment plan was altered from intravenous antibiotics to oral trimethoprim sulfamethoxazole (30 mg/kg, twice daily). Altrenogest was discontinued at a rate of 2 ml/day. The mare spontaneously delivered a live filly and a deceased colt on day 325 of pregnancy. Gross evaluation of the twins indicated that they were full term. Physical examination of the filly indicated no abnormalities, but it was decided to give intravenous frozen-thawed plasma based on the assumption that the mare had lost most if not all colostrum due to premature lactation. It was noted that the filly was progressively becoming less active. The filly was sedated with intravenous diazepam (0.1 mg/kg) and butorphanol (0.08 mg/kg) and placed in lateral recumbency for catheter placement, during which time respiration stopped; all efforts to resuscitate were unsuccessful. The filly and fetal membranes were submitted for necropsy; the pathologist identified no signs of sepsis. Fetal membranes were dichorionic with the filly's fetal membrane being unremarkable and the colt was greenish grey in color and diffusely thickened. Although this case appeared as a typical case of placentitis, it emphasized the importance of considering twins as a differential. Twins should be detected via repeated thorough transabdominal ultrasonographic examinations and should be reduced early in pregnancy.

Keywords: Mare, placentitis, twins

Ovarian cysts and cystic endometrial hyperplasia in a young breeding bitch

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An intact, 2-year maiden Labrador Retriever bitch, was presented for evaluation of scant, persistent white vaginal discharge of ~ 3-months duration, immediately following a clinically normal estrus. Repeated vaginal cytology revealed cornified vaginal epithelium consistent with persistent cytologic estrus; however, no external physical or behavioral clinical signs were observed during this time. Suspicions for this bitch's persistent cytologic estrus were hormonally

active ovarian cysts or exposure to exogenous hormones. However, there was no known exposure to any hormones or phytoestrogens. Abdominal ultrasonography was performed and bilateral ovarian cysts (right 2.2 cm; left 0.8 cm) and cystic endometrial hyperplasia were diagnosed. Transabdominal ultrasound-guided aspiration of 3.2 ml of fluid from the right ovary was performed, and cyst fluid and serum were submitted for paired hormonal analysis. There were marked elevations of estradiol and inhibin B within the cyst fluid compared to serum (estradiol 3,689.7 pg/mL versus 21.8 pg/ml; inhibin B 155,700.0 pg/ml versus 6.0 pg/ml). Progesterone (15.1 ng/ml versus 0.75 ng/ml) and Antimullerian hormone (2.2 ng/ml versus 0.18 ng/ml) were additionally elevated within cyst fluid compared to serum. Hyperestrogenism is the hallmark clinical appearance of hormonally active follicular cysts.[1] No external or behavioral clinical signs associated with hyperestrogenism were seen in this particular bitch, though cystic endometrial changes were likely due to their prolonged hormonal influence. While the reported treatment for ovarian cysts and cystic endometrial hyperplasia has historically been ovariohysterectomy, medical management was elected due to this bitch being young and a breeding prospect.[1] Cystorelin (75 µg) was given intramuscularly for 3 days in addition to aspiration of the large right ovarian cyst to cause luteinization and allow for uterine remodeling. Recheck examination performed 2 months later revealed resolution of vaginal discharge with noncornified vaginal epithelium, and follow-up ultrasonography showed a marked improvement in uterine pathology with almost complete resolution of all endometrial cysts.

Keywords: Canine, cystic endometrial hyperplasia, ovarian cyst, estrus

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Hysteroscopy on a Southern Tamandua

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Currently, there is no report available on the use of a flexible scope in Tamandua. We used a flexible multipurpose video endoscope (model 60714 NKS) with a 7.9 mm probe. A 12-year, intact female Southern Tamandua (*Tamandua tetradactyla*) was presented for a hysteroscopy examination. Several cystic endometrial glands were observed during ultrasonography. Hysteroscopy was performed under general anesthesia with a flexible scope. Approximately 3 to 4 cm of the flexible scope was introduced transvaginally. Vestibule, vaginal vault, cervix, and uterus were identified and evaluated. Several hyperplastic endometrial cysts were observed, confirming ultrasonographic findings. This examination suggested that a flexible large animal scope can be successfully used in a Tamandua to observe uterus for evaluation and diagnosis of uterine pathologies (e.g., cystic endometrial hyperplasia).

