ABSTRACTS

OPENING SESSION

Cytoplasmic blebbing as an indicator of cryodamage in bovine embryos

Soon Hon Cheong, Abdallah Wagih Abdelhady, Yoke Lee Lee, Mariana Diel de Amorim, Luis Henrique de Aguiar

Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

Cryopreservation is an important tool for bovine embryo transfer. However, cryopreservation results in 10 - 13% lower pregnancy rates compared to fresh embryo transfer. The mechanisms and cellular response of embryos to cryodamage have not been fully elucidated. Most blastocysts have a collapsed blastocoel after cryopreservation that will reform if the blastocyst survives and undergoes hatching. Using time-lapse videography, we observed a unique cellular response of blastocysts to cryopreservation, where the cytoplasm formed large clear blebs in many cryopreserved blastocysts. This cytoplasmic blebbing has been reported in human stem cells in response to dissociation and is thought to be a precursor to apoptosis but has not been reported in embryos or as a response to cryoinjury. Objective was to determine if cytoplasmic blebbing is associated with poor postthaw outcomes in bovine embryos. We hypothesized that embryos with cytoplasmic blebbing have decreased likelihood of hatching and increased risk of degeneration. Slaughterhouse-derived bovine embryos were randomly assigned to a sham treatment where the embryos were exposed to cryopreservation media and loaded into straws but not cryopreserved (No-Freeze group), or cryopreserved using the direct-transfer slow cooling method (Frozen) group. Embryos were then placed in time-lapse culture dishes and observed for 48 hours for blebbing, hatching, or degeneration. Differences in proportions between groups were evaluated using logistic regression ANOVA in JMP Pro v16. Blebbing was observed in only 3/88 (3.4%) blastocysts in the No-Freeze group, whereas most of the Frozen blastocysts 77/102 (77.5%) had blebbing ($p < 0.0001$) suggesting strong collinearity between treatment group and blebbing. Postthaw hatching rate was lower 63/102 (61.8%) for the Frozen group compared to the No-Freeze group 68/88 (77.3%; $p = 0.0203$). A similar outcome was observed for blebbing where hatching rate was lower in Bleb 46/82 (56.1%) compared to the No-bleb 85/108 (78.7%; $p = 0.0009$). Interaction between treatments and blebbing was different ($p = 0.0204$) with hatching rate. Degeneration rate was higher ($p = 0.0026$) for the Frozen group (26/102, 25.5%) compared to No-Freeze group (8/88, 9.1%). A similar outcome was observed for blebbing where degeneration rate was higher ($p = 0.0004$) for Bleb 24/82 (29.3%) compared to No-bleb 10/108 (9.3%). Interaction between blebbing and treatment was not significant for degeneration. As treatment and blebbing were collinear, when both were offered to the model, treatment became not significant and was dropped. This is the first description of cytoplasmic blebbing as a response of embryos to cryodamage. Blebbing was associated with lower hatching rates and higher degeneration rates. Blebbing is thought to be a precursor to apoptosis; however, some embryos that exhibit blebbing can recover, hatch, and avoid degeneration. Time-lapse monitoring of embryos for blebbing postthaw may be a useful and noninvasive indicator of cryodamage to select embryos for transfer.

Keywords: Cytoplasmic blebbing, cryodamage, bovine, embryos

Shifts in uterine microbiome associated with pregnancy outcomes at first insemination and clinical cure in dairy cows with metritis

Caio Figueiredo,[a,b] Hugo Monteiro,[c] Federico Cunha,[a] Fábio Lima,[c] Klibs Galvão,[a] Rafael Bisinotto[a]

[a]Department of Large Animal Clinical Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, FL, USA
[b]Department of Veterinary Clinical Sciences, Washington State University, WA, USA
[c]Department of Population Health and Reproduction, University of California, CA, USA

Reproductive efficiency is a key component for sustainability of dairy herds as daily costs associated with nonpregnant cows increase across lactation stages. Metritis is a major contributor to reduced reproductive efficiency, as cows with metritis have reduced risk of pregnancy per artificial insemination (AI), greater risk of pregnancy loss, and require more time to achieve pregnancy than unaffected cows. Despite the detrimental impacts on uterine health, cyclicity, and reproductive performance associated with metritis, 30% of cows with metritis establish pregnancy after first AI. These data suggest the existence of mechanisms linked to fertility loss that are not present in every cow with metritis. Clinical cure failure of metritis has also been associated with negative impacts on subsequent uterine health and fertility. Differences in uterine microbiome associated with clinical cure failure in cows with metritis were reported and shifts associated fertility outcomes in cows with endometritis. Therefore, the hypotheses of the present study were: 1. the uterine microbiome in cows with metritis that become pregnant at first AI differs from cows with metritis that fail to become pregnant; 2. the microbiome of cows with metritis that become pregnant at first AI is similar to cows without metritis that achieve pregnancy; and 3. clinical cure failure is associated with differences in uterine microbiome. Objectives were to assess differences in uterine microbiome associated with clinical cure failure and subsequent pregnancy outcome in dairy cows treated for metritis. Lactating Holstein cows diagnosed with metritis (n = 43) characterized by the

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

presence of reddish-brownish, watery, and fetid vaginal discharge were paired with counterparts without metritis (n = 42) based on parity and days postpartum. Uterine contents were collected through transcervical lavage at the time of diagnosis (day 0); 5 days later, after completion of antimicrobial therapy for cows with metritis (day 5); and at 40 days postpartum. Vaginal discharge in cows with metritis was evaluated on day 5 for definition of clinical cure. Uterine microbiome was evaluated by sequencing of the 16S rRNA gene. Despite similar alpha-diversity on day 0 based on Chao1, Shannon, and inverse Simpson indexes, metritis was associated with differences in beta-diversity. Prevalence of *Porphyromonas*, *Bacteroides*, and *Veillonella* was higher in cows with metritis, whereas, *Streptococcus*, *Sphingomonas*, and *Ureaplasma* among other genera were more prevalent in cows without metritis. Differences in beta-diversity between cows with metritis and counterparts without metritis persisted on day 5; however, no differences in uterine microbiome were observed between cows with metritis that cured after antimicrobial therapy and those with clinical cure failure on day 0 or 5. Although differences in richness and diversity in the uterine microbiome 40 days postpartum associated with metritis and pregnancy were observed, no differences in beta-diversity were associated with pregnancy outcomes or metritis 40 days postpartum. In summary, no relationship between the uterine microbiome and pregnancy outcomes or clinical cure was observed, indicating that there are other mechanisms associated with recovery of fertility, and clinical cure aside from uterine changes in bacterial community.

Keywords: Uterus, microbiome, fertility, cure, metritis, dairy

Increased piezo drilling intensity does not appear to adversely affect cleavage or blastocyst rates following equine intracytoplasmic sperm injection

Charles Scoggin,[a] Alaina Barhorst,[a] Etta Bradecamp,[a] Peter Sheerin,[a] Maria Schnobrich,[a] Stephanie Walbornn,[b] Erin Lohbeck,[a] Ashley Buchanan,[a] Anne Sheerin[a]

[a]Rood and Riddle Equine Hospital, Lexington, KY, USA
[b]Rood and Riddle Equine Hospital, Wellington, FL, USA

Piezo intracytoplasmic sperm injection (Piezo-ICSI) utilizes pulses generated from a piezo drill (PD) for capture and immobilization of sperm, and for breaching of the zona pellucida and oolemma to deposit a sperm into the ooplasm. A blunt and smooth micropipette is utilized during Piezo-ICSI that differs from Conventional-ICSI wherein a beveled and spiked injection tip is used. The boring power of a PD can be adjusted by changing the intensity, speed, and number of the pulses. Studies in humans and horses suggest improved embryonic development rates using Piezo-ICSI compared to Conventional-ICSI. However, Piezo-ICSI requires added expenses, training, and fine-tuning. In our laboratory, the learning curve with the PD was steep and hampered by limited information on proper drill settings. We thus performed a retrospective study to evaluate cleavage and blastocyst rates using 2 different intensities (I) of piezo pulses for equine Piezo-ICSI using an Eppendorf PiezoXpertTM PD. One setting (PI10) utilized I = 10 whereas the other (PI30) used I = 30. The speed (10) and number of pulses (infinity) remained constant across the 2 different settings. Oocytes were collected via transvaginal aspiration (TVA), held overnight in a commercial embryo holding media (EmCareTM; ICPbio Reproduction, Spring Valley, WI, USA) and placed in a commercial maturation media (EQ-IVM, IVF-BioSciences, Falmouth, Cornwall, UK) for ~ 30 hours. Oocytes were then denuded and evaluated for the presence of a polar body. Mature oocytes were fertilized with a single, motile and morphologically normal sperm using either PI10 or PI30. These presumptive zygotes (PZs) were placed back in maturation media for ~ 40 minutes and transferred to 500 µl of 1 of 2 culture medias (EQ-IVC-1, IVF BioSciences; or Global®, Life Global Group) under oil at 38.3°C in an atmosphere of 6% CO2 and 6% O2 and 88% N2. On day 5, PZs were assessed for cleavage, and cleaved embryos were moved to 1 of 2 sequential culture medias (EQ-IVC-2, IVF BioSciences; or DMEM/F-12 [Sigma-Aldrich #D6421] supplemented with 6 ml/liter 1 N NaOH and 10% FBS) and cultured under oil and at the same temperature and atmosphere. Blastocyst development was assessed from days 7 through 10. Data were analyzed using Chi-Square. A total of 845 immature oocytes were collected from 1,422 follicles punctured (59.4% recovery rate). Of these, 457 oocytes matured (54.0% maturation rate); 289 oocytes were fertilized using PI10, and 168 oocytes were fertilized using PI30. Cleavage rates between PI10 (176/289; 60.9%) and PI30 (104/168; 61.9%) were similar (p = 0.91706). Blastocyst rates were also similar between PI10 (24/289; 8.3%) and PI30 (17/168; 10.1%). In conclusion, increasing the intensity of piezo pulses during Piezo-ICSI may not hinder embryonic development rates; however, controlled studies are needed to determine the optimal settings of the PD during equine Piezo-ICSI.

Keywords: In vitro equine embryo, intracytoplasmic sperm injection, piezo drill

Horse (*Equus caballus*) and mule (*Equus mulus*) embryo morphometry

Giorgia Podico, Kianna Spencer, Igor Canisso

Department of Veterinary Clinical Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

Donkeys are used worldwide to produce mules that are considered prized animals for leisure, show, and fieldwork. The different number of chromosomes fits the hypothesis that aneuploidy and other nuclear abnormalities stand behind the higher rate of early pregnancy losses in mule pregnancies. This study aimed to compare the morphometry of equine and mule embryos. The hypothesis was that the micronuclei and nuclear fragmentation indexes are higher in mule embryos than in horse embryos. Twenty-two mares were randomly assigned in a crossover design to receive semen from both species. Twelve horse and 13 mule embryos were obtained from 44 estrous cycles of mares. All mares were bred by a fertile small standard jack or a fertile Quarter Horse stallion. Embryos were recovered 8 days after ovulation and classified regarding the stage of development and quality (compactness of the blastomeres, size, appearance of perivitelline space, color, presence of extruded cells, cell granulation, and cytoplasmic fragmentation) with a score from 1 (excellent) to 5 (degenerate). Embryos were stained with Hoechst33342, and images were acquired with a fluorescence microscope coupled with a 60 x objective (Keyence BZ-X, Keyence Corporation of America, Itasca, IL, USA). Nuclei were categorized as compact, mitotic (prophase, metaphase, anaphase of mitosis), or fragmented; micronuclei were identified as small extranuclear DNA bodies within the same cell. The fragmented, mitotic,

and micronuclei indices were calculated based on their proportion over the total amount of nuclei counted. Embryo size and nuclear morphometry were assessed through ImageJ. Data were analyzed with GraphPad using ANOVA and Student's t-test; significance was set at $p < 0.05$. Mule embryos were numerically higher ($p > 0.05$) than horse ones. One set of twins was recovered from a mare bred to the stallion that had a double ovulation; a mule and horse embryo were both recovered from 8 mares. There were no differences ($p > 0.05$) in size between mule and horse embryos (915.5 ± 288 versus 575.8 ± 69.6 µm). The mule embryos scored grade 1 (n = 8) or grade 2 (n = 5); similarly, the horse embryos scored grade 1 (n = 7) or grade 2 (n = 6). The evaluation of the nuclear morphometry revealed that horse and mule embryos had a similar number ($p > 0.05$) of compact nuclei per sector (148.7 ± 6.8 versus 156.5 ± 8.5); however, the number of mitotic nuclei tended to be higher in mule embryos (5.2 ± 0.82) than in horse embryos (3.3 ± 0.3). The fragmented nuclei index was similar ($p = 0.3818$) between mule (0.25 ± 0.1%) and horse (0.22 ± 0.1%) embryos, the mitotic nuclei index was higher ($p = 0.0209$) in mule embryos (3.2 ± 0.4%) than in horse embryos (2.2 ± 0.2%) and the micronuclei index was similar ($p = 0.7666$) between mule (0.02 ± 0.02%) and horse (0.01 ± 0.01%) embryos. This is the first study to compare the embryo morphology of mares bred with donkeys and horses. Equid embryos shared similar nuclear ultrastructure features, except that mule embryos had a higher mitotic index.

Keywords: Hybrid embryos, nuclear morphometry, micronuclei index, mitotic index

Metabolome and proteome of the seminal plasma and semen of horses and donkeys

Giorgia Podico, Igor Canisso

Department of Veterinary Clinical Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

Clinical experience suggests that mares bred with donkey semen (donkey-S) develop a milder postbreeding inflammatory response and achieve higher pregnancy rates than when they are bred with horse semen (horse-S). The presence and effect on the endometrium of pro and antiinflammatory molecules (e.g. prostaglandins, prostaglandin E2, 19-hydroxyPGE, secretory leukocyte protease inhibitor) contained in the seminal plasma have been demonstrated in other species; however, literature about this topic in equids is scant. This study aimed to investigate the metabolome and proteome of donkey-S and seminal plasma (donkey-SP), and horse-S and seminal plasma (horse-SP). We hypothesized that donkey-SP contains more antiinflammatory molecules than horse-SP. Semen (n = 9) and seminal plasma (n = 9) were collected from a single adult Quarter Horse stallion and a single adult jack; 3 pooled samples were submitted for extraction and quantification by liquid chromatography-tandem mass spectrometry. Statistical analyses were carried out with Metaboanalyst; data were normalized by sum, not transformed, and then auto-scaled. Gene ontology was evaluated with STRINGS and PANTHER. A total of 118, 80, 333, and 251 proteins were identified in donkey- and horse-SP, and donkey- and horse-S, respectively. There were 69 proteins in common between donkey- and horse-S, and 16 proteins in common between donkey- and horse-SP. Noteworthy findings; there were 42 (52%) proteins in common between horse-S and horse-SP and 57 proteins (49%) in donkey-SP were in donkey-S. Binding was the main molecular function in horse-S and horse-SP whereas catalytic activities were the main functions of proteins in donkey-S and donkey-SP. Cluster analysis revealed that the metabolomic composition of donkey- and horse-S and donkey- and horse-SP was significantly different. Metabolomic fingerprinting revealed a higher number of anti-inflammatory metabolites (e.g. choline, epinephrine, palmitoleic acid, and palmitic acid) in donkey-SP compared to horse-SP. Further in vitro and in vivo studies are warranted to evaluate their interactions with the mare endometrium.

Keywords: Horses, donkeys, seminal plasma, endometritis, proteins

Uterine edema and fluid accumulation associations with progesterone concentrations in early pregnant mares

Ilaria Colombo,[a,b] Humberto Magalhaes,[a] Giorgia Podico,[a] Gaetano Mari,[b] Igor Canisso[a]

[a]*Department of Veterinary Clinical Medicine, University of Illinois Urbana-Champaign, IL, USA*
[b]*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Italy*

Uterine edema is a useful clinical parameter to determine the readiness of a follicle to respond to induction agents and as a uterine infection/inflammation parameter. The number of days in estrus promotes a favorable environment for the establishment of a pregnancy. Conversely, uterine fluid accumulation 48 - 96 hours after breeding has been linked with persistent breeding induced endometritis in mares. Progesterone has a pivotal role during early pregnancy in maintaining and preparing the uterus to support embryo growth and development. Objective was to assess the relationships between uterine edema, and fluid accumulation in mares and progesterone in early pregnant mare. We hypothesized that uterine edema and fluid accumulation positively and negatively, respectively, affect progesterone concentrations in early pregnant mares. A retrospective study was conducted with 174 cycles. When in estrus, mares were assessed for the presence or absence of uterine edema and fluid accumulation, induced to ovulate with deslorelin acetate, and bred with cooled shipped semen. Mares had pregnancy diagnosis at 15 days after ovulation, with measurements of the embryonic vesicles and assessment of progesterone concentrations with a chemiluminescent assay (Immulite 1,000). Mares were reexamined for pregnancy on days 25, 45, and 60 days after ovulation. End points considered during the retrospective analyses included, mare, number of days with uterine edema, uterine fluid accumulation after breeding, progesterone concentrations, and embryo size. Linear mixed-effect models were used to determine the influence of all the factors on progesterone concentrations and embryo vesicle size. Postbreeding (0 - 96 hours) uterine fluid accumulation was associated with a decrease (-1.13 ng/ml; $p = 0.009$) in progesterone concentrations. In addition, mares experiencing early pregnancy loss (< 65 days) presented with lower (-2.96 ng/ml; $p = 0.05$) progesterone concentrations than mares with normal pregnancies. Longer periods of uterine edema, were associated with higher ($p = 0.0003$) progesterone concentrations at the first pregnancy diagnosis Interestingly, mares presenting with low endogenous

progesterone concentrations (< 5 ng/ml) that were supplemented with progestins had an increase (p = 0.09) in average conceptus size (3.19 mm) from 10 to 45 days after ovulation. In conclusion, the number of days with uterine edema positively influenced progesterone concentrations in early pregnancy whereas uterine fluid accumulation negatively influenced progesterone concentrations. Finally, these findings provided an intriguing observation on the positive impact of progestins on the embryo vesicle size that could be related to an improved uterine environment despite low endogenous progesterone concentrations. The results of this study need to be confirmed under controlled conditions.

Keywords: Mares, estrus, endometritis, embryo, progestins

Evaluation of aerobic culture results and the expression of embryonic transcripts from in vivo derived embryos exposed to media with and without antibiotics

Jessica Cowley,[a] Rachel Hollingsworth,[b] Paul Dyce,[b] Jessica Klabnik,[a] Richard Hopper,[a] Lamia Briand-Amirat,[c] Julie Gard Schnuelle[a]

[a]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA
[b]Department of Animal Sciences, College of Agriculture, Auburn University, Auburn, AL, USA
[c]ONIRIS, Nantes-Atlantic College of Veterinary Medicine and Food Science, Nantes, France

Centers for Disease Control and Prevention reports that more than 2.8 x 10^6 antibiotic-resistant infections occur in the United States each year leading to 35,000 deaths. Usage of antibiotics in embryo collection media may be unnecessary and detrimental to in vivo-derived embryos. Objective was to determine if embryo collection media with (AB+ treatment) or without antibiotics (AB- treatment) impacted the number of embryos recovered, bacterial growth, and abundance of embryonic transcripts related to apoptosis and free radical scavenging. We hypothesized that there are no differences in embryo recovery with and without antibiotic treatments but an impact on embryonic transcripts regulating apoptosis and free radical scavenging. In a crossover study design, 6 superovulated Angus cross cows underwent 2 conventional (day 7) uterine body embryo collections 77 days apart. Commercial embryo flush media with antibiotics (ABT Complete Flush, ABT 360, Pullman, WA, USA) was utilized when cows were assigned to the AB+ treatment. Commercial flush media without antibiotics (ABT Complete Flush without antibiotics, ABT 360) was utilized for the AB- treatment. During each embryo collection procedure, 5 samples were submitted to the Thompson Bishop Sparks Alabama State Diagnostic Lab for aerobic culture. The highest-grade embryos available were used for mRNA RT-qPCR analysis. RNA from individual embryos was isolated utilizing the PicoPure™ RNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA) and quality was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA samples, passing quality checks, were used to synthesize cDNA using a qScript cDNA synthesis kit (QuantaBio, Beverly, MA, USA). Subsequent molecular analysis using SYBR based Real-time RT PCR was completed (PerfeCTa SYBR, QuantaBio). The relative fold change was determined for abundance of Superoxide dismutase 1 (SOD-1), BCL2-associated X (BAX) and Tumor Protein P53 (TP53) normalized to Histone 2A (H2A). Treatment differences were determined by unpaired Student's t-test with significance set at $p < 0.05$. Total number of embryos recovered between treatments was not different ($p = 0.09$) nor the relative fold change of expression of SOD-1, BAX, and TP53 ($p > 0.05$). No bacterial growth was detected in samples, except for 1 sample from a single cow. This sample from the unopened bag of embryo flush media grew *Microbacterium testaceum*, a suspected environmental contaminant. Based on study findings, utilization of embryo flush media without added antibiotics may be a viable option for reducing antibiotic use in food producing species with no detrimental effects on embryo production.

Keywords: Antibiotics, embryo, superovulation, cows

Hypoluteoidism in a pedigree of Portuguese Water Dogs

Lily Lewis, Aime Johnson, Robyn Wilborn

College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Hypoluteoidism is a rare cause of pregnancy loss in dogs in which progesterone decreases to < 2 ng/ml during mid to late pregnancy with no apparent underlying cause. A heritable component has been theorized for the broad range of progesterone concentrations reported throughout normal pregnancy (e.g. sporting and working groups have lower concentrations throughout pregnancy).[1] Two Portuguese Water Dog (PWD) dogs, a dam and female offspring, were diagnosed and treated for hypoluteoidism during 4 successful pregnancies (2 litters each). First dog was presented for a workup of unexplained infertility;[2] breeding was attempted on 3 previous estrous cycles with no resulting pregnancy. Serum progesterone concentrations (P_4) and vaginal cytology were used to determine the day of ovulation and peak fertile window; transcervical insemination (TCI) was performed on days 2 and 4 using fresh-extended semen. Pregnancy was confirmed on day 26 via transabdominal ultrasonography. Due to a family history of suspected hypoluteoidism, serum progesterone concentrations were obtained throughout the second half of pregnancy. Twice weekly P_4 values were obtained starting on day 26 (16.9 ng/ml), with a steady declined observed. Supplementation with oral altrenogest (Regu-Mate®, Merck Animal Health, USA) at 0.088 mg/kg once a day began on day 45 (6.5 ng/ml). Serum P_4 concentrations continued to decline, reaching a nadir of 1.4 ng/ml on day 53. Cesarean surgery was performed on day 60 and a litter of 4 viable pups was delivered. Dog was bred again the following year using fresh extended semen via vaginal insemination (on days 2 and 3) and TCI (on day 4). Serum P_4 concentrations were monitored from day 33 (12 ng/ml), and a similar decreasing trend was observed. On day 43, P_4 concentrations were 5.3 ng/ml and altrenogest treatment was initiated, with declining concentrations reaching a nadir of 2.3 ng/ml on day 47. Cesarean surgery and ovariohysterectomy were performed on day 61, delivering a healthy litter of 6 pups. Upon sexual maturity, a female from the second litter (previously mentioned) was presented for ovulation timing and breeding management. Breeding was performed with cool-shipped semen via vaginal insemination (on day 1) and TCI (on day 4). Pregnancy was confirmed on day 32, and P_4

concentrations were 11.2 ng/ml. Similar to the aforementioned pregnancies, P_4 was monitored throughout the second half of pregnancy and altrenogest treatment was initiated on day 51 (P_4 concentrations were 3.4 ng/ml). Serum P_4 concentrations continued to decline (1.6 ng/ml on day 56 and 0.7 ng/ml on day 60). A healthy litter of 9 pups was delivered via cesarean surgery on day 63. A subsequent litter was successfully managed in the same dog, with pregnancy confirmed on day 35 (P_4 concentrations were 14.2 ng/ml). Treatment with altrenogest began on day 47 (P_4 5.9 ng/ml). During her final few days of pregnancy, P_4 concentrations continued to decline with values reaching 2.3 ng/ml on day 60 and 1.1 ng/ml on day 63. Cesarean surgery was performed on day 63, delivering a litter of 8 pups. Although other cases of hypoluteoidism have been reported,[3-5] to our knowledge this is the first confirmation of hypoluteoidism diagnosed in first-degree related individuals. Thus, investigation into the heritability of this condition should be strongly considered.

Keywords: Hypoluteoidism, progesterone, pregnancy, abortion, pedigree

References

1. Hinderer J, Ludeke J, Riege L, et al: Progesterone concentrations during canine pregnancy. Animals (Basel) 2021;11:7-9.

2. Shumack B, Wilborn RR, Johnson AK: Management of a high-risk pregnancy in a Portuguese Water Dog. Clinical Theriogenology 2017;9:486.

3. Görlinger S, Galac, S, Kooistra, HS, et al: Hypoluteoidism in a bitch. Theriogenology 2005;64:213-219.

4. Zedda MT, Bogliolo L, Antuofermo E, et al: Hypoluteoidism in a dog associated with recurrent mammary fibroadenoma stimulated by progestin therapy. Acta Vet Scand 2017;59:1-6.

5. Johnson CA: High-risk pregnancy and hypoluteoidism in the bitch. Theriogenology 2008;70:1424-1430.

Bovine oviductal organoids and their extracellular vesicles as a biomimetic model to develop a novel contraceptive

Riley Thompson,[a] Ahmed Gad,[b] Mindy Meyers,[a] Nico Menjivar,[b] Dawit Tesfaye,[b] Fiona Hollinshead[a]

[a]Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

[b]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

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. Extracellularvesicles (EVs) are nanoparticles containing bioactive molecules (e.g. lipids, proteins, and nucleic acids) that are secreted by cells to induce molecular changes in recipient cells. EVs produced by organoids are more similar to those produced in vivo because organoids retain more in vivo-like properties than other cell culture models. EVs have been exploited for use in assisted reproductive technologies to create a more biomimetic environment for embryo production and for their natural delivery mechanisms by loading them with therapeutic cargo to treat various pathologies. EVs loaded with CRISPR-cas9 ribonucleoproteins (RNPs) may be a novel method for delivery of a permanent contraceptive by localized knockout of genes governing fertility. Our objectives were to: 1. characterize bovine oviductal organoids and their secreted EVs; and 2. load EVs with RNPs targeting the progesterone receptor (PGR) gene. We hypothesized that: 1. bovine oviductal organoids maintain similar protein expression and function as their in vivo counterpart; and 2. EVs produced by oviductal organoids can be successfully loaded with specific CRISPR-cas9 RNPs. Bovine oviductal organoids (n = 13 cows) were cultured for up to 77 days (passaged every 7 - 14 days) and spent culture medium was collected for isolation of EVs via differential ultracentrifugation. First, organoids were assessed via brightfield imaging, histology, and immunohistochemistry (IHC). EVs were characterized using nanoparticle tracking analysis (NTA), western blot (WB), transmission electron microscopy (TEM), and uptake into epithelial cells of the oviductal organoids after fluorescently labelling EVs with PKH26. Then, EVs were loaded with RNPs targeting PGR using ExoFect™ transfection kit or electroporation, and size exclusion chromatography was performed to remove any unloaded RNPs. WB was then performed to assess cas9 protein in the EVs compared to PBS control. Brightfield imaging and histology demonstrated round cellular clusters with a central lumen, consistent with other oviductal organoid reports. IHC findings were positive immunostaining for PGR, oviduct-specific glycoprotein (OVGP1), FOXJ1 (cell ciliation), and periodic acid-Schiff (secretory function). EVs assessed by NTA and TEM were 50 - 150 nm in diameter, and WB indicated the presence of EV-specific surface proteins (CD63, FLOT1, TSG101) and the absence of cell-specific cytochrome C (CYCS). EV uptake was confirmed by fluorescence signal in recipient organoid cells following co-culture of organoids with PKH26-labelled EVs. Furthermore, EVs loaded with RNPs using ExoFect™ demonstrated a dark band of cas9 protein on WB, indicating EVs were loaded at a high rate, compared to low or absent expression in PBS control or after electroporation. These results demonstrated for the first time that bovine oviductal organoids display in vivo-like morphology, protein expression, and function, indicating they are a biomimetic model of the bovine oviduct to assess EV interaction with oviductal cells. Additionally, EVs secreted by organoids can be loaded with RNPs to potentially facilitate transfection of oviductal cells as a novel next generation contraceptive in various species.

Keywords: Cows, cell culture, in vitro, extracellular vesicles, CRISPR-cas9

Cryopreservation of stallion sperm using commercially available cryoprotectant combinations

Brittany Middlebrooks,[a] James Graham,[b] Paula Moffett,[a] Patrick McCue[a]

[a]*Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA*
[b]*Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA*

Glycerol has been the sole sperm cryopreservation agent in several species. Due to toxic effects of glycerol at high concentrations, researchers have evaluated alternate cryoprotectants (e.g. amides for stallion sperm). The use of amides, alone or in combination with glycerol, improved postthaw sperm motility and fertility compared to glycerol alone. Objective was to compare 4 commercially available freezing extenders on postthaw semen parameters and sperm quality. We hypothesized that Lactose-EDTA extenders containing the amides methylformamide (MF) or dimethylformamide (DMF), alone or in combination with a low concentration of glycerol, yield higher postthaw sperm motility and viability than Lactose-EDTA extender containing only glycerol. Ejaculates from 10 stallions were collected using a Colorado model artificial vagina and diluted 1:1 in a skim milk glucose extender. Diluted ejaculates were transferred to conical tubes and a centrifugation cushion was added below the extended semen. Samples were centrifuged at 1,000 x g for 25 minutes. The supernatant was removed, and the sperm pellet was diluted to 200 x 10^6 sperm/ml in each of the 4 freezing extenders (LE, 5% glycerol; CMLE, 2% glycerol plus 3% MF; CMLEF1, 5% MF; CMLEF2, 5% DMF; Animal Reproduction Systems, Ontario, CA). Extended samples were loaded in 0.5 ml straws, cooled to 5 - 8°C for 30 minutes, floated over liquid nitrogen for 15 minutes, then plunged into liquid nitrogen. Straws were stored in liquid nitrogen for a minimum of 24 hours prior to evaluation. Three straws from each freezing extender for each 10 ejaculates were thawed in a 37°C water bath for 30 seconds. The contents of the straws were transferred into individual test tubes and allowed to incubate at 37°C for 10 minutes. The following parameters were evaluated by computer-assisted semen analysis (CASA; SpermVision; MOFA, Verona, WI): percent total motility (TM), percent progressive motility (PM), percent hyperactivity (HYP), curvilinear velocity (VCL), path velocity (VAP), straight-line velocity (VSL), and linearity (LIN). Results for the 3 straws were averaged and recorded. Data were analyzed using one-way ANOVA, Tukey's HSD test, and Chi-Square analysis (JMP Pro 16.2). Data were considered significantly different at $p < 0.05$. Mean TM and PM percent were higher in CMLE (70.5 and 60.8%, respectively) and CMLE1 (70.3 and 59.2%, respectively) compared to LE (60.2 and 49.6%, respectively). There were no differences in mean TM and PM between LE and CMLEF2 (66.6 and 54.6%, respectively). Additionally, there were no differences between extenders regarding HYP, VCL, VAP, VSL, and LIN. Commercially available freezing extenders with a combination of glycerol and amide (CMLE) or an amide alone (CMLEF1) had higher postthaw total and progressive motility compared to a freezing extender containing glycerol as the only cryoprotectant (LE). It is recommended that a test freeze is performed on all stallions to determine the optimal extender prior to banking frozen semen.

Keywords: Cryopreservation, stallion, sperm, motility, amide, glycerol

Is decline in prepartum progesterone concentrations associated with litter size in dogs? A retrospective study of 42 cases

Daniela Cortes, Robyn Ellerbrock, Patricia Xavier, Maria Ferrer

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Accuracy to predict fetal readiness for birth is essential for proper timing of elective cesarean surgery and for subsequent increase in neonates' survival rate. In clinical practice, transabdominal ultrasonography and determination of serum progesterone concentrations have been used to decide when to safely intervene during full term canine pregnancies. Prepartum decreases in maternal serum progesterone concentrations is induced by prepartum elevation of maternal cortisol concentrations and is associated with fetal readiness for birth. Aim of this retrospective study was to determine the effect of litter size on serum progesterone concentrations during late pregnancy in dogs undergoing cesarean surgery. It was hypothesized that maternal serum progesterone concentrations did not decrease below 2 ng/ml in dogs carrying 1 or 2 pups. Medical records from 42 pregnant dogs presenting for elective or emergency cesarean surgery between 2014 and 2022 were evaluated. The information retrieved included age, pregnancy length, progesterone concentrations on the day of the cesarean surgery, and litter size. Progesterone concentrations were determined using chemiluminescence immunoassay. Dogs were assigned into 3 groups based on litter size as follows: 1. G1: 1 pup (n = 6), 2. G2: 2 pups (n = 4), and 3. G3: > 3 pups (n = 32). Progesterone concentrations of < 2 ng/ml were considered to be associated with the prepartum decline and consistent with fetal readiness for birth. The frequency of dogs that experienced a prepartum progesterone decline prior to surgery was compared among groups using Chi Square analysis. Pregnancy length was compared among groups using one-way ANOVA. Mean age of dogs was 2.9 years (range;1.5 - 8 years). Pregnancy length at surgery was 63.2 ±1.5 days

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

(mean ± SD) after ovulation. Pregnancy length did not differ ($p > 0.05$) between dogs with and without a decline in progesterone concentrations at surgery. Eighty percent of dogs carrying 1 or 2 pups (G1 and G2) did not have a decrease in progesterone concentrations at surgery, whereas 81% of dogs with > 3 pups (G3) had marked decreases ($p < 0.05$) in progesterone concentrations < 2 ng/ml. All pups were considered mature at birth and there were no neonatal deaths associated with prematurity. These data suggested that the littler size affected prepartum progesterone concentrations during late pregnancy in dogs. Furthermore, prepartum progesterone concentrations cannot be used reliably to predict fetal readiness for birth in dogs carrying 1 or 2 pups.

Keywords: Dog, pregnancy, progesterone, litter size

Development of a porcine oocyte collection and maturation protocol for intracytoplasmic sperm injection utilizing stallion sperm

Elizabeth Patton, JoAnne Stokes, Emily May and Jennifer Hatzel

College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

Assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), are commonly utilized in clinical equine practice for infertile mares and stallions. In ICSI, a mare's oocyte is injected with 1 sperm from the desired stallion. Embryonic development to the stage of transfer remains lower and research advancements are stifled due to lack of equine oocyte availability. We proposed using readily available porcine oocytes as a model to evaluate stallion fertility and semen processing techniques. We hypothesized that porcine oocyte reach maturity using media and protocols utilized for equine oocyte. We further hypothesized that early embryonic development is achieved with porcine oocytes after ICSI with equine sperm. We collected porcine ovaries from a local abattoir, aspirated oocytes from the follicles using the vacuum suction technique previously validated for processing bovine ovaries, and evaluated various maturation process components to optimize maturation indicated with polar body (PB) extrusion. Comparisons were made utilizing Student's t-test with significance established at p 0.05. Follicular aspiration provided 760 total oocytes. Each week, the total collection of oocytes was batched and evenly distributed into 2 groups: control maturation medium (cMM) utilized in the clinical equine program and the experimental maturation medium (either containing 10 or 20% fetal calf serum [FCS]; 0, 33, or 50% porcine follicular fluid [PFF]). All experiments were conducted at 38.2°C unless otherwise noted. Initially, we determined difference ($p = 0.004$) in PB extrusion when oocytes were placed directly in cMM after aspiration compared to holding at room temperature for 24 hours in a commercial embryo holding medium (14/58 versus 2/60). A difference ($p = 0.019$) was also identified in PB formation when evaluating cMM containing 50% PFF compared to cMM (10/38 versus 5/44). There were no differences ($p = 0.938$) in PB formation when only 33% PFF was added to cMM compared to cMM (18/76 versus 14/58) or ($p = 0.753$) when cMM contained 20% FCS versus 10% FCS (17/89 versus 15/88). Most promising results ($p = 0.028$) were obtained when 33% PFF was added to cMM in comparison to cMM and both groups incubated at 39°C (34/90 versus 25/91). Finally, we injected 6 mature porcine oocytes with stallion sperm from the same frozen ejaculate and observed an 83% (5/6) cleavage rate and 17% (1/6) early embryonic development rate. Additionally, 5 matured oocytes were selected for a sham injection resulting in a 20% (1/5) parthenogenetic activation. Therefore, porcine oocytes can be collected and successfully matured in an equine-based maturation medium with the addition of porcine follicular fluid and incubated at a higher temperature in preparation for ICSI. We achieved early embryonic development after stallion sperm injection. Although we cannot rule out parthenogenic activation due to limitations of assessing pronuclear fusion, these pilot data were intended to determine the optimal conditions for establishing a porcine oocyte maturation protocol for future studies.

Keywords: Equine oocyte, porcine oocyte, intracytoplasmic sperm injection, assisted reproductive technologies

Determining the most effective method to remove red blood cells in cases of hemospermia

Hannah Maxwell,[a] Carly Turner-Garcia,[b] Dale Kelley[a]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]Lazy E Ranch, Guthrie, OK, USA

Hemospermia has been associated with infertility, subfertility, and poor semen longevity in stallions. Regardless of the cause of hemospermia, there is lack of data regarding how to process semen containing red blood cells (RBCs). Purpose of this study was to compare the efficacy of EquiPure™, cushion centrifugation, and sperm filter in removing RBCs from stallion semen. We hypothesized that EquiPure™ is the most effective method. Six ejaculates were collected from 1 stallion; immediately after each collection, 3 ml of venous blood was drawn. Blood was added to 12 ml of semen to create a standardized level of 20% hemospermia (%V/V). The initial concentration of the blood semen mixture was obtained via Nucleocounter® SP-100™. Blood-semen mixture was loaded into a microhematocrit tube and centrifuged at 10,030 x g for 5 minutes and packed cell volume (PCV) was determined using a hematocrit reader chart. The 20% hemospermic sample was divided into 3 aliquots, each extended 1:1 with INRA 96. The PCV of the initial extended sample was measured as described. One aliquot of extended blood semen was added to an equal volume of EquiPure™ in a 15 ml conical tube and centrifuged at 400 x g for 20 minutes. Pellet was suspended with INRA to the preprocessed volume. Second aliquot of extended blood semen was centrifuged with cushion for 20 minutes at 1,000 x g, and the pellet was resuspended to the initial volume with INRA. Third aliquot of extended blood semen was poured into the sperm filter and processed according to package directions. INRA was then used to wash the filter and return the sample back to its original volume. For each of the processed aliquots, the resultant PCV was measured for comparison and the final concentration was obtained to determine recovery rate. SAS (version 9.4) MIXED procedure modeled the change in PCV and recovery rate, with ejaculate and treatment as independent variables. There were no effects of ejaculate on change in PCV or recovery, so data were combined for analysis. Data

are represented as LS means ± SEM. EquiPure™ processing lowered ($p < 0.0001$) the PCV on an average of 4.8 ± 0.5%. Cushion centrifugation lowered ($p = 0.004$) the PCV on an average of 1.7 ± 0.5%. Sperm filter processing did not have an effect ($p = 0.32$) on PCV. There were differences ($p = 0.0004$) in PCV change of EquiPure™ compared to cushion and sperm filter ($p < 0.0001$) with no differences ($p = 0.11$) between cushion to sperm filter. Recovery rates of cushion (94.3 ± 2.9%) and sperm filter (93.6 ± 3.2%) processing were comparable ($p = 0.87$). Recovery rate of EquiPure™ was 12 ± 2.9% and was less ($p = < 0.0001$) compared to cushion and sperm filter. In conclusion, EquiPure™ was effective in removing RBCs from semen whereas cushion centrifugation and sperm filter were not effective. However, the low recovery rate of EquiPure™ processing on hemospermic ejaculates may render the method impractical for most situations.

Keywords: Hemospermia, stallion, semen processing

Mammary gland secretions conductivity alone or coupled with pH is a novel and accurate predictor of foaling and its associations with electrolytes

Humberto Magalhaes, Ilaria Colombo, Kianna Spencer, Giorgia Podico, Igor Canisso

Department of Veterinary Clinical Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

Accurate prediction of parturition is paramount to providing assisted delivery and preventing foaling-induced complications. Assessment of pH and electrolyte concentrations of mammary gland secretions (MGS) is useful to detect impending parturition. We hypothesized that changes in electrolytes and pH alter the conductivity of MGS, thus, conductivity is a more accurate foaling predictor than pH and electrolytes. In Experiment 1, periparturient mares (n = 23) had MGS assessed daily for pH, conductivity, and electrolytes (Ca2+, Na+, and K+) using bench-top analyzers (LAQUA-Twin D 771, Horiba LTDA, Kyoto Japan for pH and conductivity; and Hitachi 911 Roche Diagnostics, Basel Switzerland for electrolytes) and handheld devices with the aim to validate portable handheld devices worldwide available from one manufacturer (LAQUAtwin B-722 Na+ meter, K+ meter LAQUAtwin B-731 K+ meter, LAQUAtwin B-751 Ca2+ meter, LAQUAtwin pH meter and conductivity; Horiba, Ltd., USA) LAQUA-Twin, Horiba). Mixed-model analyses revealed significant reductions in pH, conductivity, Na+, and increases in Ca2+, and K+ for impending parturition. Bland-Altman analyses revealed no significant deviation from the linearity for all devices. In Experiment 2, periparturient mares (n = 114) had daily MGS assessed for conductivity, pH, and electrolytes using hand-held devices (Laqua-Twin). Mixed-model analyses suggested effects of time ($p > 0.05$) or interaction (group*time) ($p > 0.05$) for all analytes. Conductivity was strongly associated with pH ($r = 0.90$) and moderately associated with Na+ ($r = 0.77$), Ca2+ ($r = -0.60$), and K+ ($r = -0.70$). Analyses of receiver operating characteristics (ROC) were performed to determine the cutoffs for foaling in 24 hours for all analytes. Then, the cutoff values were used to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The results of conductivity for sensitivity, specificity, PPV, and NPV (0.82, 0.80, 0.82, 0.60, respectively), pH (0.80, 0.78, 0.81, and 0.70, respectively), Ca2+ (0.92, 0.35, 0.68, and 0.76, respectively), Na+ (0.65, 0.28, 0.53, and 0.39, respectively), and K + (0.67, 0.25, 0.50, and 0.41, respectively). The sensitivity, specificity, PPV, and NPV for conductivity coupled with pH, were 0.84, 0.85, 0.81, and 0.90, respectively. In conclusion, the handheld devices tested herein were as accurate as bench-top analyzers for pH, conductivity, and electrolytes. The conductivity of MGS presented similar results to pH to predict foaling, and the combination of pH and conductivity enhanced the prediction of parturition in mares.

Keywords: Mares, parturition prediction, mammary gland, secretions, electrolytes

Improving the art of semen banking in Amur Leopard (*Panthera pardus orientalis*)

Julie Barnes, Lindsey Vansandt

Center for Conservation and Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden, Cincinnati, OH, USA

Amur leopard (*Panthera pardus orientalis*) is a critically endangered subspecies of the leopard taxon (*Panthera pardus*). The IUCN Red List estimated[1] that < 40 individuals are left in the fragmentated habitat, making in situ conservation efforts pivotal for this species. Semen cryopreservation is a critical component of their conservation management strategy; however, current methods to collect and freeze sperm have yet to be assessed in this felid species. Our objectives were as follows: 1. evaluate the ability of a newer alpha-2 agonist, medetomidine, to facilitate semen collection via urethral catheterization; 2. compare soy lecithin-based cryomedium to the standard egg yolk-based cryomedium for sperm cryopreservation; and 3. compare ultra-rapid freezing (URF) of semen to traditional straw-freezing. Adult male Amur leopards (n = 10) located in zoos (accredited by the Association of Zoos and Aquariums) were anesthetized with various combination protocols that each included a moderate dose of medetomidine. At 20 - 40 minutes atter injection, a urinary catheter was inserted 20 cm into the urethra to recover semen (2 males were not able to be catheterized until 55 -75 minutes after injection). Immediately after catheterization, males were subjected to 1 - 3 series of electroejaculation (EEJ). Semen was either immediately placed in soy lecithin URF medium with 0.2 M sucrose (URF1) or split into 3 aliquots, centrifuged, and resuspended in soy lecithin cryomedium with 4% glycerol (SOY), TEST-egg yolk cryomedium with 4% glycerol (TEY), or soy lecithin URF medium with 0.2 M sucrose (URF2). Sperm extended in glycerol-based cryomedia were sealed in liquid nitrogen vapor. Sperm extended with URF were frozen by pipetting ~ 30 µl droplets directly into liquid nitrogen. Samples from 5 males were thawed and assessed for percent progressive and rate of progressive motility (0 - 5 scale), and heterologous IVF was performed using in vitro matured domestic cat oocytes (n = 10; 24/replicate). At 48 hours after insemination, Hoechst33342 staining was used to determine oocyte maturation stage. Data were analyzed with either paired t-test or repeated measures ANOVA and reported as mean ± SEM. On average, 120.7 ± 41.5 x 10^6 total sperm were recovered via catheter; however, more sperm (285.8 ± 56.3 x 10^6) were recovered during successive EEJ. Postthaw motility only differed ($p < 0.05$) between TEY and URF2 and the rate did not differ among other groups (TEY: 31.0 ± 3.3% motile, 1.8 ± 0.3 rate; SOY: 16.0 ± 4.3%, 2.2 ± 0.1; URF1: 18.0 ± 5.6%, 2.0 ± 0.2, or URF2: 12.0 ± 2.6%, 2.0 ± 0.2. Similarly, embryo cleavage percentage (TEY: 37.4 ± 9.0; SOY: 31.7 ± 8.9; URF1: 22.5 ± 7.2;

URF2: 10.9 ± 2.9) did not differ among treatments. In conclusion, medetomidine allowed recovery of a substantial number of sperm using urethral catheterization in Amur leopards; however, (if the technology is accessible), EEJ can significantly increase the number of sperm collected. Both SOY and URF cryomedia were comparable animal protein-free alternatives to TEY, with URF providing a more rapid, simplified cryopreservation option to standard straw freezing methods. We determined the current standards of cryopreservation for Amur leopard semen that can aid in conservation management on the individual and the population level of this iconic imperiled felid.

Keywords: Semen collection, cryopreservation, ultra-rapid freezing, in vitro fertilization, Amur leopard

Reference

1. Stein, AB, Athreya, V, Gerngross, P et al: Panthera pardus (amended version of 2019 assessment). The IUCN Red List of Threatened Species: 2020.

Early diagnosis of ascending placentitis in mares

Lorena Feijo,[a] Karen Wolfsdorf,[b] Igor Canisso,[c] Julia Felippe[a]

[a]Equine Immunology Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
[b]Hagyard Equine Medical Institute, Lexington, KY, USA
[c]College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, USA

Pregnancy loss and neonatal mortality have significant economic impact on the equine industry. Placentitis is the most common cause of abortion, stillbirths, and neonatal infections.[1] Early diagnosis of placentitis allows prompt treatment intervention and increases the chances of term pregnancy. Our study was designed to identify blood parameters that are diagnostic of imminent placentitis in natural conditions. We hypothesized that differences from physiological parameters of a healthy pregnancy indicate imminent placentitis. Blood sample collections and reproductive examinations were prospectively performed monthly, from 180 to 320 days of pregnancy in mares from 2 large broodmare herds in Lexington, KY. From this cohort, we analyzed samples collected from 12 mares before ultrasonographic detection of ascending placentitis (samples named preonset), and from the same mares when they developed signs of placentitis at subsequent examination (samples named early onset). In addition, samples from 12 mares with healthy pregnancies (pregnancy-matched controls) were collected at the same time points. Blood concentrations of serum amyloid A (SAA), alpha-fetoprotein (AFP), and estradiol-17β were measured and the values were compared between groups and timepoints using parametric (Student's t-test) and nonparametric (Mann-Whitney U test) analyses, and significance was set at $p < 0.05$. Based on the stage of pregnancy, the interval between preonset timepoint and the diagnosis of placentitis (early onset) was ~ 40 days (227 ± 6 days and 267 ± 5 days, respectively). Blood concentrations of SAA and AFP in the maternal circulation were within their respective normal range with no differences between groups and timepoints. Both proteins increased in maternal circulation after induction of experimental placentitis.[2,3] Plasma estradiol-17 β concentrations were different ($p = 0.08$) in mares at early onset of placentitis (408 ± 35 pg/ml) compared to control group (498 ± 35 pg/ml) mares. In conclusion, early events of placentitis did not alter inflammatory and immunological parameters in the maternal circulation. Plasma estradiol-17β concentrations may be further investigated as an early indicator of placental dysfunction in mares with naturally occurring placentitis.