Keywords: Tamandua, hysteroscopy, endometrial cysts

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Spontaneous asymmetrical mammary gland fibroadenomatous hyperplasia in a bitch

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Mammary gland fibroadenomatous hyperplasia (MFH) is a nonneoplastic, progesterone-dependent proliferative process of the mammary glands. MFH is commonly observed in pregnant and nonpregnant queens, usually as a sudden, diffuse, and symmetrical enlargement of the mammary glands with no evidence of lactation. In this report, an 11-month, nulliparous intact female Maltese dog, was presented with a 7-day history of asymmetrical mammary gland enlargement, mild anorexia, and lethargy. Dog had reportedly undergone her first estrous cycle ~ 33 days prior to presentation. There was no history of exogenous exposure to sex steroid hormones. Right caudal thoracic mamma presented severe ecchymosis and a 15 x 10 x 8 cm (length x width x depth) subcutaneous mass, extending towards the right cranial thoracic mamma. Left caudal thoracic mamma presented mild ecchymosis and a smaller mass, measuring ~ 7.5 x 6 x 3 cm. Mammary papillae were juvenile, and no secretion could be expressed. Differential diagnoses included mastitis, abscessation, MFH, mammary neoplasia, and paraneoplastic mammary gland enlargement. Noncornified vaginal cytology and blood progesterone concentrations (12.24 ng/ml) were consistent with diestrus. There were no remarkable findings on complete blood count and serum chemistry. Ultrasonographically, the masses presented architecture characteristic of mammary tissue. There was no free or encapsulated fluid. Fine needle aspirates of the right and left caudal thoracic mammae were submitted for cytology and culture. A 48-hour culture and cytology result in no bacterial growth, suggestive of either epithelial hyperplasia or complex neoplasia. As there was no breeding intention, ovariectomy and biopsy of the mammary glands was elected. Histopathological examination of the mammary tissue

was consistent with MFH: the specimens were comprised of abundant spindle cells expanded by mild to moderate edema, and multifocal to coalescing embedded basophilic apocrine glandular ducts with very narrow lumen. Enlarged mammary tissue gradually reduced in size after ovariectomy, with minimal enlargement of the right caudal thoracic mamma after 25 days surgery and complete resolution ~ 60 - 80 days after surgery. Complications (e.g., skin necrosis, ulceration, and mastitis) did not occur in this case. Although MFH is a condition commonly observed in young female cats, it has also been reported in older male and female cats exposed to exogenous progestogens, rats, rabbits, goats, and a sea lion. Upregulation of progesterone receptors in the mammary tissue has been speculated as a predisposing factor for MFH. This condition is rarely observed in bitches, with asymmetrical MFH reported in 1 bitch after progestogens treatment. To date, this appears to be the first published report of asymmetrical MFH in a young bitch with no history of exposure to exogenous sex steroids.

Keywords: Dog, fibroepithelial hyperplasia, mammary fibroadenoma, fibroadenomatosis

Double CIDR induced anestrus in Boer doe

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Since its introduction in 1984, CIDR usage in parous ruminants has continued to be a mainstay of reproductive synchronization and management for operations of all sizes. Many protocols have been described and published over the years, with most indicating a return to estrus ~ 48 hours after removal, and insemination within the window of 48 hours after device removal. Does treated with CIDR protocols had similar reproductive performance parameters compared to those treated with FGA sponges,[1] without the reported effects of malodorous discharge as documented in ovine counterparts.[2] However, these results are typically associated with removal of the device from the vagina. A 6-year multiparous Boer doe was presented after failing to conceive via laparoscopic AI and natural cover by 2 proven bucks. Prior to both breedings, CIDRs were placed, but when the time came for removal, neither were detected. Doe's general physical examination was unremarkable apart from mild bilateral serous nasal discharge. On genital evaluation, there was mild bloody serous discharge coming from the vagina and the animal appeared painful on external manipulation. Utilizing a SonoScape A6V Expert E1V with a rectal probe, a transrectal ultrasonography was performed that revealed 2 distinct cylindrical foreign bodies, in sequential linear fashion, with the most proximal observed in the cranial vagina near the cervix, and a fluid filled uterus. Vaginal speculum examination revealed a proliferative scar tissue and serosanguinous discharge (submitted for histopathology). On digital palpation of the vagina, the string of the most distal CIDR was felt and removed with minimal manipulation. The more proximal CIDR required gentle traction and consistent pressure. At original presentation, the full extent of the soft tissue damage could not be assessed due to substantial swelling. Doe was started on a course of meloxicam 20 mg/kg every 24 hours and oxytetracycline 200 mg/kg every 48 hours for 3 doses of each. Initial follow up was scheduled for after 2 weeks to provide adequate period for the inflammation to decrease. During this visit, a vaginal speculum was easily passed to 23 cm, and there was no gross evidence of cervical or vaginal scarring. Doe seemed much more comfortable on digital evaluation, and the owners were advised to attempt breeding on her following cycle.