Keywords: Mare, pregnancy, placentitis, diagnosis, estradiol

References

1. Giles RC, Donahue JM, Hong CB, et al: Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). J Am Vet Med Assoc 1993;203:1170-1175.
2. Canisso IF, Ball BA, Scoggin KE, et al: Alpha-fetoprotein is present in the fetal fluids and is increased in plasma of mares with experimentally induced ascending placentitis. Anim Reprod Sci 2015;154:48-55.
3. Canisso IF, Ball BA, Cray C, et al: Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. Am J Reprod Immunol 2014;72:376-85.

Comparing sperm concentrations for cooled semen use in dogs

Nicole Sugai, Julie Cecere, Orsolya Balogh

Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Stallion semen is shipped cooled at sperm concentrations of 25 - 50 x 10^6/ml with a minimum of 1:3 (volume) dilution with an extender. Optimal sperm concentration for cooled semen transport in the dog is unknown. Canine semen is commonly diluted to a 1:3 - 5 (volume) without standardization for concentration. Therefore, the purpose of this study was to compare various sperm concentrations during cooled shipment. We hypothesized that lower sperm concentrations result in higher sperm quality during cooled storage for 24 hours in dogs, similar to stallions. Healthy, client-owned stud dogs (n = 7, 2 - 6 years of age, > 15 kg, 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 a control (CON) aliquot (≥ 300 µl) that was extended with CaniPlus Chill LT at 1:3 (volume) dilution. Remaining sample was centrifuged at 720 g for 10 minutes in a 15 ml Falcon conical tube, the sperm pellet extended to 200 x 10^6/ml (C200) with CaniPlus Chill LT, followed by serial dilutions to arrive at 100, 50, and 25 x 10^6/ml sperm concentrations (C100-25). All aliquots were then cooled for 24 hours according to standard packaging technique in a Minitube canine shipping box. Afterwards, they were all recentrifuged and reextended to initial aliquot concentration. This last step was conducted to evaluate whether shipping at larger volumes with lower concentrations needing further processing for insemination would give adequate results. Semen evaluations for

concentration and viability (plasma membrane integrity) by Nucleocounter® SP-100™, subjective total motility (STM), total and progressive motility (TM, PM) by CASA (SpermVision™, Minitube) were performed on the raw semen (T0), immediately after extension (T1), after 24 hours of cooling (T2), and after centrifugation and reextension at 24 hours (T3). Data were analyzed by one-way ANOVA or Kruskal-Wallis test to evaluate viability and motility (STM, TM, PM) at T1, T2, and T3 by treatment. Viability was lower for C25 compared to the C200, C100 and CON groups for time points T1 and T2 ($p \leq 0.04$), and compared to C50 at T1 ($p = 0.01$). At time T1, viability for CON was higher ($p \leq 0.03$) among treatment groups. Also, viability for CON was higher compared to C50 and C25 at time T2 ($p \leq 0.01$), and compared to C25 at time T3 ($p = 0.01$). For motility evaluations (STM, TM, and PM) there were no differences among treatment groups at time T1 ($p \geq 0.17$) or T2 ($p \geq 0.18$). At time T3, STM was lower ($p = 0.001$) for C25 compared to CON and CASA TM and PM were lower ($p \leq 0.02$) for C25 compared to C200 and CON. In conclusion, cooling canine semen for 24 hours at 100 - 200 x 10^6/ml concentration after processing or extending 1:3 (volume) without processing, resulted in better sperm viability compared to lower concentrations and having similar motility parameters.

Keywords: Dogs, chilled semen, concentration, viability

Canine endometrial organoid generation and response to exogenous hormones

Alexandria Horner, Riley Thompson, Mindy Meyers, Fiona Hollinshead

Department of Clinical Sciences, Colorado State University, Fort Collins, CO, USA

Pyometra is a life-threatening reproductive pathology that commonly affects middle-aged to older, nulliparous, intact female dogs.[1] No studies have yet focused on developing an in vitro 3D culture system to analyze this disease complex that would eliminate the need for an in vivo model to improve welfare. Recent technological developments in 3D organoid cell culture may provide an innovative method for studying pyometra and other reproductive pathologies that affect dogs. Unlike traditional 2D monolayer culture systems, organoids are capable of long-term growth while maintaining similar structure and function to their organ of origin.[2] Due to these features, organoids are an ideal in vitro model for evaluating disease processes and investigating potential therapeutic options that may improve the overall health and welfare of intact dogs, domestic and wild. Therefore, our objectives were to: 1. generate organoids from endometrial tissue of diestrous dogs; and 2. evaluate the gene and protein expression response of these endometrial organoids to exogenous steroid hormones. We hypothesized that estrogen and progesterone receptor gene and protein expression increase in estradiol and decrease in progesterone presence.[3,4] Discarded diestrous canine reproductive tracts (n = 6) were collected after ovariohysterectomy at a local spay and neuter clinic. Endometrial epithelial glands then were isolated and established in organoid culture by adapting a protocol described for equine endometrial organoids.[2] After 20 days in culture (passaged every 6 days), the following treatments were initiated: 1. 30 ng/ml progesterone (P_4) over 6 days; 2. 100 pg/ml estradiol (E2) over 6 days; 3. 100 pg/ml E_2 for 2 days followed by 30 ng/ml P_4 for 4 days; and 4. control (no steroid hormones added). After 6 days of hormonal treatments, organoids were removed from culture for transmission electron microscopy (TEM), histology, immunohistochemistry (IHC), and gene expression analysis. Brightfield imaging, histology, and TEM demonstrated, for the first time, round cell clusters with a central lumen and intact tight junctions that is consistent with endometrial organoid reports of other species.[2] IHC demonstrated positive immunostaining for cytokeratin, a marker for epithelial cells. Preliminary findings reflected the structural similarity of canine endometrial organoids to their in vivo counterpart (with molecular and functional data pending). Findings also indicated that organoids may be a functional model that can be used to study canine uterine diseases to reduce reliance on research animals and the associated financial and welfare costs. Funding provided by the AKC CHF.

Keywords: Dogs, cell culture, in vitro, endometrial, organoid

References

1. Verstegen J, Dhaliwal G, Verstegen-Onclin K: Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: Advances in treatment and assessment of future reproductive success. Theriogenology 2008;70:364-374.
2. Thompson RE, Johnson AK, Dini P, et al: Hormone-responsive organoids from domestic mare and endangered Przewalski's horse endometrium. Reproduction 2020;160:819-831.
3. Vermeirsch H, Simoens P, Hellemans A, et al: Immunohistochemical detection of progesterone receptors in the canine uterus and their relation to sex steroid hormone levels. Theriogenology 2000;53:773-788.
4. Vermeirsch H, Simoens P, Lauwers H, et al: Immunohistochemical detection of estrogen receptors in the canine uterus and their relation to sex steroid hormone levels. Theriogenology 1999;51:729-743.

Epididymal sperm acquisition, cryopreservation, and use of epididymal frozen semen in Armenian gampr dogs

Andrea Hesser

Genesis Canine Reproduction, Carrollton, TX, USA

An intact 2-year, Armenian gampr male, was presented for reproductive consultation and semen collection after initial diagnosis of cervical trauma by a local veterinarian. Animal's injury caused both cervical pain and rear limb ataxia. Prognosis for return to full musculoskeletal and nervous function was poor. A working livestock dog that is not suitable for a companion lifestyle, the owner opted to pursue semen freezing prior to euthanasia. Manual attempts to collect semen were unsuccessful. Owner elected to castrate the dog and have an epididymal flush performed to obtain semen for freezing. An intravenous catheter was placed and the dog was anesthetized with intravenous propofol. Animal was castrated, giving special attention to obtain maximum length of the vas deferens within the spermatic cord. After castration, patient was euthanized with pentobarbital. Epididymes were dissected to remove all tissues except for the cauda epididymis. Vas deferens was flushed in retrograde manner using canine semen extender to obtain an ejaculate in a conical tube. Semen was

evaluated for sperm quantity, motility, quantity, and morphologic defects, and was in excellent form. Semen was cryopreserved in straws, and the sample yielded multiple frozen semen doses. After several months, the same owner presented a 2-year, Armenian gampr dog, in estrus and opted to use the frozen semen from the epididymal flush sample. Ovulation timing was determined via vaginal cytology, progesterone concentrations, and vaginoscopy findings; dog was bred (when maximal vaginal crenulation was observed) with 200 x 10^6 progressively motile sperm by transcervical insemination twice at 24-hour intervals. After breeding, ultrasonography was not performed. Near term radiographs revealed 2 fetuses. Planned cesarian surgery was opted, and 2 healthy pups were delivered. This case demonstrated many procedures of advanced reproductive technology that could be applied in dogs. Epididymal flush is performed in dogs by some practitioners, although successful pregnancy using epididymal semen in dogs has not yet been described. Epididymal frozen semen success is still not well understood in dogs, and recommended insemination dosing of total progressively motile sperm may vary from the standard approaches in traditionally acquired frozen semen samples.

Keywords: Epididymal flush, semen cryopreservation, transcervical insemination

Pregnancy outcome with uterine serosal inclusion cyst in a dog

Fabio Pinaffi, Jacob Rother, Sheila Megehee

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA

A 6-year, female German Shepherd that had previously whelped 3 times, was presented for failure to conceive. She had her first litter via cesarean surgery, second by natural whelping, and third by a combination of natural whelping and emergency cesarean surgery. During her last cesarean surgery, an increased amount of space was noticed between pups that required excessive manipulation of the uterus to clear. Outer (serosal) surface of the uterus had multiple cysts. Thereafter, 2 breedings (transcervical insemination [TCI] with > 300 x 10^6 progressively motile sperm [PMS] from days 2 to 4 after ovulation) attempts were unsuccessful. Ovulation was defined as the day when vaginal cytology reached > 90% superficial epithelial cells and serum progesterone concentrations were 4 to 10 ng/ml. Transabdominal ultrasonography was performed on day 30 after ovulation that revealed a slight amount of fluid within her uterus with no fetuses and multiple cysts at the distal part of uterine horns. The third breeding management included 2 breedings by TCI on days 2 and 4 after ovulation with > 300 x 10^6 PMS, intravenous oxytocin treatment (2 IU) 6 hours after each breeding and repeated every 12 hours for 24 hours, and uterine lavage using 60 ml saline by TCI scope the day after each breeding. Oxytocin treatment and uterine lavage were aimed to improve uterine clearance by inducing uterine contractions and decreasing bacterial contamination, respectively. Transabdominal ultrasonography performed on the day after breeding revealed no intraluminal uterine fluid. Dog whelped 1 pup naturally and 7 pups via cesarean surgery. The smallest pup had difficulty breathing and failed to thrive. It received intensive care but was pronounced dead within a few hours. During cesarean surgery, multiple cysts were observed grossly on the serosal surface of the distal part of uterine horns. Additionally, a uterine laceration that occurred during manipulation of the uterus (matched with the scar from the previous cesarean surgery incision) was repaired. Uterine serosal inclusion cysts (SIC) are incidental findings in multiple domestic animal species including pigs, cats, and dogs. Clinical importance is still unknown (commonly localized at uterine horns' tip), lack endocrine activity, and are not associated with the estrous cycle, and mostly occur in old multiparous dogs of large breeds. It is suggested that SIC may cause adhesion of the uterus to other organs or peritonitis by rupturing. Potential causes of SIC are overstretching of the uterus which can occur in big litters, rapid uterine contractions of the myometrium during involution, genetic predisposition, and excessive handling of the uterine horns during cesarean surgery. In the present case, SIC could have affected uterine contractility and, consequently, reduced uterine clearance after estrus and/or breeding. Outcome of this case suggested that fertility could be achieved without a major decrease in litter size using tools to improve uterine clearance (e.g. oxytocin and uterine lavage).

Keywords: Serosal inclusion cysts, uterine clearance, pregnancy, dog

Disorder of sexual development due to XX/XY chimerism in a Siberian husky female dog

Jenna Dockweiler, Takeshi Kawakami, Adam Boyko

Embark Veterinary, Inc., Boston, MA, USA

A 22-month, intact female Siberian husky, underwent genetic testing as part of a prebreeding screen. Her owner reported that she never exhibited signs of estrus. However, her littermate sister had experienced at least 2 apparently normal estrous cycles. A review of veterinary records indicated that her external genitalia appeared normal, albeit juvenile, female. She had no remarkable medical history other than an episode of nocturnal urinary incontinence that resolved without treatment. A commercially available direct-to-consumer saliva-based genetic test was performed (Embark® Breed & Health Dog DNA Test) at 3 separate times. Genotyping samples were assayed using a canine-specific research-grade microarrayplatform with > 230,000 probes, including probes to detect loci on both the X and Y chromosomes. All 3 samples failed, calling diploid genotypes across all chromosomes, suggesting that she was a chimeric dog by having 4 copies of each autosome. Furthermore, probe signals on the X and Y chromosomes suggested that she had 3 copies of X chromosome and 1 copy of Y chromosome, the pattern expected in an XX/XY chimera. This chromosomal abnormality was the likely cause of her primary anestrus. This patient will be sterilized at 24 months of age, and her gonads will be submitted for histopathology at that time. Although chimerism is not associated with known major health concerns in the dog, affected patients are likely infertile. Therefore, this patient is no longer considered a breeding candidate. This case illustrated a cost-effective method for screening for chromosomal disorders that affect sexual development in the dog.

Keywords: Genetics, disorder of sexual development, chimera, primary anestrus

Medical management of follicular cysts in a dog

Lily Lewis, Candace Lyman, Robyn Wilborn

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL

A 14-year, nulliparous English Setter dog, was presented for evaluation of persistent vulvar bleeding of 3 months duration. Despite her geriatric age, no other health concerns were reported, previous estrous cycles and interestrus intervals were consistent. Patient was bright, alert, and responsive, with an ideal body condition score and vital parameters within normal limits. Vulva was moderately enlarged. A small amount of frank blood was dripping from the vulva intermittently throughout the examination, and excessive blood staining was noted on the hind limbs. A vaginal swab cytology was performed that revealed a population of 95% superficial and anuclear cells, suggesting cellular hyperplasia secondary to estrogen. Serum progesterone concentrations were 0.2 ng/ml. Based on these diagnostic tests and clinical findings, hyperestrogenism (exogenous or endogenous) was suspected. A more detailed history of the patient's diet and environment made exogenous estrogen less likely as the cause. Duration of vulvar discharge exceeded the timeframe of expected proestrus and estrus. Thus, additional diagnostic testing was recommended to investigate an endogenous source of estrogen. Abdominal ultrasonography revealed bilateral ovarian enlargement with cystic changes noting that the left side was more severely affected than the right. Specifically, the left ovary had a large, hypoechoic, thin-walled structure within the mid ovarian body, measuring 2.25 x 1.47 cm. Right ovary had a smaller hypoechoic structure, measuring 0.33 x 1.47 cm. These structures were determined to be cystic in nature as they measured larger than expected for a normal canine ovarian follicle (2 - 8 mm). Other changes observed included generalized cystic endometrial hyperplasia and ill-defined hypoechoic splenic nodules within the splenic head. Remarkably, no evidence of bone marrow suppression was evident on a complete blood count. Thus, ovariohysterectomy was recommended, noting that a laparotomy would allow for evaluation of the splenic nodules, with a possibility for additional diagnostics or treatments (e.g. splenic biopsies, splenectomy). Although the owners declined surgical treatment, they did inquire about medical management possibility. Intramuscular human chorionic gonadotropin (Chorulon®, Merck Animal Health, USA) treatment was discussed, noting that the success rate could vary. Patient was treated with 1,000 units every 24 hours for 3 days. Seven days after treatment, the owner reported substantial decreases in the amount of vulvar bleeding. Approximately 30 days after treatment, the owner reported that the bleeding had resolved completely. Throughout this period, no adverse side effects were reported by the owner. A final follow-up was made ~ 60 days after treatment and no relapse of bleeding was reported.

Keywords: Follicular cyst, estrogen, hyperestrogenism

Effects of pituitary abscess and subsequent treatment on cyclicity in a Thoroughbred broodmare

Rachel Doenges, Maria Schnobrich, Brad Tanner

Rood and Riddle Equine Hospital, Lexington, KY, USA

A 7-year, Thoroughbred broodmare, was presented for malodorous, left-sided nasal discharge in February. Tooth 210 (left maxillary second molar) was nonvital with an open pulp horn. The tooth was extracted, and a sinus trephination performed. Antibiotic therapy was started based on culture. Mare developed intermittent fever, anisocoria, and ataxia. She was readmitted and was treated with intravenous potassium penicillin, gentamicin, flunixin meglumine, and dimethyl sulfoxide. Signs persisted despite therapy. Magnetic resonance imaging (MRI) was performed that revealed an enlarged pituitary gland with heterogeneous signal intensity and irregular margins, indicative of a pituitary abscess. Additionally, inspissated material was still present in the sinus, and there was increased signaling at the basisphenoid bone. Horses and ruminants are prone to bacterial meningitis and brain abscess secondary to systemic infection. However, brain abscess secondary to local extension has also been reported in horses. Main routes for local extension are as follows: via cranial nerves; direct inoculation, as observed with a penetrating wound; extension from adjacent structures; or hematogenous spread.[1] Based on MRI findings, we speculated that local extension occurred from the left sinus through the basisphenoid bone to the pituitary gland. A right retrobulbar abscess developed, MRI findings supported that this abscess was secondary to the pituitary abscess draining via the optic nerve. An enucleation was performed. The neurological deficits improved, and she was transitioned to oral chloramphenicol. An MRI was repeated 2 months later. Pituitary had returned to normal size, and other signs of pituitary abscess were resolved. Prior to the mare's initial presentation, the mare's reproductive tract was monitored in preparation for breeding. Transrectal ultrasonography findings were consistent with vernal transition until January 18th, when a normal ovulation was confirmed. During her hospitalization, regular reproductive ultrasonography was not performed. However, transrectal evaluation (performed after she was discharged) revealed small inactive ovaries with no uterine edema or cervical softening. Dominant follicles did not develop. This was documented from late February until August. On August 17th, a dominant follicle (38 mm) with appropriate uterine edema and cervical softening was noted, indicating return to cyclicity. The following year, the mare conceived but pregnancy was lost between 60 and 120 days. She had a successful pregnancy the subsequent year. In human medicine, hypopituitarism is commonly observed with pituitary abscess and is treated with hormone replacement therapy, as needed. Pituitary abscess in domestic animals is usually fatal. In human medicine, in addition to antibiotics and management of the primary infection site, neurosurgical drainage is normally performed.[2] Neurosurgical drainage is generally not feasible in large animals. However, in this case, apparently the drainage occurred via the optic nerve and may have contributed to the positive outcome. A bull was successfully treated for pituitary abscess; however, the bull was slaughtered due to low testosterone and teratozoospermia that did not resolve with treatment.[3] This case is notable, not only in that the mare survived but she also returned to reproductive soundness.

Keywords: Pituitary abscess, tooth root abscess, hypopituitarism, sinusitis

References

1. Smith JJ, Provost PJ, Paradis MR: Bacterial meningitis and brain abscesses secondary to infectious disease processes involving the head in horses: seven cases (1980-2001). J Am Vet Med Assoc 2004;224,739-742.
2. Zhu H, Gu XM, Hong J, et al: Successful treatment of pituitary abscess with intravenous antibiotics: a case report. Genet Mol Res 2014;13,10523-10528.
3. Braun U, Malbon A, Kochan M, et al: Computed tomographic findings and treatment of a bull with pituitary gland abscess. Acta Vet Scand 2017;8,59.

Differences in uterine bacterial community compositions and gene expression between normal and metritic Holstein dairy cows at 5 - 12 days postpartum

Tal Raz,[a,b] Ron Sicsic,[a] Shaked Druker,[c] Tamir Goshen,[c] Maor Kedmi,[c] Nahum Shpigel[a]

[a]Koret School of Veterinary Medicine; Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
[b]Krieger School of Arts & Sciences, Advanced Academic Programs (Bioinformatics), Johns Hopkins University, Baltimore, MD, USA
[c]Hachaklait, Mutual Society for Veterinary Services, Caesarea Industrial Park, Israel

Metritis is the most prevalent postpartum uterine disease in dairy cows that results in tremendous economic losses to farmers. Postpartum contamination of the uterus probably occurs in all dairy cows; however, not all animals develop metritis. Nevertheless, the pathophysiology of this disease is still not clear. In Israel, cows are routinely examined during 5 to 12 'days in milk' (DIM) and may be diagnosed with metritis if clinical findings include fetid mucopurulent vaginal discharge and transrectal palpation findings include large, gas, or fluid-filled uterus. Our objective was to utilize a large data set of 16s amplicon sequences and mRNA-Seq gene expression to: 1. describe and compare uterine bacterial load and community composition; and 2. analyze and compare endometrial gene expression, between cows with and without metritis, and between cows with different bacterial community compositions. Postpartum Holstein-Frisian dairy cows (n = 139) were examined routinely at 5 - 12 DIM, and the luminal endometrium was sampled aseptically using a transcervical sterile double-guarded cotton swab. As expected, reproductive performance parameters after the voluntary waiting period were superior in healthy cows. Uterine bacterial load (uBL), quantified by 16S rRNA gene qPCR, did not differ between healthy (n = 70) and metritis cows (n = 59). Endometrial cytological analysis revealed a bimodal distribution of polymorphonuclear cell (PMN) percentage within each group, with most cows having either very low or very high PMN (30% of cows < 4% PMN; 30 % of cows > 88% PMN), regardless of the disease status (healthy or metritis). However, the PMN percentage was correlated to the uBL. Metagenomic analysis of 16S sequencing revealed that in metritis cows, there was a typical bacterial community with higher relative abundances of the phyla Bacteroidetes (metritis: 28.1 ± 2.9%, healthy: 9.1 ± 1.6%; $p < 0.001$) and Fusobacteria (metritis: 28.3 ± 2.4%, healthy: 19.0 ± 2.8%; $p < 0.001$). In contrast, the bacterial community composition of healthy cows was more diverse. However, some healthy cows had a 'metritis-like' bacterial community, with no clinical signs of metritis. Analysis of gene expression by mRNA-seq methods indicated 152 differentially-expressed genes (DEGs) when metritis (n = 5) and healthy cows (n = 10) were compared; most were immune-related DEGs. Furthermore, comparing metritis cows (n = 5) with typical bacterial community composition to healthy cows (n = 4) with similar 'metritis-like' bacterial community composition indicated 27 DEGs, suggesting variation in uterine response to similar bacterial composition, resulting in different clinical outcomes. Further understanding of the pathophysiology of uterine diseases may lead to new preventive and therapeutic approaches.

Keywords: Metritis, dairy cows, 16s metagenomics, RNA seq, gene expression

Spontaneous pneumothorax resolved via pneumocentesis in a neonatal Alaskan Malamute

Tiffany Hoffman,[a] Morgan Agnew[b]

[a]Rutgers University, New Brunswick, NJ, USA
[b]Philadelphia Animal Hospital, Philadelphia, PA, USA

A neonatal male Alaskan Malamute pup was presented after developing acute respiratory distress shortly after delivery. Pup was delivered via cesarean surgery (performed at 66 days after LH surge). Five live pups were delivered; however, 1 with schistosomus reflexus was euthanized immediately after owner consent. Physical examination was initially unremarkable; however, shortly after resuscitation, the patient's mucous membranes and foot pads became pale blue, he started gasping and a small amount of clear fluid bubbled from his nose. Initially the patient's respiratory effort improved with oxygen supplementation and CPR. Radiographs revealed severe pneumothorax displacing the patient's heart cranially and laterally against the left lateral thorax. Pneumocentesis was performed with a 25 gauge butterfly with a 20 ml syringe attached; 24 ml air was removed from the right lateral thorax. Radiographs were repeated that revealed decreases in air in the pleural space and lungs appeared to reinflate. Oxygen therapy was continued. GV26 was stimulated using a 25 gauge needle. CPR was discontinued to avoid a perceived risk of reintroduction of air into pleural space from the force used with CPR. Radiographs were repeated (for the last time) that revealed further improvement with only a small amount of air visible around pericardium and diaphragm. Patient's clinical picture improved with a return to normal vocalization, respiratory rate, muscle tone, and mucous membrane color. Patient was discharged with the instruction to keep him separated from other pups and to monitor closely for any signs of respiratory distress. Patient was empirically treated with ceftiofur at a dose of 2.5 mg/kg, twice daily, for 7 days and has been doing well. Although this condition is widely studied in human neonates, there is limited research on acute pneumothorax and treatment in neonatal pups, In humans, symptomatic spontaneous pneumothorax (SP) occurs in 0.05 - 1% of all live born infants, and presents within the first hours of life.[1] In dogs, primary spontaneous pneumothorax (PSP) is the most common form of spontaneous pneumothorax and is typically associated with the rupture of bullae or subpleural blebs.[2] PSP is the most commonly diagnosed in healthy, younger-middle aged dogs, and is overrepresented in Siberian Huskies.[2] Additional research on the resolution of PSP in canine neonates is

warranted. This case illustrated the importance of radiographs in determining a cause for respiratory distress in neonates to help improve neonatal outcomes.

Keywords: Pneumothorax, Alaskan Malamute, respiratory distress, neonatal, pneumocentesis

References

1. Clark S, Saker F, Schneeberger M, et al: Administration of 100% oxygen does not hasten resolution of symptomatic spontaneous pneumothorax in neonates. J Perinatol 2014;34: 528-531.
2. Gilday C, Odunayo A, Hespel AM: Spontaneous pneumothorax: Pathophysiology, clinical presentation and diagnosis. Top Companion Anim Med 2021;45:100563.

Reducing the incidence of endometritis in dairy cows by hormonal regimen given to shorten the interval from calving to the first postpartum ovulation

Tal Raz,[a,b] Shaked Druker,[a,c] Ron Sicsic,[a] Alon Kaplan,[c] Maor Kedmi[c]

[a]Koret School of Veterinary Medicine; Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel
[b]Krieger School of Arts & Sciences, Advanced Academic Programs (Bioinformatics), Johns Hopkins University, Baltimore, MD, USA
[c]Hachaklait, Mutual Society for Veterinary Services, Caesarea Industrial Park, Israel

Postpartum uterine inflammatory diseases, such as metritis and endometritis, are prevalent in bovine dairy herds, resulting in major economic losses. Endometritis can be diagnosed from 21 day-in-milk (DIM) onward by evaluating the percentage of polymorphonuclear cells (PMN) in endometrial cytology, typically obtained by cytobrush and, therefore, is commonly referred to as cytological endometritis (CEM).[1] Previous studies suggested that spontaneously ovulating cows early in lactation have reduced endometritis incidence later in their lactation. Our objective was to evaluate the efficacy of 2 hormonal regimens for hastening ovulation in early lactation (treatment initiation at 24 - 27 DIM) and explore the incidence of CEM later in lactation. Holstein dairy cows (n = 450) were included in a prospective cohort study. Based on transrectal ultrasonographic examination (presence of corpus luteum, CL) combined with milk progesterone cow-side test (mP_4) at 24 - 27 DIM, cows were assigned; positive control: cows spontaneously ovulated by 24 - 27 DIM, no treatment. Cows that did not spontaneously ovulate were randomly assigned to following groups (n = 101 cows/group): Select Synch: GnRH analog and $PGF_{2\alpha}$ 7 days later; Select Synch-CIDR: GnRH analog and $PGF_{2\alpha}$ 7 days later, with 7 day CIDR; negative control: no hormonal treatment (2 saline injections 7 days apart). Cows were examined by transrectal ultrasonography 5 times (24 - 27 DIM; 31-34 DIM; 38 - 41 DIM; 45 - 49 DIM; 66 - 69 DIM). CEM diagnosis was made based on endometrial cytobrush at 38 - 41 DIM and 66 - 69 DIM, according to the criteria we recently defined.[1] At 24 - 27 DIM, 92.7% (417/450) of the cows were clearly defined as ovulating or not (CL + high mP4 versus no CL + low mP4). Of these, 114 (27%) spontaneously ovulated by 24 - 27 DIM, with overall, a higher (p = 0.0003) proportion among multiparous versus primiparous cows (33 versus 17%; OR = 2.44, 95% CI 1.49 - 4.00). Both hormonal protocols efficiently induced ovulation and increased percentage of cows ovulating during the 70 DIM voluntary waiting period (Select Synch 92.1, Select Synch-CIDR 89; negative control 71.3; p = 0.0466). Furthermore, Select Synch was associated with a lower risk for CEM than the negative control, particularly in cows without postpartum ketosis (18.6 versus 35.4%; OR = 0.42, 95% CI 0.18 - 0.90; p = 0.0295). We concluded that simple hormonal treatments given at early lactation stimulated ovarian activity and reduced the risk for subsequent endometritis in postpartum Holstein dairy cows.

Keywords: Endometritis, dairy cows, postpartum, uterus, ovulation

Reference

1. Druker SA, Sicsic R, van Straten M, et al: Cytological endometritis diagnosis in primiparous versus multiparous dairy cows. J Dairy Sci 2022;105:665-683.

Induction of parturition in embryo recipient camels carrying calves produced by somatic cell nuclear transfer

Ahmed Tibary,[a] Irfan Khan,[b] Abdelhaq Anouassi[b]

[a]Department Veterinary Clinical Science, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[b]Advanced Scientific Group, Camel Embryo Transfer Laboratory, Sweihan, UAE

Embryo transfer is an established reproductive technology in camels.[1] Pregnancies from in vitro produced embryos by somatic cell nuclear transfer (SCNT) have been reported. However, very limited data are available regarding parturition complications and calf survival. Pregnancy duration in camels is extremely variable and averages 386 days. Perinatal mortality in camels is high, and dystocia is the major predisposing factor.[2] Monitoring of pregnant recipients during parturition is essential for early veterinary intervention and calf resuscitation if needed. Induction of parturition is performed to ensure adequate monitoring during labor. Here we report preliminary results on a protocol of induction of parturition in recipients carrying calves from embryos obtained by SCNT. Parturition was induced at 380 days in 22 pregnant embryo recipients. Each recipient received an intramuscular injection of 750 µg of cloprostenol. The females were monitored hourly for onset of the second stage of parturition (appearance of the amniotic sac), duration of second stage of labor (fetal expulsion), and duration of the third stage of parturition (expulsion of the placenta). Additionally, sex, weight and health of calves were recorded. All data are reported as mean ± SEM. The interval from cloprostenol treatment to the beginning of the second stage of labor was 25.33 ± 2.18 hours (range 6 to 32 hours) with 70% of the recipients calving between 24 and 32 hours after treatment. Duration of the second stage of parturition was 25.33 ± 4.49 minutes. The

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

duration of the 3rd stage of labor was 3.31 ± 0.81 hours. One female required obstetrical assistance and retained the fetal membranes (> 12 hours). The mean weight of calves was 34.23 ± 1.47 kg (33.54 ± 1.64 for males and 35.78 ± 3.2 for females). Calves were standing and nursing withing 2.73 ± 0.6 hours after birth. The duration of the stages of parturition, the incidence of dystocia and of retained fetal membranes were similar to those reported previously by the authors for pregnancies from in vivo produced embryos.[3] These preliminary results suggested that induction of parturition using cloprostenol is a good strategy to ensure the presence of veterinary help at parturition. The majority of females are expected to calve between 24 and 32 hours after induction. Therefore, treatment should be performed early in the morning in order to obtain birth during the daylight.

Keywords: Embryo, cloning, parturition, neonatal survival

References

1. Anouassi A, Tibary A: Development of a large commercial camel embryo transfer program: 20 years of scientific research. Anim Reprod Sci 2013;136:211-221.

2. Nagy P. Reiczigel J, Barua R. et al: Pregnancy and parturition in dromedary camels III. Incidence, timing and factors affecting abortions and perinatal mortality under intensive management. Theriogenology 2023;197:322-333.

3. Tibary A, Anouassi A: Reproductive physiology in the female camelidae. In Tibary A, Anouassi A. Theriogenology in camelidae, 1st Edition, Rabat; Actes Editions: 1997. p. 208-222.

Are leukocytes in semen and poor semen quality indicators of *Brucella ovis* infection in rams?

Dinesh Dadarwal, Abby Toews, Roshan Fernandopulle, Fritz Schumann, Brooklin Hunt, Kamal Gabadage

Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon SK, Canada

Brucella ovis (B. ovis) is a nonzoonotic, infectious reproductive pathogen of sheep known to cause subfertility and infertility in rams due to inflammation of genital organs and accessory sex glands. Our objectives were to assess 1. the prevalence of *B. ovis* in rams in Saskatchewan and 2. the association between naturally occurring *B. ovis* infection and leucocyte and/or semen quality parameters. Fifty-eight rams (various breeds) from 5 producer-owned flocks in various locations in Saskatchewan were enrolled for the study carried out in June 2022. On the day of the visit, a breeding soundness evaluation (BSE) was performed. The BSE included a brief physical examination (including body condition score, 5-point scale), scrotal content assessment, scrotal circumference measurement, and semen quality (motility and sperm morphology). Gross motility of good and very good (10 x objective), individual progressive motility of > 60% (40 x objective), and > 70% morphologically normal sperm (using eosin-nigrosin stained semen smears under 100 x objective) indicated a satisfactory semen sample. Semen samples were aliquoted and submitted to Prairie Diagnostic Services for the culture of *B. ovis.* The *B. ovis* isolates were confirmed by whole genome sequencing. Continuous and binomial outcomes were compared by linear and logistic regressions (odds ratio), respectively, using Stata/BE17. P values < 0.05 were considered significant. A total of 15/58 (25.9%) rams from 2/5 flocks were positive for *B. ovis* on bacteriological culture. The scrotal circumference was, on average, 4.7 cm lower (p < 0.01, 95% confidence interval [CI] 2.2 - 5.7) in rams with BCS < 2.5 than in those with BCS > 2.5. The scrotal circumference tended to differ (p = 0.08) among breeds. Rams with leukocytes in semen had 16 times higher odds (p = 0.01, 95% CI 1.9-133.5) of diagnosed with *B. ovis* than those with no leukocytes. The *B. ovis* positive rams had only 7.5 and 6.8% odds (p < 0.01) of having > 70% morphologically normal sperm and sperm motility characterized as fair to very good, respectively. Further, the semen ejaculate of *B. ovis* positive rams had 29 times higher odds (p < 0.01, 95% CI 18.1 - 40.4) of having detached normal sperm. In conclusion, 1 in every 4 tested rams was positive for *B. ovis* on culture. Leukocytes in semen and poor semen quality (motility and morphology) are valuable indicators to identify rams for *B. ovis* testing.

Keywords: Brucellosis, ram, infectious epididymitis, infertility

Fetal ultrasonographic assessment to predict days prepartum in unsynchronized ewes

Jamie Stewart, Nicole Sugai, Sherrie Clark-Deener, Kevin Pelzer

Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Parturition induction is the treatment of choice for ewes diagnosed with pregnancy toxemia. With unknown breeding dates, parturition induction is frequently delayed due to the owner's desire to preserve fetuses' life. There is a need to better predict fetal maturity, as this may hasten the decision to induce parturition. Objective was to assess parameters that can predict when healthy ewes are near term. A group of 33 haired ewes were confirmed pregnant by transabdominal ultrasonography after an unsynchronized breeding to a single ram over a 30-day breeding period. Sample collection and evaluation began at 4 days prior to the first possible due date. Milk pH (digital pH-meter), milk protein percentage (Brix refractometer), and serum progesterone concentrations (RIA) were measured twice weekly until parturition. Transabdominal ultrasonography to assess fetal heart rate (HR), renal corticomedullary distinction (CMD), and gastrointestinal peristalsis (GIP) was performed once weekly until parturition. Parturition dates for each ewe were used to calculate the days prepartum (DPP) for each parameter measured at each collection point. Data were analyzed using R. Continuous parameters (milk pH/protein, serum progesterone concentrations, and fetal HR) were analyzed by ANOVA with a fixed variable of DPP grouped into ≤ 5, 6 - 10, 11 - 15, or 16 - 22 days. For categorical data (fetal GIP and CMD), data on DPP were analyzed as a continuous variable using ANOVA with the categorical score included as the fixed variable. Ewe was included as a random variable for all analyses. Milk pH did not differ (p = 0.72) in relation to DPP but tended to differ (p = 0.08) based on collection date which we speculate may have resulted from differing ambient temperatures. Milk protein also did not differ (p = 0.12) in relation to DPP but there were no similar effects (p = 0.86) of collection date. Fetal HR at 16 - 22 DPP (166 ± 7.2 bpm) was greater than at 6 - 10 DPP (140 ± 3.9 bpm; p = 0.007) and ≤ 5 DPP (138 ± 3.4 bpm; p = 0.001) and tended (p = 0.09) to be greater than at 11-15 DPP. Fetal HR did not differ (p ≥ 0.35) among 11-15, 6 -10, or ≤ 5 DPP. Mean DPP

for absent/occasional fetal GIP (13 ± 1.2 days) was greater than mean DPP for frequent (8.1 ± 0.8 d; p = 0.005) or continuous (5.4 ± 0.9 d; p < 0.01) GIP, which did not differ (p = 0.15) from each other. Mean DPP for absent fetal CMD (13 ± 2.0 days) was greater than mean DPP for distinct CMD (7.7 ± 0.9 d; Mean DPP for indistinct CMD (8.6 ± 0.9 d) did not differ (p = 0.04) from absent (p = 0.13) or distinct (p = 0.81) CMD. Results presented herein demonstrated that ultrasonographic evaluation of fetal HR, GIP, and CMD can predict when healthy ewes are within ~ 1.5 - 2 weeks from their due dates. In a field setting, milk pH proved to be an unreliable measurement; however, further investigation is necessary to assess its measurement in controlled temperature (e.g. in a laboratory).

Keywords: Ewe, fetus, milk pH, parturition, ultrasonography

Agreement between evaluators assessing bull sperm morphology by conventional methods versus video capture

Jenifer Koziol,[a] Laura Waugh,[a] Ryan Williams,[a] Leo Brito,[b] Colin Palmer,[c] Chance Armstrong[d]

[a]School of Veterinary Medicine, Texas Tech University, Amarillo, TX, USA
[b]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
[c]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada
[d]College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Sperm morphology assessment has been an important aspect of bull breeding soundness evaluation and has been reported as one of the most significant reasons that bulls fail evaluation. Objective was to determine the agreement between skilled evaluators assessing bull sperm morphology by conventional methods versus video capture. The impetus behind this study was that though intra-evaluator and inter-evaluator experiments have been conducted, no studies have been reported comparing reading of stained morphology slides via microscope versus evaluation of video captured by microscopic evaluation at the same power. For this experiment semen was collected from 6 bulls and 5 eosin-nigrosin stained slides were made per bull. One slide was utilized for video capture at 1,000 x oil-immersion utilizing a Nikon DS-Fi3 high-definition color microscope camera taking care to make sure that the slide was in focus during image capture. Once 100 cells had been video captured, the video captured sperm were labeled 1 - 100 ensuring that all sperm captured were clearly in focus and fully visible on the screen using the video capture software. Care was taken to ensure that the video captured cells were representative of the entire slide. This process was repeated for 6 bulls. Following imaging, 1 slide and 1 video per bull were sent to 4 independent board-certified theriogenologists with substantial expertise in evaluation of bovine sperm morphology. Eosin-nigrosin slides and video were coded for anonymity so that one would not influence the evaluation of the other. The 4 evaluators were requested to review slides using their preferred individual methods for reviewing stained slides and assess 100 cells at 1,000 x magnification under oil-immersion and categorize per Society for Theriogenology morphology reporting standards. For the videos, cells were numbered as described above and evaluators were asked to categorize as they did for stained slides. Evaluators had the opportunity to add additional comments to their evaluator sheets. Data were analyzed utilizing R stats package utilizing a mixed model ANOVA. There was a difference (p = 0.005) between evaluators evaluating the video captures versus using conventional methods. The coefficient value of -7.568 suggested that the video capture leads to less variability among the evaluators; with a standard error of 2.119, the results were proven to be precise. However, following comparison between conventional versus video capture, evaluators declared more cells as normal. This was most evident in 1 bull that suffered from high levels of diadem defects. This defect was described by evaluators during conventional evaluation but was not identified with the safe frequency on video capture which led to a large discrepancy in morphologic classification numbers. Consequently, it could be inferred that although video capture offers a greater precision between evaluators, conventional methods provide increased accuracy as evaluators are able to fine focus on sperm defects enabling them to identify those defects that were not as easily identified (e.g. diadem defect) for correct categorization.

Keywords: Bovine, bull, sperm morphology, assessment

Effect of season on gestation length and parturition interval in alpacas

Salman Waqas, Eduardo Arroyo, Ahmed Tibary

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

Alpacas are considered nonseasonal breeders although nutritional deficiencies and harsh environment can dictate seasonality. Gestation length in alpacas ranges from 320 to 390 days. Objective was to analyze the hypothesis that mating season (Spring: March - May, Summer: June - August, Fall: September - November, Winter: December - January) affects gestation length and subsequent parturition interval in alpacas. Records on 978 gestations from 339 Huacaya females in a large Pacific Northwest herd were analyzed. All females started their reproductive career between 13 and 16 months of age and all postpartum breeding was initiated 2 to 3 weeks after parturition. Only gestations that were between 320 and 390 days in length and resulted in an offspring that survived by 3 weeks postpartum were included. Data were analyzed by descriptive statistics and one-way ANOVA. The means were compared using Tukey's test and presented as mean ± SEM. Pregnancy rate of mated females was 82.4% with a mean number of services per pregnancy of 1.8 ± 0.2. The overall mean gestation length was 342.24 ± 0.31 days with a median of 341 days. Fifty percent of gestation lengths were between 336-348 days. The season of breeding had effect (p < 0.0001) on the length of gestation. The gestation length for spring breeding (349.27 ± 1.07 days) was significantly longer than for summer breeding (342.94 ± 0.43 days), winter breeding (341.98 ± 1.33 days) and fall breeding (339.06 ± 0.43 days). Gestation length for summer breeding and winter breeding did not differ significantly from each other. Gestation length for fall breeding was the shortest but did not differ significantly from that of winter breeding. In multiparous females, the interval between parturitions ranged from 334 to 1,412 days with a median of 367 and mean of 418.67 ± 5.87 days. Fifty percent of parturition intervals ranged from 355 to 407 days. The season of preceding parturition had no effect (p = 0.529) on subsequent

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

parturition interval. The subsequent parturition interval was 433.9 ± 11.6, 416.18 ± 9.07, 413.3 ± 12.2 and 413.46 ± 8.63 for the preceding fall, winter, spring and summer birthing, respectively. The record analysis from this alpaca herd provided information that can help in establishment of herd reproductive efficiency and productivity benchmarks. These data confirmed the effect of season on pregnancy length and illustrate that, in well managed herds, pregnancy rate and interval between parturitions are better than previously reported.[1,2] A more complete analysis of reproductive records from several large herds will allow a better characterization of areas that theriogenologists can focus on to improve herd productivity.

Keywords: Alpacas, pregnancy, parturition, seasonality, productivity

References

1. Cruz A, Cervantes I, Burgos A, et al: Estimation of genetic parameters for reproductive traits in alpacas. Anim Reprod Sci 2015;163:48-55.

2. Davis GH, Wuliji T, Moore GH, et al: Growth, reproduction and fiber production of alpacas imported from Chile. Proc NZ Soc Anim Prod 1991; p. 255-258.

Delayed insemination with sexed semen relative to estrus improved pregnancy in beef heifers

Rachel Hanson,[a,b] Ramanathan Kasimanickam,[b] Vanmathy Kasimanickam,[b,c] Kamron Ratzburg,[b,d] Kate Madsen[b,e]

[a]Animal Health Clinic, Blackfoot, ID, USA
[b]College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[c]AARVEE Animal Biotech LLC, Corvallis, OR, USA
[d]Marias Veterinary Clinic, Shelby, MT, USA
[e]Mountain View Veterinary Service, Twin Bridges, MT, USA

Sexed semen (SS) has been designed for the deliberate production of genetically superior calves of the desired sex, thereby accelerating genetic progress in a cattle operation. Initially, SS was recommended for inseminating nulliparous heifers at 12 hours after the first estrus sign. However, delayed (> 12 hours) insemination with SS relative to the onset of standing estrus resulted in an increased pregnancy rate/AI (PR/AI) in dairy cattle. In beef cattle, ovulation occurs at 32 hours after the onset of estrus. Given that oocyte lifespan is constant (~ 10 hours after ovulation), the optimal interval between insemination and ovulation is vital to allow adequate time for sperm transport and capacitation in the female reproductive tract. Objectives were to 1. determine the effect of timing of SS insemination after estrus onset and AI sire on PR/AI in beef heifers and 2. elucidate the sire differences in postthaw sperm DNA fragmentation percentage (%SDF) at hours 0, 12, and 24. Angus beef heifers (n = 891) from 3 locations were blocked by age, BCS, and RTS. The CIDR + Select Synch program was implemented to synchronize estrus in all heifers. On day 0, heifers received a 1.3 g progesterone intravaginal insert (CIDR), plus 100 µg of intramuscular GnRH (gonadorelin diacetate tetrahydrate; 2 ml). Seven days later CIDR devices were removed, estrus detection patches were applied, and 25 mg of intramuscular $PGF_{2\alpha}$ (high concentration dinoprost; 2 ml) was given. Heifers were observed thrice daily for estrus (visual + Estrus Alert patch status) until 96 hours after $PGF_{2\alpha}$ treatment. Heifers that expressed estrus were randomly assigned to receive insemination at 12, 20, or 28 hours after the first estrus expression. SS (SexedULTRA™ 4M) containing y chromosome-bearing sperm from 3 bulls was utilized. Pregnancy was diagnosed by ultrasonography between 30 and 60 days after AI. Postthaw microscopic percentages SDF at 0, 12, and 24 hours were evaluated for each bull using the acridine orange staining method. Data were analyzed by mixed model. The PR/AI percentage did differ ($p = 0.05$) among insemination time, 12 hours: 46.5 (139/299), 20 hours: 47.7 (142/298), and 28 hours: 55.8 (164/294), respectively. The PR/AI between 12 and 28 hours and between 20 and 28 hours differed ($p = 0.02$) but did not differ ($p = 0.78$) between 12 and 20 hours. No differences ($p = 0.20$) in PR/AI percentages among bulls and locations (bull 1: 47.9 [138/288]; bull 2: 47.7 [143/300]; bull 3: 54.1 [164/303]). An interaction ($p = 0.04$) of time of insemination by bull for PR/AI was detected. For bull 1, percent SDF at 0 and 24 hours postthaw did differ ($p < 0.05$), 2.2 and 6.4. For bull 2, postthaw percent SDF at 24 hours (7.9) was greater ($p < 0.05$) compared to 0 hour (1.3) and 12 hours (4.6). For bull 3, postthaw percent SDF did not differ ($p > 0.1$) among 0, 12, and 24 hours (1.2, 1.9, and 2.4, respectively). In conclusion, the time of insemination with SS when delayed to 28 hours after the first estrus expression resulted in improved pregnancy. Lower PR/AI for heifers in 12 and 20 hour groups compared to 28 hour group was plausibly due to an increase in bull's postthaw percent SDF over time and the time interval between insemination and ovulation.

Keywords: Beef heifers, sexed semen, estrus onset, insemination, sperm DNA fragmentation, pregnancy

Periparturient plasma prolactin concentrations and maternal behavior in beef cattle

Rory Nevard,[a,b] Sameer Pant,[a,b] John Broster,[a,b] Scott Norman,[a,b,c] Cyril Stephen[a,b]

[a]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia
[b]Gulbali Centre for Agriculture, Environment and Water, Wagga Wagga, NSW, Australia,
[c]Kallangur Veterinary Surgery, Kallangur, Queensland, Australia

Maternal ability of a cow contributes to calf health and survival, particularly in extensive beef production systems. Improving maternal behavior could mitigate calf wastage, and therefore, characterizing physiological drivers of superior maternal behavior, and its objective assessment thereof are of interest. Whilst the hormonal milieu around calving is complex, past studies have demonstrated that periparturient blood prolactin concentrations are a key component. It is yet to be established whether plasma prolactin concentrations can be used to predict maternal behavior postcalving in beef cattle. Use of radio telemetry and activity meters have afforded novel opportunities for objective behavioral quantification in extensive systems, and therefore the primary objective was to evaluate the relationship between periparturient prolactin concentrations (PPC) and maternal behavior of cows assessed both subjectively via maternal behavior scores (MBS), and objectively via measuring cow-calf contact using proximity loggers. Measures of maternal behavior and PPC were also compared against transfer of passive immunity (TPI) and calf

performance measures such as weaning weights (WW) and average daily gain (ADG). It was predicted that periparturient plasma prolactin concentrations are correlated to cow-calf contact in the postpartum period and calf health parameters. Thirty-one multiparous Angus cows (age 9 - 11 years) were selected after a single-sire controlled mating period. A month prior to the expected calving dates, cows were fitted with ultra-high frequency proximity logger collars provided by SIRTRACK® (now LOTEK, Havelock North, NZ) and had blood collected for assessment of PPC. On the day of calving, calves were fitted with proximity logger collars and MBS was recorded based on the cow's response to calf handling. Throughout the calving period, cows were yarded, and blood was continually collected every second or third day. A commercial bovine prolactin ELISA kit (AssayGenie) was used to measure PPC. Area Under the Curve (AUC) analysis was performed to quantify PPC, which was then used to assess correlation with MBS and cow-calf proximity logger contact data. The loggers recorded between 702 - 1,893 contacts (mean 1,394, SD 294, ± SEM 54) and between 47,884 - 350,360 seconds of total contact time (mean 178,694, SD 97,366, ± SEM 17,777), Cow-calf contact over 12 days was negatively correlated with PPC ($r = -0.45$; $p = 0.012$). Precalving PPC was also weakly negatively correlated with MBS ($r = -0.31$; $p = 0.043$). Neither cow-calf contact nor MBS were correlated ($p > 0.05$) with calf performance (WW, ADG) or health (Calf Vigour Scores, serum total protein 24 - 72 hours postcalving). Average weaning weights of calves grouped according to their dams' assigned MBS at birth tended to be greater for higher MBS, although not significant ($p > 0.05$). Overall, results indicated that PPC could influence cow-calf interactions and MBS. However, further research is warranted to validate these results, and to evaluate the efficacy of using cow-calf contact data as an objective measure of maternal behavior.