Keywords: Boer, CIDR, induced, anestrus

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Papillomavirus and vaginal vesicular lesions in a dairy herd

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Papillomavirus has been studied extensively.[1-5] Perianal lesions were observed in a herd of 150 Holstein cows in upstate New York. Lesions included anal warts, vaginal warts, and vaginal vesicles. Days in milk and lactation were noted to be risk factors for the presence of these lesions, but had minimal bioeconomic impact on reproduction and milk production. Cross-sectional data was taken across the herd to document the presence or absence of anal warts, vaginal warts, and vaginal vesicles. The vesicles were scored on a scale from 1 to 5 based on severity. These cross-sectional data were collected at 3 time points over the course of 6 months to gain information on incidence and persistence. Tissue biopsies from 3 cows and a vaginal swab from 1 cow were collected and submitted for in situ hybridization (ISH), virus isolation, polymerase chain reaction (PCR), and bacterial culture. Bovine papillomavirus (BPV) was identified from a vaginal wart via ISH. The histological pattern of the lesion was not consistent with BPV type-1 or -2 and samples were negative for BPV 1 and 2 via PCR. It was suspected that the BPV identified was a novel strain or a strain not previously associated with the lesions seen in this outbreak. This case raises important welfare concerns on dairies since the etiology and transmission of these lesions remain unknown. It is postulated that it may be caused by iatrogenic transmission from farm personnel involved in pregnancy detection and artificial insemination on the dairy. Treatment included surgical removal of 1 anal wart as it was interfering with palpating, breeding, and defecating. Improved care to change rectal sleeves between cows and to use more lubricant was implemented.

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Dystocia and foal survival in a mare with seven hour long second stage of labor

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An 8-year, Arabian mare, at 328 days of pregnancy was referred for failure of normal foaling progression. Owner reported that the mare had her 'water bag' ruptured 30 minutes prior but since had no contractions. Mare was comfortable and interested in feed and there appeared a second membrane at the vulva that was confirmed to be the amniotic sac via photograph and consultation with the referring veterinarian. Mare arrived ~ at 6 hours after the initial call. Upon arrival the mare was bright, alert, and responsive with mildly increased heart rate (48 beats/minute) and respiratory rate (40 beats/minute), and decreased borborygmi. Her mucous membranes were pink and moist with a CRT of < 2.5 seconds. She was straining on arrival. Transabdominal ultrasonography confirmed a live fetus with a heart rate of 64 beats/minute. Epidural anesthesia was given (4 ml 2% lidocaine), and on abbreviated vaginal examination, the fetus was partially engaged in the birth canal in cranial presentation, dorsopubic position, with both front legs extended. During the examination the mare increased abdominal effort and attempted to go down. She was immediately anesthetized for controlled vaginal delivery. Fetal maldisposition of lateral deviation of head and neck was discovered and corrected within 15 minutes and a live filly was delivered. The filly displayed normal behavior at birth. The mare retained the fetal membranes that was delivered after oxytocin treatment in lactated Ringer's drip (30 IU in 30 minutes) followed by chorioallantoic distention. Postpartum evaluation was normal except for a 30-degree cranio-ventral tilt of the vagina and vaginal vestibule. After 4 days, mare and filly were discharged. Rupture of the chorioallantois is considered the beginning of the second stage of parturition. In the mare, the second stage of parturition is rapid and lasts ~ 20 minutes 10 - 30 minutes). Foal survival decreases by 10% for each additional 10 minutes beyond 30 minutes. In this case the foal survived ~ 7 hours after initiation of the second stage of foaling. This is extremely rare. Although the reason for this delay could not be determined, failure of the foal to rotate and lack of uterine contractions may have delayed placental detachment and prevented hypoxia. This case illustrated an uncommon outcome of a delayed second stage of labor and underscores the importance of proceeding with care and optimism even if the second stage has been abnormally long.

Keywords: Mare, dystocia, chorioallantois, maldisposition