Keywords: Maternal behavior, beef cattle, calving, prolactin, proximity logger, calf health

Surgical excision of a leiomyoma in a subfertile Morgan mare

Rachel Doenges, Maria Schnobrich

Rood and Riddle Equine Hospital, Lexington, KY, USA

A 6-year, Morgan mare, was presented for subfertility. During the previous breeding season, the mare conceived but could not maintain a pregnancy. Referring veterinarian felt a mass associated with left uterine horn prior to breeding and owner was not interested in referral. On transrectal ultrasonography, a 3.2 cm firm mass was noted at the left uterine horn tip (as an intramural mass) that was observed during hysteroscopy. Owner opted for surgical resection in hopes of improving mare's fertility. Mare was placed in dorsal recumbency, and a midline incision was made. Left uterine horn was exteriorized allowing visualization of the mass at the distal end. An incision was made over the mass. This incision extended through the serosa and the myometrium down to the mass. Mass was well encapsulated, making it possible to easily dissect around it. Mass was removed from the uterine wall, except for a small portion that required incision into the uterine horn for complete removal. Histopathology confirmed the presumptive diagnosis of leiomyoma (spindle cells arranged in intersecting streams and bundles and low mitotic rate [1 per 10, 400 x fields]). Histopathology also confirmed that clean margins were achieved with normal myometrium surrounding the mass without any evidence of infiltration of neoplastic cells. Mare's recovery was uneventful. Mare was treated with intravenous flunixin meglumine, potassium penicillin, and gentamicin. She was discharged on sulfadiazine/trimethoprim. A full recovery was made with no reported complications. Occurrence of uterine tumors is low in horses;[1] however, leiomyoma is the most common uterine tumors in mares, accounting for 26% of uterine neoplasia.[2,3] Leiomyomas are usually singular masses ranging 2 - 5 cm in diameter, but multiple masses in the same mare can occur.[1,2] Surgical excision is usually curative; however, there have been instances of recurrence, including a report of 2 half-sibling Appaloosa mares that developed cervical masses despite ovariohysterectomy.[4] These cases also suggested a familial predisposition.[4] Leiomyomas are commonly pedunculated but can be intramural as in this case.[3] Although leiomyoma is a benign neoplasia, these masses can negatively affect fertility. For this reason, surgical excision is indicated in broodmares with fertility issues.[3] In contrast, removal is not necessary in mares not used for breeding as long as there is not hemorrhage or recurrent secondary endometritis.[3] With pedunculated leiomyomas, removal can be performed by incision at the base of the stalk. This has been accomplished intrauterine using a snare or electrosurgical loop.[5] Removal of intramural masses proves more difficult and often requires partial hysterectomy or ovariohysterectomy, both negatively impact reproductive potential. However, mares with partial hysterectomies have gone on to produce healthy foals.[6] In this case, preservation of the left ovary and uterine horn is a positive prognostic indicator for future reproductive success.

Keywords: Mare, leiomyoma, uterine tumor

References

1. Hurtgen JP: Uterine abnormalities. In: McKinnon AO, Squires EL, Vaala WE, et al: editors. Equine Reproduction. 2nd edition, Ames; Wiley-Blackwell: 2011. p. 2671.
2. Card CE: Endoscopic examination. In: McKinnon AO, Squires EL, Vaala WE, et al: editors. Equine Reproduction. 2nd edition, Ames; Wiley-Blackwell: 2011. p. 1947-1948.
3. McCue PM: Neoplasia of the female reproductive tract. Vet Clin North Am Equine Pract 1998;14:510-511.
4. Romagnoli WE, Momont HW, Hilbert BJ, et al: Multiple recurring uterocervical leiomyomas in half-sibling Appaloosa fillies. J Am Vet Med Assoc 1987;191:1449-1450.
5. LeBlanc MM: Diseases of the reproductive system: the mare. In: Colahan PT, Mayhew IG, Merritt IG, et al: editors. Equine Medicine and Surgery, 4th edition, Goleta: American Veterinary Publications: 1991. p. 1036.
6. Santschi EM, Slone DE: Successful pregnancy after partial hysterectomy in two mares. J Am Vet Med Assoc 1994;205:1180-1181.

Determining the volume of blood in a stallion's ejaculate

Dale Kelley,[a] Hannah Maxwell,[a] Carly Turner-Garcia,[b] Reed Holyoak[a]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]Lazy E Ranch, Guthrie, OK, USA

Hemospermia can have a detrimental effect on fertility depending on the amount of blood present in an ejaculate, with 5% having no impact on fertility.[1] Estimating the amount of blood in an ejaculate may aid in decision making on whether to utilize the semen for breeding or attempt further processing. Purpose of this experiment was to determine a method to estimate the percent of blood contamination in an ejaculate. First portion of the experiment used a mathematical simulation to predict the packed cell volume (PCV) of a hemospermic ejaculate based on the PCV of whole blood. The PCV of the blood was assumed to be either 30, 35, or 40% and range of blood and semen dilutions from 0 to 100% was modeled for each assumed blood PCV by multiplying the percent dilution of the blood semen combination by the blood PCV to give a predicted PCV for the semen. A regression line was

plotted for each simulation, and the equation of the line calculated. This produced the following equation:

Percent of blood in ejaculate = [PCV semen+(PCV blood/100)] ÷ (PCV blood/100)

Second portion of this experiment compared the actual percent of blood in the ejaculate to predicted percent of blood in the ejaculate based on PCV of blood and hemospermic sample. Semen was collected from 1 stallion and blood was drawn from the jugular vein of 10 donor horses into 10-ml EDTA tubes using a vacutainer needle. Blood was loaded into microcapillary tubes and centrifuged (10,030 x g for 5 minutes) and PCV was determined using hematocrit reader chart. Blood from each donor was added to semen at 5, 10, 20, 40 and 50% (v/v) to create 0.5-ml aliquots. Raw semen and blood semen dilutions were loaded into microcapillary tubes, centrifuged, and measured. The above equation was used to predict the percent of blood in the ejaculate based on the PCV of whole blood and blood-semen combination. SAS (version 9.4) SGPlot procedure was used to generate a Bland-Altman plot of the difference and mean of the actual percent of blood contamination and predicted percent of blood contamination. Student's t-test was performed for zero bias using actual and predicted values and independence of bias was tested using the Corr procedure using the bias (difference between actual and predicted) and magnitude (average of actual and predicted). Outliers (n = 2) were removed and data were reanalyzed when standard deviations > 3. Semen had no evidence of hemospermia based on the color after collection and centrifugation of microcapillary tubes. The mean difference between the actual percent of blood and predicted was - 0.59 ± 0.51. The test for zero bias (actual versus predicted values) was not different ($p = 0.26$) and there was no correlation ($p = 0.80$) in the independence of bias. These data suggested that the amount of blood contamination in semen can be estimated based on the PCV of the stallion's blood and PCV of the ejaculate.

Keywords: Stallion, ejaculate, hemospermia, packed cell volume

Reference

1. Turner CE, Walbornn SR, Blanchard TL et al: The effect of two levels of hemospermia on stallion fertility. Theriogenology 2016;86: 1399-1402.

Prepartum hemorrhage in a warmblood mare

Erin Newkirk,[a] Stephanie Walbornn[b]

[a]WellGrove Equine, Loxahatchee, FL, USA
[b]Rood and Riddle Equine Hospital in Wellington, Wellington, FL, USA

Periparturient hemorrhage (PPH) is a potentially serious, life-threatening complication of pregnancy in mares. Hemorrhage usually occurs at foaling or within 48 hours. Prepartum hemorrhage has been described to occur in up to 14% of cases, with fatality range of 40 - 100%.[1,2] Early recognition of hemorrhage with prompt aggressive medical management appears to be related to successful treatment of the condition. A case of prepartum hemorrhage in a 19-year, multiparous warmblood mare, is described. Mare presented at 324 days of pregnancy for acute onset of colic that was poorly responded to routine medical management including nonsteroidals, antispasmodics, and sedation. Transrectal palpation and nasogastric intubation at presentation were within normal limits for a late-pregnant mare. Transabdominal ultrasonography using the FLASH protocol[3] was performed due to lack of response to treatment. Swirling echogenic free-fluid was noted in the left mid-abdomen that was consistent with hemoperitoneum. Supportive care was instituted on farm that included intravenous fluids, aminocaproic acid and yunnan baiyao treatment. Resolution of overt colic signs were noted within 12 hours of initial presentation. Mare was started on trimethoprim sulfamethoxazole and gentamicin sulfate for antimicrobial coverage and maintained on flunixin meglumine every 12 hours. She received intravenous fluids supplemented with calcium, potassium, dextrose, and vitamin B12 at 48 hours after initial colic due to continued depression, poor appetite, intermittent fever, and abnormalities on blood chemistry. Mare became colicky at 72 hours after initial presentation. Ultrasonography revealed distended stomach and sluggish small intestinal motility with no free intraabdominal fluid and 28L of gastric reflux. Fetus was active with heart rate between 90 -100 beats per minute. She was subsequently referred to Rood and Riddle Equine Hospital for further medical support and evaluation. On further workup, no hematoma was felt on transrectal palpation or observed via ultrasonography. Transabdominal ultrasonography findings were within normal limits with fetal heart rate were ~ 90 beats per minute. Mare was treated with intravenous fluids, gastroprotectants, and antibiotics were changed to ceftiofur and rifampin. She was discharged 5 days later. At 342 days of pregnancy, the mare entered labor and foaled without assistance or complications. No attempt was made to breed the mare the same season as foaling, however the following year the mare was managed via embryo transfer.

Keywords: Prepartum hemorrhage, periparturient hemorrhage

References

1. Dechant JE, Nieto JE, Le Jeune SS: Hemoperitoneum in horses: 67 cases (1989-2004). J Am Vet Med Assoc 2006;229:253-258.
2. Arnold CE, Payne M, Thompson JA, et al: Periparturient hemorrhage in mares: 73 cases (1998-2005). J Am Vet Med Assoc 2008;232:1345-1351.
3. Busoni V, De Busscher V, Lopez D, et al: Evaluation of a protocol for fast localised abdominal sonography of horses (FLASH) admitted for colic. Vet J 2011;188:77-82.

Assessment of methods to activate epididymal sperm motility

Giorgia Podico,[a] Victor Absalón-Medina,[b,c] Igor Canisso[a]

[a]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA*
[b]*Department of Animal Science, College of Food, Agricultural, and Environmental Science, The Ohio State University, Columbus, OH, USA*
[c]*ST Genetics, South Charleston, OH, USA*

Horse epididymal sperm have satisfactory motility activation (Mot-Act) after harvesting and cryopreservation; however, donkeys typically display poor Mot-Act despite high-plasma-membrane integrity (PMI) with high-mitochondrial-membrane-potential (ΔΨM). Mot-Act can be achieved in murine epididymal sperm with centrifugation by removal of sperm-bound seminal plasma (SP) proteins; also, in other mammals, the dilution of epididymal sperm with SP promotes Mot-Act. Dilution of cryopreserved sperm with an extender without cryoprotectants aids Mot-Act in other species. It is possible that these methods can enhance donkey and horse epididymal Mot-Act and that there might be positive interactions between methods; however, these hypotheses have yet to be tested. Single-layer colloid-centrifugation (SLC) is a powerful tool to remove sperm-bound particles. Thus, we assessed methods and potential interactions to promote Mot-Act in epididymal sperm by comparing an equid with satisfactory Mot-Act (i.e. horse) with another poor Mot-Act (i.e. donkey). Mot-Act, PMI, and ΔΨM were assessed by submitting horse and donkey cryopreserved sperm to reextension (1:3) in the same egg-yolk extender used for cryopreservation (BC), also the same extender lacking cryoprotectants (CPA), and donkey- and horse-SP. The Mot-Act, PMI, and ΔΨM were carried out 10-, 30-, and 60-minutes after incubation. Donkey and horse epididymal sperm had lower ($p < 0.05$) Mot-Act, PMI, and ΔΨM when diluted with donkey- and horse-SP. Conversely, Mot-Act was similar ($p > 0.05$) among horse-SP, BC, and CPA and increased ($p < 0.05$) after 10 minutes of incubation. PMI and ΔΨM were not different ($p > 0.05$) among groups in horse epididymal sperm. Similar processing methods were then applied before and after SLC for cryopreserved horse and donkey epididymal sperm; Mot-Act, PMI, and ΔΨM were assessed. Donkey epididymal sperm had higher ($p < 0.05$) Mot-Act when diluted in the same egg-yolk extender compared to donkey-SP or CPA. Horse epididymal sperm had lower ($p < 0.05$) PMI and ΔΨM when diluted in donkey-SP compared to BC and horse-SP. Sperm recovery after SLC was similar ($p > 0.05$) between donkey and horse epididymal sperm. Zona-pellucida binding was carried out as an in vitro functional assessment of Mot-Act, as it is necessary for fertilization. Dilution with SP from either species' sperm did not enhance ($p > 0.05$) zona-pellucida binding as in other mammals. Previously, it was suggested that donkey sperm does not bind to heterologous zona-pellucida (i.e. bovine); however, herein, it did bind similarly to horse sperm ($p < 0.05$). Findings suggested that donkey epididymal sperm can have enhanced Mot-Act by reextension with BC unlike other standard methods. Furthermore, this is the first report to demonstrate that donkey sperm binds to heterologous zona-pellucida.

Keywords: Equid epididymal sperm, single-layer centrifugation, zona-pellucida binding assay

Two manual noncommercial methods to prepare platelet-rich plasma in donkeys (*Equus asinus*)

Lorenzo Segabinazzi, Cynthia Xue, Alexis Hall, Silvia Marchi, Patrice Bernier, Robert Gilbert, Hilari French

Ross University School of Veterinary Medicine, P.O. Box 334, Basseterre, Saint Kitts

Platelet-rich plasma (PRP) is a biologic, whole blood (WB) derived, immunomodulatory therapy that has gained popularity in broodmare programs for its potential to mitigate postbreeding uterine inflammation/infection and enhance fertility rates. In jennies, increased susceptibility to postbreeding endometritis and poor fertility rates have been associated with insemination using frozen-thawed jack semen. Because many donkey breeds are threatened with extinction, frozen semen technology offers a viable chance at recovery. Thus, PRP may have potential in similarly benefitting donkey breeding programs. However, the majority of PRP protocols previously described in donkeys have not undergone critical assessment. Commercial and noncommercial PRP production methods exist, but the latter confers advantages (e.g. field applicability and decreased cost) for a species more likely to receive veterinary care in resource-limited settings. Therefore, the objective was to evaluate 2 manual noncommercial methods of producing PRP (double centrifugation [DC] and single centrifugation [SC]) in 6 healthy adult donkeys (n = 3 jennies; n = 3 johns; ages 4 - 8 years) undergoing 3 blood collections 3 weeks apart. For DC-PRP, WB collected in a 150 ml blood transfusion bag containing citrate-phosphate-dextrose solution with adenine was split into 50 ml tubes and centrifuged at 400 × g for 15 minutes. Plasma was transferred in 15 ml conical tubes for centrifugation at 1,000 × g for 10 minutes, then the bottom 2.5 ml of plasma fraction from the second centrifugation was preserved as DC-PRP with the remaining plasma supernatant as DC platelet-poor plasma (PPP). For SC-PRP, WB was collected in 2.7 ml sodium citrate vacutainer tubes, centrifuged at 120 × g for 10 minutes, and the top third supernatant plasma fraction subsequently deemed as SC-PPP with the remaining lower plasma fraction as SC-PRP. Concentrations of platelets, leukocytes (WBC), and erythrocytes (RBC), and platelet-derived growth factor-BB (PDGF-BB) were assessed in the WB and extracted PRP and PPP. Data were analyzed by Friedman and Dunn's as a post hoc test with significance as $p < 0.05$. Both protocols increased platelet concentration (1.7 - 5, 2-fold), reduced WBC (1.1 - 50, 4-fold), and decreased RBC (at least 829-fold) compared to WB ($p < 0.0001$). Activity of PDGF-BB in DC-PRP and SC-PRP increased ($p < 0.003$) by 1.2 -7.0-fold compared to WB Platelet concentration and PDGF-BB activity increased 3-fold ($p < 0.0001$) and 5.8-fold ($p = 0.0007$), respectively, in DC-PRP than SC-PRP. Leukocyte concentrations were wider ($p < 0.0001$) in DC-PRP than SC-PRP but were not different ($p = 0.61$) between WB and DC-PRP Reductions in WBC and RBC were 42-fold and 22-fold greater, respectively, in SC-PRP than in DC-PRP ($p < 0.0001$). These results indicated that noncommercial methods of PRP preparation described in horses are applicable to donkeys. Double centrifugation yielded higher platelet concentrations and PDGF-BB activity despite greater WBC and RBC contamination than PRP processing via SC. Future studies are needed to determine in vivo efficacy of PRP in donkeys.

Keywords: Endometritis, jennies, frozen semen, blood therapy

Colostrum quality associations with passive immunity transfer in foals

Humberto Magalhaes, Gustavo Araujo, Ilaria Colombo, Kianna Spencer, Giorgia Podico, Igor Canisso

Department of Veterinary Clinical Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA

Equine epitheliochorial placentation precludes the transfer of passive immunity (TPI) from mare to fetus; thus, colostrum serves as the sole source of TPI. Colostrum assessment at foaling with a Brix-refractometer is the primary method used in practice to determine colostrum quality and its potential for a complete TPI (> 800 mg/dl); however, guidelines have not been critically established. In addition, as new tools become available to monitor mammary gland secretions to detect impending foaling (e.g. handheld pH and conductivity devices), it would be helpful to determine if those devices can also add value to the colostrum evaluation at foaling. Objective was to determine whether pH, conductivity, and Brix-index at foaling can be used to predict TPI. Colostrum of periparturient mares (n = 114) were assessed at foaling for pH, conductivity, and Brix-refractometer (Laqua-Twin-Horiba, Japan). Foaling was assisted, and time to stand and time to the nurse were recorded. At 24 hours after foaling, foals had a complete blood cell count and TPI assessment using a commercial analyzer (ARS, Foal-IgG-analyzer). Foals were grouped based on IgG concentrations as complete-TPI (i.e. ≥ 800 mg/dl) and failed-TPI (i.e. < 800 mg/dl). Data were analyzed for normality with Shapiro-Wilk's test and multiple logistic regression. Cutoff values for the Brix-index (> 24.4%), conductivity (< 4.5), and pH (< 6.4) were determined by receiver operating characteristics (ROC). Sensitivity, specificity, positive predictive value (PPP), and negative predictive value (NPV) for the Brix-index, conductivity, and pH of the colostrum at foaling were calculated to predict the TPI. Of all foals, 74.5% (85/114) had complete TPI and 25.4% (29/114) failed. Foals with complete-TPI had IgG (1,912 ± 278.1mg/dl) four-fold higher ($p < 0.0001$) than those with failed-TPI (480.4 ± 260.6 mg/dl). Their Brix-indexes were 26.5 ± 4.3 versus 21.4 ± 5.9 ($p = 0.0007$), respectively. Conductivity and pH differed ($p = 0.007$ and $p = 0.02$, respectively) between complete- and failed-TPI (conductivity 4.4 ± 0.6 versus 5.4 ± 11.7 and pH 6.2 ± 0.2 versus 6.5 ± 0.4, respectively). There were strong associations between conductivity and pH ($r = 0.87$) and a negative and strong correlation between pH, conductivity, and the Brix-index ($r = -0.87$; $r = -0.78$). IgG Concentra rations were moderately correlated with RBC ($r = 0.70$) and the Brix-index ($r = 0.71$). Sensitivity, specificity, PPP, and NPV of the Brix-index, conductivity, and pH of colostrum at foaling to predict the TPI were 0.80, 0.76, 0.95, and 0.38; 0.77, 0.71, 0.93, and 0.38; 0.75, 0.37, 0.71, and 0.42, respectively. TPI of Brix-index coupled with conductivity or pH presented a sensitivity, specificity, PPP, and NPV of 0.85, 0.60, 0.89, and 0.40 ($p = 0.02$), and 0.83, 0.50, 0.89, and 0.37 ($p = 0.01$), respectively. Combining conductivity and pH resulted in a sensitivity 0.91, specificity 0.91, PPP 0.33, and NPV 0.33. In conclusion, colostrum's Brix-index, pH, and conductivity are closely linked with TPI in foals; ROC-values could indicate if a foal could benefit from colostrum supplementation or will likely need hyperimmune plasma.

Keywords: Equine colostrum, quality, immunoglobulin G

Commensal virome of the equine uterus

Lulu Guo,[a] Udaya Desilva,[b] Reed Holyoak[a]

[a]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]Department of Animal and Food Science, Oklahoma State University, Stillwater, OK, USA

Our aim was to classify and characterize the resident uterine virome of healthy mares. We determined the presence of a resident virome in the healthy uterus with the objective to elucidate the association between the microbiome and the virome within the uterus. Although the vaginal microbiome of mares has been well studied, very few investigations of the healthy uterine microbiome have been conducted due to the uterus long being considered a sterile environment. We recently established that the healthy equine uterus harbors a rich and diverse microbiome. As an obvious next step in elucidation of the equine uterine microbiome, we searched for the presence of an endometrial virome within healthy mares' uterus. We were also interested in the presence of a bacteriophage community within the uterus that could be harnessed to control pathogenic bacteria in lieu of commercial antibiotics to which many organisms have developed a resistance. Small volume lavage samples were filtered through 0.45- and 0.22-µm filters to remove bacteria, DNAse and RNAse treated to remove free nucleic acids, and proteinase treated to dissolve viral capsids. The remaining nucleic acids were whole genome amplified and subjected to metagenomic Next Generation Sequence analysis (mNGS). Subsequent bioinformatic analysis revealed the presence of a robust virome including several hitherto unknown bacteriophages. We also established some host microbe bacteriophage relationships. A preliminary analysis of the identified viruses is presented. Our analysis demonstrated the presence of both RNA and DNA viruses belonging to 11 classes, 11 orders, 14 families, 8 genera, 7 phyla, and 29 species. The core host species predicted for the viruses identified in this study perfectly correspond to the uterine microbiome described by us in a previous study. Our research not only revealed the existence of a native virome, but also the presence of antibiotic resistance genes involved with resistance to 1 or more antibiotics. We anticipate that our research initiative will provide a foundation for researchers to explore the reproductive tract virome in other mammalian species.

Keywords: Virome, bacteriophage, horse, endometrium, uterus, microbiome

Antiluteogenic effects of cloprostenol in cycling mares

Sabrina Cousseau,[a] Erin Oberhaus,[b] Carlos Pinto[a]

[a]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[b]School of Animal Sciences, College of Agriculture, Louisiana State University, Baton Rouge, LA, USA

The aim of this study was to assess the effects of serial treatment of the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthetic analogue, cloprostenol, on early luteal development and gonadotropins profiles. It was hypothesized that cloprostenol treatment would prevent

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

luteal development and affect gonadotropins concentrations. Eight cycling mares were arranged in a crossover design being studied during 1 control (luteal) cycle (CTRL) and 1 aluteal cycle (AL). Serial transrectal ultrasound examinations were performed, mares were detected in estrus with ≥ 35 mm follicles, and ovulation was induced with intramuscular deslorelin acetate (1.8 mg). At detected ovulation (Day 0), mares were randomly allotted to a CTRL group or an AL group induced by 500 µg of cloprostenol treatment twice daily on Days 0, 1, and 2 and once daily on Days 3 and 4. Daily blood samples for RIA determination of plasma progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations were collected. Interovulatory intervals (IOI) were recorded and compared using a paired t-test. Hormone plasma concentrations were compared between groups until seven days postovulation (Day 7) using a repeated measures ANOVA. The significance level was set as $p \leq 0.05$. Mean (± SD) IOI of AL cycles was shorter ($p < 0.0001$) than CTRL cycles (12.88 ± 1.35 versus 21.38 ± 1.30 days). Concentrations of plasma progesterone remained below 1 ng/ml in the AL group throughout the period evaluated. Aluteal cycles presented sustained LH concentrations that became higher ($p \leq 0.04$) than CTRL cycles starting 6 days postovulation. Day-by-day plasma FSH concentrations did not differ ($p \geq 0.1416$) between groups at any of the time points evaluated. In conclusion, serial treatment of cloprostenol during the early stages of luteal development had a similar antiluteogenic effect to what was previously reported with the use of the $PGF_{2\alpha}$ natural analogue dinoprost.[1] Cloprostenol-induced aluteal cycles were characterized by a shortened interovulatory period, persistently low (< 1 ng/ml) plasma progesterone concentrations, sustained elevated concentrations of LH, and no day-by-day differences on plasma FSH.

Keywords: Mares, antiluteogenesis, $PGF_{2\alpha}$, LH, progesterone

Reference

1. Coffman EA, Pinto CR, Snyder HK, et al: Antiluteogenic effects of serial prostaglandin F2α administration in cycling mares. Theriogenology 2014;82:1241-1245.

Conservative medical management of a grade III rectal tear in a mare

Alex Wittorff, Reed Holyoak

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA

A 9-year, American Paint mare, was presented for routine breeding management; previous examination by a local veterinarian the week prior revealed that the mare was not in estrus. Additionally, the owner noted that the mare appeared lethargic and was rapidly losing body condition. On visual examination the mare was quiet and alert. Transrectal palpation quickly revealed an unanticipated grade IIIa rectal tear (serosa intact with disruption of mucosal, submucosal, and muscularis layers). The defect was located laterally at 3 o'clock, 20 cm cranial to the anal sphincter and included a 30 cm diverticulum. A presumptive abscess was diagnosed via transrectal ultrasonography when a firm structure, 7 cm in diameter, was palpated perirectal and ventral to the diverticulum opening. Edges of the diverticulum opening had begun to fibrose, but safe removal of inspissated purulent material, sloughing necrotic tissue, and impacted feces was achieved. Due to the thinness of the cranial diverticulum wall and continued fecal accumulation with resulting diverticulum distention, rupture into the peritoneum was impending if not treated immediately. Referral for surgical treatment was discussed with the owner, but due to financial constraints, the owner elected to donate her to the veterinary teaching program where she was treated with conservative medical treatment. Mare received a dose of intramuscular ceftiofur crystalline free acid (Excede® 6.6 mg/kg) on days 1 and 4 of treatment and a dose of intravenous N-butylscopolammonium bromide (Buscopan, 0.3 mg/kg) and flunixin meglumine (0.25 mg/kg) on days 1 to 5. On day 1, manual removal of diverticulum contents was performed prior to lavaging the defect. A 3/4" diameter nasogastric tube was guided into the diverticulum and water infused by a stomach pump until the diverticulum was full, being careful not to overfill and stress the thin diverticulum wall. Feces and debris were evacuated within the efflux. This was repeated until the efflux drained clear. Packing of the diverticulum was performed with 6-inch brown gauze soaked in a 10% sugar-betadine solution to promote granulation tissue formation and for antimicrobial effects. Lavaging and packing was repeated daily from days 2 to14, then every other day between days 14 and 21. Early on during treatment, fecal and purulent material was adhered to the gauze, but by day 14 very little debris was present. By day 21, the diverticulum had reduced to a size too small for continued packing; localized treatment was discontinued at that point and a perirectal abscess could not be identified. Examination of the remaining defect was performed every 2 - 3 days to ensure complete reduction of the diverticular space. Scar formation and complete healing of the defect was achieved by week 4 of treatment. She was successfully utilized for transrectal palpation laboratories by veterinary students. This case demonstrated the resolution of a life-threatening, high-grade rectal tear with conservative medical treatment. Survival rates for grade III rectal tears vary from 75% (surgical treatment) to 38% (medical treatment). Practitioners presented with grade IIIa or b rectal tears can successfully implement conservative, medical treatment plans for these life-threatening injuries.

Keywords: Rectal tear, medical management

Seminal vesiculitis in a Zangersheide stallion

Anne Sheerin, Maria Schnobrich, Peter Sheerin

Rood and Riddle Equine Hospital, Lexington, KY, USA

Seminal vesiculitis is the most common abnormality affecting accessory sex glands of the stallion. It is characterized by changes in seminal characteristics and inflammation of the glandular mucosa. It is typically caused by pathogenic bacteria resulting in purulent excretions. Seminal vesiculitis has varying effects on the stallion's fertility but typically results in reduced fertility, sperm longevity, and motility.[1] This report describes a case of seminal vesiculitis in a 2009 Zangersheide stallion. He was presented to the Theriogenology Service at Rood and Riddle Equine Hospital for routine semen freezing. The ejaculate from the first collection contained malodorous, thick, red tinged mucoid material. This material contained blood and neutrophils from the gel portion. A second collection was obtained 2 hours later, and the ejaculate was fractionated into the sperm-rich and sperm-poor fractions. Due to the abnormal semen the stallion was examined via transrectal ultrasonography. Transrectal ultrasonography of the accessory sex glands revealed thickened walls with edema and hyperechoic flecks in the seminal vesicles. It was determined that the purulent material was from the gel portion of the ejaculate. A culture was performed on the semen, which resulted in the growth of *Pseudomonas aeruginosa* that was sensitive to amikacin, enrofloxacin, gentamicin, imipenem, polymyxin B, ticarcillin, and timentin. Twice daily clean out collections and systemic enrofloxacin were recommended. However, the infection persisted. Therefore, subsequent collections were obtained by fractionating the ejaculate to collect the sperm rich fraction and dispose of the gel fraction. Further collections successfully obtained semen that had no growth in culture. Fractionated ejaculates were frozen using a commercial extender containing antibiotics and no growth was noted in postthaw cultures. A total of 50.75 commercial quality doses of semen were collected in 9 collections. Mean postthaw progressive motility was 62.1% (range 32.6 - 81%). Stallion was able to continue his show career throughout collections and

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

his performance was not affected. Seminal vesiculitis can presumptively be defined by semen characteristics, namely, pyospermia and hemospermia. Further diagnostics include transrectal ultrasonography of the seminal vesicles, culture of semen, and endoscopy. Most definitive diagnosis is by culturing seminal vesicle contents obtained via urethral endoscopy. Various treatments for seminal vesiculitis exist, but most have limited success. Systemic antimicrobial treatments often do not reach therapeutic concentrations within the glands. Treatments frequently must be repeated as the infections can be recurrent.[1] Fractionating the ejaculate into sperm rich and gel portions is a useful technique to obtain a sperm rich fraction with no bacteria.

Keywords: Stallion, seminal vesiculitis, semen collection

Reference

1. Scheeren VFC, Sancler-Silva YFR, Ali H, et al: Update on seminal vesiculitis in stallions: J Equine Vet Sci 2020;94:103234.

Neonatal pup death due to hyperthermia in a malfunctioning incubator

Bruce Christensen, Alyssa Shelby

Kokopelli Assisted Reproductive Services, Sacramento, CA, USA

A 4-year, female French bulldog, was presented for breeding with chilled, shipped semen from a proven male French bulldog. Serum progesterone and LH assays determined the LH surge date and transcervical insemination was performed 5 days later with 600 x 10^6 sperm. At day 31 of pregnancy, transabdominal uranography revealed 3 embryos with heartbeats and appropriate development, and 1 site of embryonic resorption. Serum progesterone concentrations were 33.94 ng/ml (Tosoh AIA-360 automated immunoassay analyzer; South San Francisco, CA, USA). Patient was given fenbendazole and amoxicillin/clavulanic acid. An evaluation conducted a week later confirmed no further signs of fetal losses. Transabdominal ultrasonography (64 days after LH surge) revealed 3 fetuses with heart rates ranging from 167 - 188 bpm. All fetal kidneys had marked corticomedullary distinction. Fetal intestines had mild degree of peristalsis. Serum progesterone concentrations were 8.18 ng/ml. Two female and 1 male neonate were delivered via elective cesarean surgery and resuscitated without complications; however, 1 of the females was markedly smaller than the other 2 neonates. Each neonate was placed in an incubator with the thermostat set at 90°F (32.2°C) (PuppyWarmer ProSeries Transport Incubator [NOTE: numbers not credible] (325 x 450 x 350 mm (Battle Creek, MI, USA). An oxygen concentrator was set to deliver 1 liter of oxygen/minute into the incubator (PuppyWarmer Pro Series, Richland, MI, USA). After 30 minutes in the incubator, it was noticed that the pups had fluid pooling around their faces. Temperature on the incubator thermostat was turned down to 85°F (29.4°C). Pups were evaluated, they had good movement, were dried off and turned to their other sides. Pups were visually monitored every few minutes through the incubator window and the temperature was observed on the thermostat that dropped to 85°F (29.4°C). Labored breathing from pups was observed and 30 minutes after turning down the incubator to 85°F, 2 pups ceased breathing. Upon removal, it was noted that these 2 pups had dry mucous membranes. The incubator subjectively felt very warm. A rectal digital thermometer was placed in the anus of the remaining neonate that read > 109°F (42.8°C; maximum temperature capacity for the rectal thermometer). Alcohol was placed on the ventrum and feet of the neonate, but it ceased breathing within 2 minutes. A digital thermometer placed in the incubator also read > 109°F (42.8°C). The incubator thermostat still read 85°F (29.4°C). Following the death of the neonates, an independent mechanical engineer was able to repeat the malfunction. Wide temperature fluctuations occurred independent of the positioning of the oxygen tube or oxygen flow. Neonatal pups and kittens are poikilothermic and highly sensitive to environmental temperatures, unable to compensate using physiologic thermoregulatory mechanisms. Much attention is placed on preventing hypothermia, but little has been focused on the dangers of hyperthermia. It is recommended that veterinarians and breeders do not rely on incubator thermostat readings alone, but should closely monitor environmental temperatures using independent devices, such as thermocouples, infrared sensors, and other thermometers. Neonates should be closely monitored for signs of hypo- or hyperthermia.

Keywords: Neonate, hyperthermia, resuscitation, incubator, thermoregulation

Semen parameters, gross and histopathological findings of *Brucella ovis* outbreak in rams

Devinda Wickramasingha,[a] Fritz Schumann,[a] Soraya Sayi,[b] Abby Toews,[a] Roshan Fernandopulle,[a] Kamal Gabadage,[a] Dinesh Dadarwal[a]

[a]Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon SK, Canada
[b] Department of Veterinary Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon SK, Canada

Brucella ovis (*B. ovis*) is a gram-negative bacterium that primarily causes infectious epididymitis, mainly in mature rams. Objective was to describe the clinical signs and pathological lesions in the reproductive tract of rams affected by a natural outbreak of *B. ovis* infection in a commercial sheep flock. Eleven rams of various breeds, aged 1 - 5 years, were enrolled in a more extensive study on the breeding soundness evaluation (BSE) criteria. Rams were used for breeding in a multi-sire group during the 2021 - 2022 natural breeding season. During the summer of 2022, semen samples of all 11 rams were submitted to Prairie Diagnostic Services, Saskatoon for bacteriological culture. *Brucella ovis* was isolated from all 11 rams and further confirmed by whole genome sequencing on the bacterial isolates. A complete BSE was performed before euthanasia, followed by necropsy, giving special attention to reproductive organs. Scrotal circumferences were satisfactory (36 ± 3 cm) for all rams. Based on sperm morphology, 1 yearling ram was classified as questionable and others as unsatisfactory. Two of 11 rams had unilateral palpable lesions in epididymis tail. Rams that failed BSE (n = 10) had good semen density but poor gross and individual motility (23.9 ± 17%). Sperm morphology had low proportion of normal sperm (41 ± 12%) and high proportion of detached heads (40 ± 15%) and mid-piece defects (14.6 ± 14%). At necropsy, 6/11 rams had grossly visible lesions in testes (focal hemorrhages or mineralization), tunics (adhesions), and epididymides (epididymitis with purulent or caseous exudate). However,

histopathological evaluation revealed inflammatory lesions in all rams in multiple reproductive organs, specifically, in epididymides (11/11, head n = 8, body n = 9, and tail n = 11), ductus deferentia (10/10), ampullae (11/11) and seminal vesicles (11/11) and to a lesser degree in testes (9/11, parenchyma n = 6 and rete testes n = 6). Lesions in these tissues were mainly lymphoplasmacytic and neutrophilic infiltrates and fibrosis. Other histopathologic lesions were vasculitis, mineralization, and spermatic granuloma formation. In conclusion, subclinical infections with *B. ovis* may be missed during a routine BSE. This is evidenced by only 2 of 11 rams having abnormalities on physical examination whereas all rams had internal lesions.

Keywords: *Brucella ovis*, rams, breeding soundness evaluation, epididymitis, histopathology

Testicular interstitial cell tumor in a cryptorchid horse

Eduardo Arroyo,[a] Salman Waqas,[a] Julie Cecere,[b] Thomas Cecere,[b] Phillip Sponenberg,[b] Ahmed Tibary[a]

[a]Comparative Theriogenology, Department of Veterinary Clinical Science, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[b]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA

A 6-year, American Quarter Horse gelding, was presented for evaluation of suspected cryptorchidism due to stallion-like behavior. The horse was purchased at an auction 10 months prior to presentation. Owner reported occasional stallion-like behavior, going so far as to mount mares. Referring veterinarian reported the presence of scrotal scar that suggested prior castration. Anti-Mullerian hormone serum concentrations were 10.2 ng/ml (geldings < 1 ng/ml) and testosterone serum concentrations were > 500 pg/ml (geldings < 100 pg/ml). The horse did not have any known medical problems, vaccination and deworming status were not known. Physical examination was within normal limits on presentation. Results of palpation of the inguinal area were compatible with those reported by the referring veterinarian. Further diagnosis by transrectal palpation and ultrasonography revealed a soft mass that was suspicious of a testis in the right inguinal region. The mass was easily movable and located over the internal inguinal ring. Percutaneous ultrasonography revealed a 5 cm diameter testis just cranial to the right external inguinal region. Testicular parenchyma contained multiple 1 cm diameter echolucent areas that were suspected to be due to neoplasia. Cryptorchidectomy was performed by laparoscopy in the standing horse under heavy sedation, and premedication with antibiotics (intravenous gentamicin 6.6 mg/kg, once daily and intravenous cefazolin 11 mg/kg, 3 or 4 times daily) and tetanus toxoid. Retained testis was removed with the aid of a morcellator. Mild emphyema was noted around the surgical sites, but no major complications were observed, and the horse was discharged 2 days after the surgery. Histopathological examination of testis was inconclusive, but a diagnosis of interstitial cell tumor was made after consultation with a second group of pathologists. Histomorphology features were different from the classic Leydig cell tumor and included multinucleated spindleoid neoplastic cells that surrounded seminiferous tubules. The tumor was suspected to be hormonally inactive because Leydig cells surrounding the Sertoli cell-lined seminiferous tubules remote from the neoplasm were not atrophic. Germinal and nongerminal cell tumors are rare, and the frequency of occurrence is difficult to ascertain in horse populations. Most male horses are castrated at an early age. It is hypothesized that retained cryptorchid testes are more likely to develop a neoplasia due to the constant exposure to normal body temperature. Most Leydig cell tumors do not result in overproduction of steroidogenic hormones (estrogen and androgens). Usually, these tumors do not metastasize, so prognosis is excellent after retained testis removal. This case highlighted the need of accurate diagnosis of location, size and echotexture of the testis in case of suspected cryptorchidism in hemicastrated horses.

Keywords: Leydig cells, gelding, seminiferous tubule, hemicastration

Disorder of sexual development: 64 XX, SRY negative phenotypical mare with bilateral retained testes

Gail McRae,[a] Erin Runcan,[a] Marco Coutinho da Silva,[a] Christopher Premanandan[b]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA
[b]Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA

A 4.5-year, Percheron mare, was presented for unilateral ovariectomy. Referring veterinarian reported that the mare was displaying stallion-like behavior and had elevated serum testosterone (298.2 pg/ml) concentrations suggestive of a granulosa cell tumor (GCT). Examination of the mare's external genitalia revealed a mildly elongated vulva and a slightly enlarged clitoris with no other abnormalities. Vaginoscopy revealed normal vagina and cervix. Complete palpation of her gonads was not possible due to the size of the mare. Transrectal ultrasonography revealed gonads that had homogenous, moderately hyperechoic echotexture bilaterally. Gonads measured 3 x 3 cm (left gonad) and 3 x 4 cm (right gonad). No follicles were observed within the parenchyma of the gonads and uterine horns were connected to the uterine body. Additionally, the uterus was flaccid and contained a moderate amount of anechoic fluid within the lumen and absence of endometrial edema. Due to the abnormal appearance of gonads and the owner's eventual lack of desire to breed the mare, bilateral gonadectomy was recommended. Surgery was performed laparoscopically with the mare sedated and restrained in stocks. After surgery, the gonads were transected along the sagittal plane, fixed in formalin, and submitted for histopathology. A sample of whole blood was submitted to the Texas A&M Molecular Cytogenetics Laboratory for karyotyping. Grossly, the parenchyma of the gonads had a homogenous texture more consistent with testes, with no visible follicles. Histopathological findings were Sertoli-only tubules with tissue suggestive of ductus deferens and absence of ovarian tissue. Karyotype results revealed a normal female genotype (i.e. 64, XX SRY (-), inconsistent with the presence of testes. This case is a good demonstration of a disorder of sexual development (DSD) that is not characteristic of those that are more often described in the literature. In this case, the reason for the development of testes is unclear. There is evidence in other species that an upregulation of the SOX9 gene may be involved.[1] Currently there is no clear answer, but this case exemplified an abnormal presentation of a DSD with an otherwise normal phenotypic presentation.

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

Keywords: Disorder of sexual development, mare, karyotyping

Reference

1. Peretti V, Katiuska S, Ciotola F, et al: An unusual case of testicular disorder in sex development of Arabian mare (64, XX SRY - negative). Animals 2020;10:1963-1975.

Persistent endometrial cups in a Thoroughbred mare

Mariah Slack,[a] Carol McLeod,[b] Maria Schnobrich[a]

[a]*Rood & Riddle Equine Hospital, Lexington, KY, USA*
[b]*Carol McLeod Private Practice, Lexington, KY, USA*

Endometrial cups in the mare are believed to regress by 140 days of pregnancy. These fetal tissues from the chorionic girdle invade the endometrium at ~ 35 days and secrete equine chorionic gonadotropin (eCG), which facilitates formation of accessory corpora lutea that provide progesterone support for pregnancy. Maternal lymphocytic immune response is responsible for the natural destruction of endometrial cups from days 100 to 140 of pregnancy. Failure of endometrial cup regression results in elevated eCG concentrations, prolonged diestrus, and continuous luteinization of follicles with failure of ovulation. This case describes a 12-year-old Thoroughbred mare that had persistent endometrial cups for 2 years. Initial confirmation of endometrial cups was performed after diagnosis of bilateral twin death at 53 days of pregnancy. Transrectal ultrasonography of the reproductive tract revealed multiple corpora lutea on both ovaries and twin nonviable fetuses located at the base of each uterine horn. Manual removal of the conceptuses was performed, and hysteroscopy confirmed numerous endometrial cups circumferentially located at the base of each uterine horn. Serum eCG concentrations were 100.0 IU/ml. Mare had numerous treatments to induce regression of the endometrial cups with marginal decreases in eCG each time. Following treatments (eCG concentrations at treatment, day postovulation) were performed: 1 vial mycobacterium cell wall fraction immunostimulant (Settle®, MCWFI) injected hysteroscopically (100 IU/ml, 53 days); 1 vial amnion product (StemWrap D™) injected hysteroscopically (12 IU/ml, 293 days); laser ablation (8.3 IU/ml, 319 days); kerosene infusion (4.2 IU/ml, 335 days); 4 vials of MCWFI injected hysteroscopically in both uterine horns (3.4 IU/ml, 379 days; 4 vials intrauterine MCWFI of infusion (3.1 IU/ml, 410 days); and 4 vials of MCWFI injected in the region of previous cups hysteroscopically (1.3 IU/ml, 545 days). Final regression of the cups was achieved at ~ 18 months after pregnancy loss. This case highlighted how endometrial cups can persist despite aggressive treatment. It is possible that the twin pregnancy and mechanism of removal was associated with the persistence of these tissues and further work is needed to identify why some mares have such difficulty with natural regression of endometrial cups.

Keywords: Mare, endometrial cups, equine chorionic gonadotropin, persistent endometrial cups

Persistent Müllerian duct syndrome and enlarged prostatic utricle in a male dog

Peter Welsh,[a] Kaylyn McDaniel,[a] Elizabeth Goldsmith,[b] Joshua Ramsay,[b] Eduardo Arroyo,[a] Alan Conley,[c] Tina Owen,[a] Yoko Ambrosini,[a] Michela Ciccarelli[a]

[a]*Veterinary Clinical Sciences, Washington State University, Pullman, USA*
[b]*Veterinary Microbiology and Pathology, Washington State University, Pullman, USA*
[c]*School of Veterinary Medicine, University of California Davis, Davis, USA*

A 1-year, male intact Miniature Schnauzer mix, was presented for chronic intermittent hematuria. Abdominal ultrasonography revealed a large, fluid-filled cystic structure dorsal to the prostate that extended cranially. Computed tomography imaging revealed that the fluid-filled cavity resembled uterus, with both uterine horns entering scrotum through the inguinal canal adjacent to testes. Dog had homozygote mutation of the anti-Müllerian hormone receptor type 2 consistent with persistent Müllerian duct syndrome (PMDS). Intraoperative fluoroscopy examination revealed a communication between uterus and urinary bladder via an enlarged utricle, explaining hematuria and urine in the reproductive tract (urometra). Gonadohysterectomy was performed, and the tissue was submitted to the Washington Animal Disease Diagnostic Laboratory for assessment. On histologic examination, the rudimentary uterine body and uterine horns (uterus masculinus) consisted of tubular structures lined by round to columnar epithelial cells and multifocal ulcerations. Rudimentary vagina was lined by thick squamous epithelium. Cross sections of rudimentary ductus deferens were randomly interspersed throughout the smooth muscle layers of the rudimentary uterus and vagina and within the connective tissue adjacent to right testis and epididymis. Within the testes, < 10% of the seminiferous tubules had evidence of complete spermatogenesis, < 10% were atrophied, and the remaining had evidence of incomplete spermatogenesis. Few mature and immature spermatids were within the epididymal lumen. Several other cases of PMDS in Miniature Schnauzers have been described in the literature, often being an accidental finding at cryptorchidectomy or associated with reproductive neoplasia like leiomyoma or Sertoli cell tumors. To our knowledge, this is the first clinical report of a phenotypically intact male dog with PMDS and urometra due to an enlarged prostatic utricle. This case illustrated a combination of a disorder of sex and urogenital sinus development.

Keywords: Uterus masculinus, disorder of sex development, hematuria, urometra

Parturition induction in a late-term pregnant alpaca based on fetal maturity with unknown breeding timing

Nicole Sugai, Sherrie Clark-Deener, Jamie Stewart

Department of Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

A 4-year, intact female alpaca, was presented on 2/26/22 for pregnancy diagnosis and gestational aging due to unknown breeding history after the owner received her from a camelid rescue group. At this initial evaluation, transabdominal ultrasonography revealed a healthy fetus. Thoracic height was measured 5.04 cm and fetal biparietal diameter measured 4.97 cm. Using equations derived from a previous publication,[1] the gestational age was determined to be between 229 and 247 days, with a due date between 8/1 and 8/20/22. Reevaluation was performed on 8/3, due to the owner not observing any impending signs of parturition, such as behavioral alterations, vaginal discharge, or mammary gland development. Both transabdominal and transrectal ultrasonography were performed to assess fetal viability and positioning. Ability to visualize only caudal abdomen prevented diagnosis of a possible fetal posterior presentation. Owner was instructed to wait another week before considering induction of parturition due to the unknown breeding date and lack of signs for impending parturition. Client returned on 8/18 for a single lateral abdominal radiograph to better assess the fetus' positioning. Radiologist reported that the fetus was in posterior presentation with dorso-sacral posture, flexion of forelimbs and hindlimbs, and cervical ventroflexion. Fetal viability appeared normal with an appropriate heartbeat visualized on ultrasonography, so the decision was made to continue monitoring. Owner returned on 8/23 with concerns that the dam was acting lethargic and losing weight. The tail head ligament was slightly more relaxed than it was at the previous visit, and thick colostrum was expressed from the udder. Using a hand-held pH meter, the milk pH was determined to be 6.44, consistent with impending parturition in mares.[2] Transabdominal ultrasonography revealed a strong fetal heartbeat (124 beats per minute) and good cortico-medullary distinction of the fetal kidney. At this time, the owner elected to induce parturition with 250 µg of intramuscular cloprostenol treatment at ~ 1:00 pm. Owner took the dam home to monitor overnight with a plan to bring her back next day morning for further monitoring and parturition assistance in the clinic. Dam began showing signs of active labor by ~ 9:30 AM (~ 20.5 hours after induction). Cria began to present cranially and dorsosacral with both front limbs extended by ~ 10:00 AM. A healthy male cria (5.63 kg) was delivered with minimal assistance at 10:15 AM. Fetal membranes passed at 10:52 AM and was evaluated to confirm complete expulsion. This case is important due to the ability to successfully induce parturition in an alpaca with an unknown breeding date when there was concern for the dam's wellbeing. The information collected was extrapolated from other species and appeared to be an adequate predictor of fetal maturity and survival in this case. Findings highlighted the need for further studies to better understand prepartum ultrasonographic and radiographic findings and milk composition changes in normal versus abnormal camelid pregnancies to facilitate such management decisions.

Keywords: Alpaca, fetal maturity, parturition induction, gestational aging

References

1. Gazitúa FJ, Corradini P, Ferrando G, et al: Prediction of gestational age by ultrasonic fetometry in llamas (Lama glama) and alpacas (Lama pacos). Anim Reprod Science 2001;66:81-92.
2. Canisso IF, Ball BA, Troedsson MH, et al: Decreasing pH of mammary gland secretions is associated with parturition and is correlated with electrolyte concentrations in prefoaling mares. Vet Rec 2013;173:218.

Successful embryo transfer after large colon displacement laparotomy

Patricia Xavier, Maria Ferrer, Daniela Cortes, Amy Brandon, Naomi Crabtree, Robyn Ellerbrock

Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA, USA

An 8-year, Appendix Quarter Horse mare, was presented in January 2023 for embryo transfer. Mare had an extensive history of embryo transfer attempts, with no embryos recovered in 2021, and 4 embryos recovered in 2022 that were shipped and transferred, resulting in no pregnancies. On presentation, the mare had a no bacterial growth on uterine culture and was in diestrus. She received intramuscular dinoprost tromethamine (5 mg once), and ovarian activity was monitored via transrectal ultrasonography. Ovulation was induced with deslorelin at insemination, when the mare had 4 follicles on the right ovary (29, 34, 43, and 44 mm), grade 3 uterine edema, and no intrauterine fluid. She was inseminated with 1.8×10^9 progressively motile sperm from a Quarter Horse stallion, and ovulated 2 follicles 44 hours later. Due to a history of breeding-induced endometritis, the uterus was lavaged 6 hours after insemination with 0.9% saline and DMSO, and was infused with 2 grams of ampicillin. The following day, the mare received an infusion with platelet rich plasma after uterine lavage. Thereafter, she received intramuscular oxytocin (20 IU, once daily), oral firocoxib (0.1 mg/kg, once a day), oral altrenogest (0.44 mg/kg, once a day) and oral trimethoprim sulfamethoxazole (20 mg/kg, twice a day). At 60 hours after ovulation, the mare developed severe colic characterized by severe large intestinal gas distention, unresponsive to medical management (intravenous fluid therapy, flunixin meglumine [1.1 mg/kg, twice daily]), gastric decompression and lavage, sedation with detomidine and butorphanol, and 2 right-sided percutaneous large intestinal trocarisations). Exploratory celiotomy via a ventral midline incision was elected 12 hours after onset of colic signs, and a right dorsal displacement of the large colon was diagnosed and corrected after pelvic flexure enterotomy, with a duration of 5 hours total anesthetic time. After surgery, the mare was managed with intravenous potassium penicillin G (20,000 IU/kg, once daily), intravenous gentamicin (6.5 mg/kg, once daily), intravenous flunixin meglumine (1.1 mg/kg, twice daily), and oral altrenogest (0.044 mg/kg, once daily) until embryo flush. She continued to colic, was febrile, and refluxed 24 - 32 liters/day for 48 hours after surgery, and was held off feed for 4 days after surgery, during which time the colic signs and reflux resolved, although the mare remained intermittently febrile. Embryo flush was performed 8 days after ovulation (5 days after surgery), and a grade 1 blastocyst was recovered. The embryo was immediately transferred to a 14-year, maiden Warmblood mare. Recipient mare ovulated 2 follicles 6 days

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

prior to transfer, and was treated with oral firocoxib (0.1 mg/kg, once daily), oral trimethoprim sulfamethoxazole (20 mg/kg, twice daily) and oral altrenogest (0.044 mg/kg, twice daily) starting at the time of transfer. Three days after transfer, a 7 mm embryo was visualized via transrectal ultrasonography, and the mare is still pregnant at 60 days. Several papers have described increased pregnancy loss after colic surgery or illness, and other retrospective studies have described a lower foaling rate when the mare underwent surgery before 40 days of pregnancy; however, there is no information on embryo recovery rates after colic surgery. To our knowledge, this is the first case report of embryo recovery from a mare after colic surgery, and successful pregnancy in a recipient mare.

Keywords: Mares, embryo, colic surgery, endotoxemia, hyperthermia

Progesterone concentrations in mares after follicular puncture

Alex Wittorff,[a] Candace Lyman[b]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Transvaginal follicular aspiration of oocytes in mares may be performed during all stages of estrous cycle. Interestingly, data are minimal, contradictory, and inconclusive when evaluating effects on serum progesterone (P_4) concentrations. Some literature suggested late formation of luteal tissue and rising P_4 concentrations after follicular aspiration whereas others indicated that follicular aspiration had no effect on luteal formation and resulting P_4 secretions. Objective was to quantify serum P_4 concentrations in unsynchronized mares undergoing follicular puncture for the purposes of either ablation or oocyte collection as part of a separate but concurrent research project. The concurrent ongoing project called for follicular puncture to take place in 6 healthy, young mares (3 - 10 years) 17 days apart. We hypothesized that luteal formation and resulting serum P_4 concentrations are not affected by follicular puncture. All follicles > 5 mm were punctured and plasma samples were collected on day 0 (the day of follicular puncture), 3 or 4, and 6. Serum P_4 concentrations indicated that puncture of ovaries with mature luteal tissue present (n = 2) was not detrimental to the already elevated P_4 concentrations present at follicular puncture. Mares documented as having a recent ovulation and, therefore, with freshly developing luteal tissue (n = 4) had P_4 concentrations > 2 ng/ml on day 3 and > 7 ng/ml on day 6, also indicating no detriment to the maturation and function of the luteal tissue. Serum P_4 concentrations in mares with a preovulatory (≥ 35mm) follicle (n = 4) were < 1 ng/ml on day 6, indicating stunted P_4 concentrations. Although testing and assay error should always be considered, delayed luteinization and corpus luteum (CL) formation due to follicular puncture must not be ruled out. All findings are further strengthened by the corresponding luteinizing hormone (LH) concentrations; if a numerical rise in LH concentrations was documented, it occurred alongside a numerical rise in P_4 concentrations, with the exception of 1 mare. Similarly, in the absence of quantitative increases in LH concentrations, P_4 concentrations also did not rise to expected concentrations. In conclusion, follicular puncture in the presence of newly developing or mature CLs did not compromise overall systemic impact of CLs and P_4 concentrations after 6 days in majority of mares (n = 8). Two mares had a stunted LH concentrations rise, and similar inadequate P_4 concentrations (P_4 < 2 ng/ml) after 6 days. We suggest that more research is needed to determine whether delayed luteinization and stunted P_4 concentrations of preovulatory follicles after follicle punction are inevitably linked with or occur independent of LH concentrations at puncture.

Keywords: Mares, follicles, transvaginal follicular ablation, oocyte, corpus luteum

Immunohistological and molecular characterization of the Hippo pathway in granulosa cell tumors of the mare

Aurore Le Breton,[a] Mouhamadou Diaw,[a] Guillaume Saint-Jean[b]

[a]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada,
[b]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada

Ovaries with honeycomb aspects are typical of granulosa cell tumors (GCT) in the mare. They reflect abnormalities in the dynamic of the proliferation of granulosa cells, even if GCTs are known to be benign neoplasms. To bring information on this poorly understood phenomenon, we focused on the Hippo pathway, a series of oncoproteins (serine/threonine kinases) that have a key role in several cancers. Hippo pathway is well known to control cell turnover in a physiological and pathological context. We hypothesized that this pathway is modified in the tumor compared to control tissues. Therefore, we performed immunohistochemistry (IHC), Western Blot (WB), and polymerization chain reaction (PCR) analysis on paraffin-stored ovaries from mares with GCT and controls (12 samples each). Presence of YAP and TAZ proteins (critical effectors of the Hippo pathway) was evident in granulosa cells of both tumoral and control tissues. Nevertheless, their intracellular repartition was significantly (ANOVA) more cytoplasmic in GCT suggesting their proproliferative action was repressed compared to controls for which the immunohistological signal was also unclear. The quantitative PCR can confirm this observation proving that target genes (CTGF, ANKRD1, CYR61, and NOV) are downregulated in GCT in the mare. Those findings suggested that GCT in the mare has a regulation of granulosa cell proliferation by an antioncotic mechanism, resulting in the retention of the main effectors of the Hippo pathway in the cytoplasm of the cell. This is an important argument to understand the benign character of equine GCTs that are different from human (and most documented) ones, known to be malignant and have metastatic potential.

Keywords: Mare, granulosa cell tumor, Hippo pathway

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

Clitoral adenocarcinoma in a mixed breed, spayed female dog

Christina Moss,[a] Nicole Sugai,[b] Rebecca Persons,[b] Brittany Ciepluch,[b] Julie Cecere[b]

[a]Piedmont Equine Practice, The Plains, VA, USA
[b]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA, USA

Our case is a 9-year, mixed-breed, spayed female dog that was presented to the primary care veterinarian for dermatologic signs on the nasal planum consistent with pemphigus. A clitoral mass was an incidental finding at this examination and benign neglect was chosen as the mass was not currently causing any distress. Multiple other masses were removed from the body at the initial visit with a high suspicion of neoplasia. Subsequently, the patient was diagnosed with pemphigus foliaceus and was maintained on immunosuppressive doses of steroids. Dog was presented to the emergency service after 5 weeks for prolapse of the clitoral mass and hemorrhagic discharge. In a follow up appointment, theriogenology department identified a 2 x 2 cm, multi-lobulated, highly vascular, nonexfoliating mass on the ventral wall of the vestibule around the clitoris; the urethra was not involved and could be easily catheterized. Blood work revealed normal total calcium levels with evidence of hepatopathy attributed to the chronic steroid treatment for the dermatologic disease. No lymph node enlargement was detected on physical examination nor on abdominal ultrasonography. Anal glands were of normal appearance during ultrasonographic examination. Surgical removal of the mass was elected and performed. Histopathology revealed an unencapsulated, infiltrative, densely cellular mass with cells arranged in sheets, ducts, and rosettes supported by a fibrous stroma. There were 15 mitotic figures identified per 10 fields (400 x). Diagnosis was made of clitoral adenocarcinoma. A follow up examination with the primary care veterinarian ~ 1 month after mass removal revealed no gross evidence of local regrowth and no evidence of local lymph node metastasis. Canine clitoral carcinoma is a rare neoplasm of the female genital tract. Fewer than 12 cases have been reported and the first case was reported in 2010.[1] Clitoral carcinomas share a variety of clinical, histological, and immunohistochemical features with apocrine gland anal sac adenocarcinomas with normal anal glands.[1-5] This suggests that the apocrine glands of the clitoris are the true primary site of the tumor versus metastasis from the anal glands. A review of 6 cases of clitoral adenocarcinoma described the condition as occurring in spayed females with a median age of 9.5 years.[4] Individual case reports of the condition involved animals of similar signalment.[1-3,5] Presenting clinical signs included vulvar licking, perineal swelling and irritation, hemorrhagic vulvar discharge, lower urinary tract signs, and presence of a visible or prolapsed soft tissue mass. Clitoral masses have also been an incidental finding during physical examination, as observed in this case.[4,5] Previous case reports suggested that hypercalcemia of malignancy was present in up to half of the cases of canine clitoral carcinoma, potentially due to primary tumor production of parathyroid hormone-related protein.[4] If not addressed, metastasis to locoregional lymph nodes may occur and has occurred, with histologic confirmation, in 60% of the previously reported cases.[1,3-5] Canine clitoral carcinoma remains a rarely reported condition requiring continued study and reports to determine nature, associated complications, and prognosis.

Keywords: Clitoris, prolapse, neoplasia, adenocarcinoma, calcium, canine

References

1. Neihaus SA, Winter JE, Goring RL, et al: Primary clitoral adenocarcinoma with secondary hypercalcemia of malignancy in a dog. J Am Anim Hosp Assoc 2010;46:193-196.
2. Mitchell KE, Burgess DM, Carrigan MJ: Clitoral adenocarcinoma and hypercalcemia in a dog. Aust Vet Pract 2012;42:279-282.
3. Rout ED, Hoon-Hanks LL, Gustafson TL, et al: What is your diagnosis? Clitoral mass in a dog. Vet Clin Pathol 2016;45:197-198.
4. Verin R, Cian F, Stewart J, et al: Canine clitoral carcinoma: A clinical, cytologic, histopathologic, immunohistochemical, and ultrastructural study. Vet Path 2018;55:501-509.
5. McAloney CA, Taikowski KL, Cronin JP, et al: Pathology in practice. J Am Vet Assoc 2021;259:1-3.

Vaginal lipoma in a cow

Eduardo Prado, Tulio Prado, Elizabeth Whitt, Alisson Bradley, Pierre-Yves Mulon

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

Lipoma is a benign tumor due to fat accumulation. It was observed in different parts of the body altering normal function of an affected organ. Except for a report,1 vaginal lipoma in cows have not been documented. A 5-year, mixed breed cow, was presented on 01/09/2023 with cervicovaginal and rectal prolapse. On 12/2022, owner noticed a mass in the vaginal caudal aspect. Cow last calved in August of 2022. On presentation, the cow was bright, alert, and responsive, and all vital parameters were within normal limits. Cow had a body condition score of 8 out of 9. Cow was restrained in a chute, and prolapses were washed with water and dilute iodine solution. Rectal prolapse was resolved, and then the cervicovaginal prolapse was visualized. Mass was observed in the vaginal left caudal aspect. Attempts to replace the cervicovaginal prolapse were unsuccessful due to the mass blocking the ability of the reproductive tract to return to its normal anatomical position. On 01/10/2023, a resection of the mass was performed. Mass was removed by electrocautery and closed in routine fashion. After mass removal, the cervicovaginal prolapse was replaced after several attempts and Buhner stitch was applied. Mass was submitted to histopathology for further evaluation and was interpreted to be a vaginal lipoma with secondary infection.

Keywords: Cow, prolapse, lipoma, vagina

Reference

1. El-Maghraby HM: A retrospective study on some surgical affections of the perineum and vagina in farm animals. Zag Vet J 2002;30:84-100.

Blastomycotic prostatitis

Marthina Greer

Veterinary Village and ICSB- WI/IL/IN Lomira, WI, USA

Blastomycosis should be included in the differential diagnosis when a dog from a blastomycosis endemic area presents for signs and symptoms of prostate disease. Prostate disease is a relatively common finding in intact male dogs. Forms of prostate disease described in the literature include: benign prostatic hyperplasia/hypertrophy (BPH), bacterial prostatitis, neoplasia of the prostate, and paraprostatic cyst(s). Fungal prostatitis is not frequently described in the literature. Of the fungal systemic infections noted, blastomycosis is the most commonly reported infection of the canine reproductive tract. Blastomycosis is a fungal disease from soil fungus primarily in the upper Midwest near a body of water and other regions in the United States. Infrequently, prostate and testes are involved in blastomycosis making it as a systemic disease. Organs more commonly affected by blastomycosis include lungs (in the form of fungal pneumonia), eyes, and skin. Four dogs were diagnosed with blastomycosis prostatitis in our practice in 20 years. Three of 4 dogs were presented for primary prostate disease whereas 1 was presented with a diagnosis of blastomycosis with primary ocular disease. Consistent with reports in the literature, where sporting and hunting dogs are most commonly affected by blastomycosis, the dogs diagnosed were German Shorthaired Pointer, a Boykin Spaniel, a Curly Coated Retriever, and the outlier, a Samoyed. Also consistent with the literature, 2 were under 3 years of age (Curly Coated Retriever and Samoyed) whereas the other 2 were > 7. First dog, the German Shorthaired Pointer, presented at age 7 with the only symptom of prostate disease. His ejaculate was submitted for bacterial culture. He returned 1 week later with respiratory distress and radiographs revealed evidence of fungal pneumonia. Culture had grown blastomycosis; laboratory personnel were understandably distressed to find out that a fungal culture had been submitted. Dog was euthanized due to the advanced form of pneumonia and lack of good treatment options at this time. Second dog, a Samoyed, presented as with the first, with the only symptoms being enlarged prostate. At surgery, a draining skin lesion was noted on a rear foot, suggesting blastomycosis on the differential list. Third dog, a Boykin Spaniel, presented after examined by an ophthalmologist for retinal lesions, for castration and enucleation of both eyes. Owner, a retired physician, elected to remove only the affected testes and both eyes so the dog could continue to serve as his hunting buddy. He was successfully treated with oral antifungal drugs. Fourth dog, a Curly Coated Retriever, was 3 years. His age was not consistent with the age most often associated with BPH. He was recently seen enough that the Mira Vista urine blastomycosis test was used for diagnosis. He was treated with oral itraconazole, managed by an ophthalmologist to spare his vision, and was later castrated. He is still alive at this abstract submission.

Keywords: Blastomycosis, fungus, prostate, prostatitis

Pregnancy in a Warmblood mare inseminated with frozen-thawed-extended semen on-farm

Justin McNaughten,[a] Karen Wolfsdorf[b]

[a]Palm Beach Equine Clinic, Wellington, FL, USA
[b]Haygard's Equine Medical Institute, Lexington, KY, USA

Frozen-thawed semen provides numerous advantages for breeders; however, for veterinarians this modality can create organizational and logistical challenges (more intensive mare management, specialized equipment to thaw and transport frozen semen, and the hazards of working with liquid nitrogen). To ameliorate these challenges, breeders are encouraged to utilize purpose-built facilities for breeding management and insemination. When a haul-in scenario is not an option, practitioners breeding onfarm with frozen-thawed semen must decide how to overcome the aforementioned challenges efficiently and safely. Researchers have reported pregnancies after insemination with frozen semen that was thawed, extended, and cooled for 24 hours.[1] In this case report, the authors describe the use of frozen-thawed-extended semen on-farm. A 12-year, multiparous Warmblood mare (with a foal at foot), was examined on-farm for breeding management. Owner requested that the mare is managed and inseminated on-farm with a single multi-straw dose of frozen semen. To manage expectations, the authors outlined a breeding management plan utilizing a timed-insemination protocol and splitting the multi-straw dose of frozen semen. Serial reproductive examinations were performed. Once a preovulatory follicle and moderate uterine edema were detected, the mare was given an intramuscular GnRH analogue (Sucromate®, 1.8 deslorelin acetate mg/ml). Treatment timing was selected ensuring the pre and postovulation inseminations were no more than 12 hours before and no more than 6 hours after ovulation, respectively. On the day of insemination, 0.5 ml straws were immersed in a water bath at 37°C for 30 seconds. A commercial semen extender was added diluting the frozen-thawed semen to a final concentration of 50 x 10^6 total sperm/ml. To create equivalent insemination doses, the total volume was halved, transferred into 2 latex-free syringes, and packaged in individual styrofoam semen shipping containers with ice packs. First dose was utilized within 1 hour after thawing whereas the 2nd dose was chilled and stored at 5°C for 14 hours. Prior to each insemination, transrectal palpation and ultrasonography were performed confirming that the 1st and 2nd insemination occurred pre and postovulation, respectively. Mare was assessed next day and postbreeding management was routine. Transrectal ultrasonography on day 15 postovulation confirmed pregnancy. Although the need for specialized equipment and the hazards of working with liquid nitrogen will not be eliminated, this case report demonstrated that some of the logistical issues may be overcome while breeding on-farm with frozen-thawed semen.

Keywords: Equine, insemination, frozen, semen, breeding management

Reference

1. Prell MJ, McCue PM, Moffett PD, et al: Motility and fertility evaluation of thawed frozen stallion semen after 24 hours of cooled storage. J Equine Vet Sci 2020;90:102983.

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

Influence of season and plasma prolactin concentrations on fertility of Bos taurus beef bulls

Rory Nevard,[a,b] Sameer Pant,[a,b] John Broster,[a,b] Scott Norman,[a,b,c] Cyril Stephen[a,b]

[a]*School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia*
[b]*Gulbali Centre for Agriculture, Environment and Water, Wagga Wagga, NSW, Australia*
[c]*Kallangur Veterinary Surgery, Kallangur, Queensland, Australia*

Prolactin is known to influence testicular function in beef bulls, but the relationship between plasma prolactin concentrations and semen quality parameters (e.g. sperm motility and morphology) is unclear. Furthermore, both seasonal variability and innate circadian rhythms can potentially influence plasma prolactin concentrations, complicating efforts to characterize the relationship between prolactin and bull semen quality parameters. Objective was to assess seasonal variability in bull plasma prolactin concentrations and determine its relationship with semen quality parameters. Twelve mixed-age beef bulls (7 Angus and 5 Limousin) were used. Bulls were yarded, had blood collected, and examined for breeding soundness on the last week (± 1 week) of each month from April to December. Reproductive parameters assessed included scrotal circumference (SC), progressive motility (PM) of sperm (assessed using computer assisted sperm analysis), and sperm morphology. Plasma prolactin concentrations were quantified using a commercial bovine prolactin ELISA kit (bPRL, AssayGenie). Results indicated that plasma prolactin concentrations were lower ($p = 0.043$) in summer compared to winter in contrast to previous findings. Plasma prolactin concentrations and daylength (measured in hours) were negatively correlated ($r = -0.23$, $p = 0.040$), but the correlations between plasma prolactin concentrations and reproductive parameters were not significant (prolactin versus SC: $r = -0.041$, $p = 0.736$; prolactin versus sperm morphology: $r = -0.03$, $p = 0.789$; prolactin versus PM: $r = 0.02$, $p = 0.923$). Bull age had influence on both percentage normal sperm ($p = 0.032$) and SC measurements ($p = 0.025$). Older bulls tended to have larger SC and more morphological sperm abnormalities. Month of sampling significantly influenced SC. Overall, plasma prolactin concentrations had influence on seasonal variability, and although no relationship was identified between plasma prolactin concentrations and male reproductive parameters, this could likely be attributed to the relatively small sample size, insufficient statistical power, and infrequent sampling. Future studies investigating this relationship in larger populations, accounting also for seasonal variability, are warranted.

Keywords: Bull, beef cattle, fertility, prolactin, season

Severe edema of the uteroplacental unit in a Quarter Horse mare

Stephanie Walbornn,[a] Erin Newkirk[b]

[a]*Rood and Riddle Equine Hospital in Wellington, Wellington, FL, USA*
[b]*WellGrove Equine, Loxahatchee, FL, USA*

Placentitisis described as inflammation of the placenta and is most commonly caused by an ascending infection through the cervix. Clinical signs of placentitis include premature mammary development and vulvar discharge. Ultrasonographic changes include thickening of the uteroplacental unit and separation between the placenta and the endometrium. Several studies have described normal values for the combined thickness of the uteroplacental unit (CTUP).[1,2] Routine monitoring throughout pregnancy via ultrasonographic examination can help to diagnose early cases of placentitis and implement treatment at the early stage of the disease process. A case of severe uteroplacental edema in a maiden 2008 Quarter horse mare is described. Mare was presented at 309 days of pregnancy for evaluation and monitoring after premature mammary development was observed and a higher CTUP was diagnosed via transrectal ultrasonography. On presentation, mammary development was observed and vulvar discharge was not noted. On transrectal ultrasonography, CTUP measured 2.5 - 3 cm and it was difficult to determine if the increase in thickness was present within the endometrium, chorion, or both. CTUP was diffusely increased both dorsally and ventrally. A small area of separation was observed close to the internal cervical os. Echogenicity of fetal fluids were within normal limits. Allantoic fluid was slightly echogenic (1 out of 4) and the amniotic fluid was more echogenic (2 out of 4) and the amniotic membrane did not appear thickened. Fetus was mobile and average fetal heart rate was 74 beats per minute. Mare was given antibiotics, antiinflammatories, and Regumate® and was monitored daily via ultrasonography for fetal and placental viability. Next day, fetal heart rate increased to 120 beats per minute and remained elevated at subsequent examinations; therefore, the mare was started on intranasal oxygen at 10 liters/hour. No changes were observed in the placenta once treatment was implemented. Mare entered spontaneous labor at 317 days of pregnancy and foaled with assistance. Filly had signs of prematurity (floppy ears, domed head, silky haircoat, and incomplete ossification of the cuboidal bones). Mare expelled most of fetal membranes within 3 hours after delivery and retained a piece in the nongravid uterine horn. Treatment was implemented. Examination of fetal membranes revealed thickening at the area of the cervical star and a few avillous areas on the chorion. Fresh and fixed samples were submitted to the University of Kentucky Diagnostic Laboratory for culture and histopathology. Pathology report indicated chronic placentitis, but no etiologic agent was identified. Postfoaling ultrasonography of the uterus revealed no severe edema within the endometrium; therefore, it was suspected that the edema was primarily within the chorion. The amount of edema present within the placenta raised the question of whether placental insufficiency or another placental abnormality also had a part in the premature foaling and the possibility of an infectious agent contributing to placentitis.

Keywords: Placentitis, uteroplacental thickness, placental insufficiency

References

1. Bucca S, Fogarty U, Collins A, et al: Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. Theriogenology 2005;64:542-557.
2. Renaudin, CD, Troedsson, MHT, Gillis, CL, et al: Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. Theriogenology 1997;47:559-573.

Fertility in a mare with pharyngeal lymphoma that was treated with doxorubicin

Stephanie Walbornn,[a] Justin McNaughten[b]

[a]*Rood and Riddle Equine Hospital in Wellington, Wellington, FL, USA*
[b]*Palm Beach Equine Clinic, Wellington, FL, USA*

Doxorubicin is a chemotherapeutic agent utilized for several kinds of neoplasia including lymphoma. In mice, doxorubicin had a negative effect on granulosa cells.[1] Limited information is available on the effect of doxorubicin treatment on mares' fertility. A case of a 12-year, Irish Draft maiden mare, with a history of doxorubicin treatment for localized pharyngeal lymphoma is described. Last treatment was performed in September 2021. Mare also had a history of recurrent respiratory infections and had been diagnosed with combined variable immunodeficiency. Breeding management began in April 2022. Mare was bred during 4 estrous cycles prior to recovery of an embryo. Normal ovarian architecture with normal ovarian activity were present. Mare was bred with frozen semen on the first estrous cycle and freshcooled semen on the subsequent 2 estrous cycles from 1 stallion and then switched to another stallion and bred over 1 estrous cycle with freshcooled semen. On day 7, grade 3 expanded blastocyst was recovered from an embryo flush. This embryo was transferred into a recipient mare on the same day and the mare was confirmed pregnant at 14 days. Heart beat was also confirmed and to our knowledge the recipient mare is still pregnant. It is difficult to discern whether the difficulty obtaining an embryo was due to subfertility from the chemotherapeutic agent or systemic immunodeficiency predisposing the mare to a decreased immune response within the uterus. To our knowledge, this is the first report of breeding management in a mare treated with doxorubicin and successful recovery of an embryo with a viable pregnancy.

Keywords: Subfertility, embryo transfer, doxorubicin

Reference

1. Ting Z, Hong H, Lin F, et al: Effect of doxorubicin-induced ovarian toxicity on mouse ovarian granulosa cells. Regul Toxicol Pharmacol 2017;86:1-10.

Diagnosis of canine herpesvirus preserves future neonatal viability

Tiffany Hoffman,[a] Casey Rubin,[b] Morgan Agnew[c]

[a]*Rutgers University, New Brunswick, NJ, USA*
[b]*Philadelphia Animal Hospital, Philadelphia, PA, USA*

A 5-year, Great Dane female dog, was presented for right hind leg lameness, lethargy, and decreased appetite of 2 days of duration at 49 days of pregnancy. Physical examination was unremarkable. Transabdominal ultrasonography revealed that the fetus immediately cranial to the cervix had no heartbeat, decreased fetal fluids, and a thickened placenta. All other fetuses appeared normal. Vaginal cytology revealed many cocci and neutrophils. Owner declined for a culture. Patient was empirically treated with 9 mg/kg cefpodoxime once a day. Patient's initial serum progesterone concentrations were 28.54 ng/ml (in-house Mini Vidas® machine); high enough to maintain pregnancy. Follow up ultrasonography (in 5 to 7 days) was recommended, but was not performed. Patient was presented 8 days later with decreased appetite and blood-tinged purulent vulvar discharge. Transabdominal ultrasonography was performed that revealed multiple deceased fetuses surrounded by echogenic fluid in both uterine horns. No viable fetuses were visualized. A complete blood count and serum chemistry were within normal limits; patient's serum progesterone concentrations were 4.39 ng/ml. Ovariohysterectomy was recommended and performed the same day. Five fetuses were easily isolated from the uterus and it was diagnosed that they had died at different stages of development. Based on the appearance of the fetuses, canine herpes virus (CHV) was suspected, and a titer was submitted to Cornell University. Patient's virus neutralization antibody titer was positive for CHV. Testing was recommended for other breeding female dogs in the same kennel, 2 of which also tested positive for CHV. For all female dogs with positive titers, following recommendations were made for subsequent pregnancies: begin oral antiviral treatment starting 1 week prior to due date; give oral plasma from donor with a positive CHV titer to all neonates at birth; give pups oral antivirals for 2 weeks after delivery. Blood was drawn from female dogs in the kennel and plasma was stored for oral treatment to neonates at birth. CVH is 1 of the most common causes of viral abortion and neonatal death in canines whereas Brucella canis and Streptococcus spp. are the most common causes of bacterial abortion and neonatal death.[1] Naive female dogs exposed to CHV during middle of pregnancy are at high risk for aborting underdeveloped fetuses.[2] CHV abortion is not common in subsequent litters after an initial exposure; however, viral recrudescence is possible.[2] Oral blood plasma (collected from patients with a positive CHV titer) treatment may suppress viremia, help prevent CHV infection, and decrease mortality in neonates.[2] This case illustrated the importance of appropriate diagnostic testing to help preserve reproductive function in female dogs and neonatal viability.

Keywords: Herpesvirus, abortion, vaginal cytology, neonatal viability

References

1. Lamm CG, Njaa BL: Clinical approach to abortion, stillbirth, and neonatal death in dogs and cats. Vet Clin North Am Small Anim Pract 2012;42:501-513.
2. Decaro N, Martella V, Buonavoglia C: Canine Adenoviruses and Herpesvirus, Vet Clin North Am Small Anim Pract 2008;38:799-814.

STUDENT CLINICAL CASE SESSION

Ovarian teratoma in a Quarter horse mare

Ashley Beyer,[a] Kylie Roux,[a] Allison Salinger,[b] Gabriel Gonzalez,[a] Christine Lopp-Schurter,[c] Kevin Walters,[a] Heath King,[a] Darcie Sidelinger[a]

[a]Mississippi State University College of Veterinary Medicine, Mississippi State, MS, USA
[b]Mid South Equine Sports Medicine & Surgery, Tioga, TX, USA
[c]Iowa State University College of Veterinary Medicine, Ames, IA, USA

A 5-year, Quarter horse, mare was presented for an ovarian mass. Left ovary appeared enlarged with abnormal texture on transrectal palpation and measured ~ 8 cm in diameter with multiple hypoechoic, cystic areas along with several hyperechoic areas with shadowing on transrectal ultrasonography. Right ovary was normal in size and contained multiple small to medium follicles. Mare was diagnosed nonpregnant. Based on the size and appearance of the left ovary, an ovarian tumor was suspected, and surgical removal was recommended. A standing-flank, hand-assisted, laparoscopic ovariectomy was performed. Because of its size, the left ovary was removed from the abdomen using a sterile bag. On gross examination, tissues that resembled hair, adipose, bone, and cartilage were noticed, fitting the description of ovarian teratoma; however, owner declined histopathology. Ovarian teratomas are reported to be the second most common ovarian tumor in mares, second only to granulosa cell tumors, and more commonly observed in younger mares.[1] As a tumor of germ cell origin, teratoma must contain at least 2 of the mature form of germ cell tissues (endoderm, ectoderm, or mesoderm).[2] Prognosis for ovarian teratoma is good as they are typically benign and hormonally inactive.[1] Ovarian teratomas are often incidental findings with no associated clinical signs.[3] Contralateral ovary is often unaffected, continues cycling normally, and pregnancy rates are acceptable.[2,4] Removal of the affected ovary is the treatment of choice.[1] Mare recovered uneventfully from surgery and was discharged next day. Owners report that the mare is doing well and they did not attempt breeding.

Keywords: Mare, ovary, teratoma, laparoscopic ovariectomy

References

1. Catone G, Marino G, Mancuso R, et al: Clinicopathological features of an equine ovarian teratoma. Reprod Domest Anim 2004;39:65-69.
2. Duarte L, Allen WR, Guimei M, et al: Equine ovarian teratomas: Diagnostic challenges illustrated by case reports. Equine Vet Educ 2021;34:393-405.
3. Singh H, Singh N, Jan MH, et al: Clinicopathological features of ovarian teratoma presented as colic in mare. Theriogenology Insight 2019;9:7-11.
4. Gündüz M, Kaşikçi G, Kiliçarslan R, et al: Reproductive performance following unilateral ovariectomy for treatment of ovarian tumors in 7 mares. Turk J Vet Anim Sci 2010;34:283-287.

Outbreak of penile warts among a group of Angus and Charolais bulls

Thomas Burke, Manuel Chamorro, Thomas Passler, Miguel Saucedo, Chance Armstrong

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

A group of Angus and Charolais bulls was presented for evaluation of penile masses. A total of 10% of the 300 bulls prepared for sale was affected. This group of bulls was reportedly eating and drinking normally and fed a diet of hay, 60% soy hull pellets, 20% distillers' grain, and 20% ground corn. Affected bulls were individually placed on a tilt table and stimulated rectally until erection was achieved. Once extended, a towel clamp was placed under the dorsal apical ligament of the penis, to maintain extension, and the penis was examined. The masses appeared to be fibrous and varying in severity from small to large, single, coalescing, or pedunculated. Masses were located laterally or circumferential with some invading towards or extending beyond the external urethral orifice on the distal third of the penis. Three bulls had necrosis of the associated penile mass. Bulls' penises were cleaned thoroughly, and a dorsal penile nerve block was placed proximally with 3 - 5 ml of 2% lidocaine. Masses were surgically resected using cautery and scalpel blades. An 8 French polypropylene catheter was placed in the urethra to prevent iatrogenic damage to urethra and urethral lumen compromise. Absorbable sutures were placed in the surgical sites of 4 bulls. Three bulls received mycobacterium cell wall fraction immunotherapeutic treatment after surgery and owner was instructed to give the injection to remaining bulls once supplies were restocked. Samples from the resected masses were sent for recombinant vaccine production for the specific strain of papillomavirus that caused this outbreak. Owner was instructed to monitor penis and prepuce for bleeding, swelling, and inflammation for 1 week and have their herd veterinarian reexamine these bulls for recurrence of the masses at 30 - 45 days after surgery. They were also informed that these bulls were not to be sold for meat for 21 days after the immunostimulant was given to allow for appropriate withdrawal time. Penile warts or fibropapillomas are caused by bovine papilloma virus.[1,2] Bovine papilloma virus lesions typically

appear on areas of the body more prone to abrasion like head, neck, and legs.[3] Fibropapillomas of the penis occur in young bulls housed together and participating in homosexual behavior.[1,3] The virus enters through small abrasions in the penile skin and causes neoplastic growth of fibroblasts on the distal portion or glans penis.[1,3] Penile warts are a common occurrence in young bulls, but the prevalence within a group tends to be low, 2 - 5%. However, some instances the prevalence was 10 - 30%.[1-3] Penile warts treatments include surgical removal via scalpel excision, CO2 laser, or cryotherapy. These therapies may be performed alone or with an immunostimulant treatment to prevent recurrence.[1] Phimosis and paraphimosis may occur secondary to large penile fibropapillomas due to the distal portion of the penis becoming too large to pass though the preputial orifice.[4]

Keywords: Fibropapilloma, bull, penis, wart

References

1. Hopper RM, Wolfe DF: Restorative surgery of the prepuce and penis. In: Hopper RM: editor. Bovine Reproduction. 2nd edition, Hoboken; Wiley-Blackwell: 2021. p. 210-211.
2. Whittier WD, Warnick LD, Eller AL: Efficacy of bovine papilloma virus vaccination in preventing papillomas in yearling beef bulls. Bov Proc 1997;30:146-148.
3. Dawson L, Bierchwal CJ: Fibropapilloma of the bovine penis. Bov Prac 1983;18:184-187.
4. Maxwell H: Inability to breed due to injury or abnormality of the external genitalia of bulls. In: Hopper RM: editor. Bovine Reproduction. 2nd edition, Hoboken; Wiley-Blackwell: 2021. p. 161-166.

Monozygotic twin pregnancy in a Quarter Horse embryo recipient mare

Emma Mullins, Humberto Magalhaes, Kianna Spencer, Giorgia Podico, Igor Canisso

College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana IL, USA

Monozygotic twinning is a rare incidence compared to dizygotic twins.[1] Development of monozygotic twins has been associated with zona pellucida hatching during intracytoplasmic sperm injection and assisted hatching protocols with in vitro embryos.[2] Thus, in vivo embryos are less likely to develop monozygotic twins. Occurrence of a monozygotic twin pregnancy in a Quarter Horse mare that was presented for premature udder development and lactation is described. Mare had a nonsurgical embryo transfer performed 8 months before. Embryo was produced in vivo and transferred immediately after collection from the donor mare. Recipient mare was diagnosed pregnant with a singleton at the initial examination with no further examinations. At ~ 7 months of pregnancy, the mare developed an engorged udder with early milk production. Premature udder development and lactation is often indicative of placentitis, a leading cause of infectious abortions in the horse.[3] Mare was given intramuscular penicillin G procaine (22,000 ui/kg, once every 12 hours), oral altrenogest (0.088 mg/kg, once daily), and intravenous flunixin meglumine (1.1 mg/kg, once every 12 hours) at the referring facility to treat the presumed placental disease. Mare's udder decreased in volume; however, purulent vulvar discharge developed, and the mare was referred. Physical examination was unremarkable. Transrectal ultrasonography revealed fetal fluids with floating hyperechoic materials. No fetal movements were detected on ballottement and transabdominal ultrasonography revealed a nonviable fetus. Intramuscular prostaglandin $F_{2\alpha}$ (cloprostenol 250 µg) was given to promote delivery of the fetus. However, 2 fetuses and 2 fetal membranes were delivered 30 minutes after treatment; the smaller fetus presented signs of umbilical torsion. Postabortion, the mare was given a large volume uterine lavage and flunixin meglumine with butorphanol to address her discomfort. As more complications occurred (i.e. metritis, laminitis), owners elected to euthanize due to poor prognosis and financial constraints. To prevent twinning complications, recipient mares should be examined between 25 and 30 days of pregnancy.[4] Although the initial pregnancy diagnosis determined a singleton, it is common that twins are not identified at the first examination, and a second examination should be conducted to confirm the diagnosis to prevent further development of both embryos.[5] This case was unique as twinning is very unlikely in in vivo embryo transfers. Studies are limited on monozygotic twinning in in vivo embryos due to reduced prevalence compared to in vitro embryos.

Keywords: Monozygotic pregnancy, embryo transfer, noninfectious abortion

References

1. Roberts MA, London K, Campos-Chillon LF, et al: Presumed monozygotic twins develop following transfer of an in vitro-produced equine embryo. J Equine Sci 2015;26:89-94.
2. Hodder ADJ, Liu IKM, Ball BA: Current methods for the diagnosis and management of twin pregnancy in the mare. Equine Vet Educ 2008;20:493-502.
3. Fedorka CE, Scoggin KE, Ruby RE, et al: Clinical, pathologic, and epidemiologic features of nocardioform placentitis in the mare. Theriogenology 2021;171:155-161
4. Crabtree JR: Management of twin horses. Br Vet J 2018;40:66-74.
5. Mancill SS, Blodgett G, Arnott RJ, et al: Description and genetic analysis of three sets of monozygotic twins resulting from transfers of single embryos to recipient mares. J Am Vet Med Assoc 2011;238:1040-1043.

Low conception rates in a breeding Standardbred stallion due to spermiostasis and seminal plasma toxicity complicated by low libido and osteoarthritis

Hayley Rossiano,[a] Kianna Spencer,[a] Giorgia Podico,[a] Igor Canisso[a]

[a]College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana IL, USA

An 11-year, Standardbred stallion, was presented for breeding soundness evaluation after a reported pregnancy rate of 5% per cycle and average of 7 jump attempts per collection. On presentation, he displayed signs of low libido and lameness localized bilaterally to both tarsi. Semen was collected in 2 jumps and subsequently extended using a skim milk-based (SC) and an egg yolk-based (EY) extender. Percentages of total motility (TM) and progressive motility (PM) were assessed with computer-assisted semen analyzer. On presentation, initial ejaculate (EI) had a volume of 45 ml with concentration of 328 x 10^6 sperm/ml and 1.5 x 10^{10} total sperm. Total motility was 64% and progressive motility was 52%. Motility decreased after extension

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

with EY (TM 56%, PM 51%) and SC (TM 30%, PM 24%). Motility decreased with EY (TM 54%, PM 50%) and SC (TM 47%, PM 39%) in 24-hour cooling test. On average, with serial collections, the first ejaculate (E1) had a volume of 73 ml, concentration of 218 x 10^6 ml, TM 48% (range: 10 - 68%) and PM 45% (range: 31 - 58%) and second ejaculate (E2) had a volume of 65 ml, concentration of 103 x 10^6 ml, TM 62% (range: 32 - 80%) and PM 53% (range: 25 - 67%). Stallion was diagnosed with sperm accumulation, seminal plasma toxicity, low libido, and osteoarthritis. Semen collection was performed, every other day, for 60 days, to control sperm accumulation.[1] Both tarsometatarsal joints and distal intertarsal joints received intra-articular corticosteroid injections. Oral analgesics included firocoxib (0.1 mg/kg, once daily), intermittent oral phenylbutazone (2.2 mg/kg), as needed, and oral gabapentin (10 mg/kg, twice a day). Stallion's grain was supplemented with extra-virgin olive oil, vitamins E and C, DHA, and alpha lipoic acid top dressing. Vitamin C and E blended with polyunsaturated fatty acids (PUFAs) reduced sperm oxidative damage and a combination of PUFAs and antioxidants stimulated overall strengthening of membrane integrity in fresh, cooled, and frozen semen.[2,3] Pentoxifylline (8.5 mg/kg) was given twice daily to increase testicular perfusion, sperm production, and increase live capacitated sperm.[4,5] Imipramine (3 mg/kg) was given orally, 1 hour prior to collection to improve erection and to lower ejaculation threshold.[6,7] An extended teasing protocol was utilized before mounting the phantom to minimize number of collection attempts and decrease orthopedic strain. Omeprazole (1 mg/kg, once a day) was given adjunctively to prevent gastric ulcerations during hospitalization. Lastly, cushion centrifugation was performed on all ejaculates to remove seminal plasma, increasing the percentage of progressively motile sperm to combat seminal plasma toxicity.[8] Semen collected after 60 days of treatment had a volume of 80 ml with concentration of 242 x 10^6 sperm/ml, 1.9 x 10^{10} total sperm, 73% TM and 68% PM. Abnormal accumulation of sperm and seminal plasma toxicity has been anecdotally described in literature but there is a lack of research in the area on standards of treatment. This case also described the unique factor of low libido and osteoarthritis that complicated increased numbers of collections on the phantom needed to increase semen parameters.

Keywords: Sperm accumulation, seminal plasma toxicity, low libido, osteoarthritis

References

1. McCue PM, Scoggin C, Moffett P, et al: Spermiostasis in stallions: A retrospective study of clinical cases. J Equine Vet Sci 2014;34:47.

2. Bazzano M, Laus F, Spaterna A, et al: Use of nutraceuticals in the stallion: Effects on semen quality and preservation. Reprod in Domest Anim 2021;56:951-957.

3. Freitas ML, Bouéres CS, Pignataro TA, et al: Quality of fresh, cooled, and frozen semen from stallions supplemented with antioxidants and fatty acids. J Equine Vet Sci 2016;46:1-6.

4. Pozor MA, Muehlhaus J, King A, et al: Effect of pentoxifylline treatment on testicular perfusion and semen quality in miniature horse stallions. Theriogenology 2011;76:1027-1035.

5. Ortgies F, Klewitz J, Martinsson G, et al: Effect of procaine, pentoxifylline and trolox on capacitation and hyperactivation of stallion spermatozoa. Andrologia 2011;44:130-138.

6. McDonnell SM, Garcia MC, Kenney RM, et al: Imipramine-induced erection, masturbation, and ejaculation in male horses. Pharmacol Biochem Behav 1987;27:187-191.

7. McCue PM: Chemical ejaculation. In: Dascanio JJ, McCue PM: editors. Equine Reproductive Procedures. 2nd edition, Ames; Wiley Blackwell: 2021. p. 347-348.

8. Brinsko SP, Crockett EC, Squires EL: Effect of centrifugation and partial removal of seminal plasma on equine spermatozoal motility after cooling and storage. Theriogenology 2000;54:129-136.

Severe hind limb lameness in a female dog with closed-cervix pyometra

Jewel Toenges,[a] Michela Ciccarelli[a,b]

[a]College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[b]School of Molecular Bioscience, Washington State University, Pullman, WA, USA

Pyometra is a life-threatening disease that is commonly presented in older intact female dogs with clinical signs of lethargy, anorexia, polyuria, polydipsia, and vaginal discharge in cases of open-cervix. Severe cases of pyometra are often associated with other signs of systemic disease such as leukocytosis, neutrophilia with a left shift, anemia, hypoalbuminemia, and other changes in liver or kidney function. Pyometra has been only reported concomitant to lameness in 2 other reports but may be an under-documented clinical sign.[1,2] A 4-year female intact, English Bulldog, was presented for sudden grade 6 left hind lameness, 20 days after transcervical insemination (TCI). Transabdominal ultrasonography revealed multiple vesicles without visible embryo proper and multiple corpora lutea on both ovaries. Complete blood count, biochemistry, and blood progesterone were completed to rule out pyometra and recent pregnancy loss. Results were unremarkable and blood progesterone concentrations were 21.2 ng/ml. She was prescribed carprofen and recommended strict kennel rest until an orthopedic examination appointment was available. Six days after the initial presentation, she was evaluated by the orthopedic surgery service. Musculoskeletal examination was performed along with stifle radiographs, both were within normal limits, presuming that the lameness was due to a soft-tissue injury. Another transabdominal ultrasonography was performed that confirmed the lack of embryos and possible mucometra/pyometra. Ovariohysterectomy (OHE) was recommended to resolve mucometra/pyometra and was performed the same day. After OHE, the patient's lameness resolved completely. Due to the sudden resolution of hindlimb lameness following surgery, it was assumed that the pyometra was the cause. Several mechanisms may have had a role in the pathogenesis such as immune mediate arthritis due to bacterial infection or toxin-induced, visceral pain, pressure, and discomfort from uterine distension, but neuromuscular pathology was not ruled out. This case demonstrated that hindlimb lameness may be included as a rare clinical sign of pyometra. Further research is needed to investigate the pathogenesis of pyometra and lameness.

Keywords: Closed-cervix pyometra, hindlimb lameness

References

1. Klainbart S, Ranen E, Glikman G, et al: Hindlimb lameness and gait abnormalities in bitches with pyometra. Vet Rec 2014;175:46.
2. Jitpean S, Ström-Holst B, Emanuelson U, et al: Outcome of pyometra in female dogs and predictors of peritonitis and prolonged postoperative hospitalization in surgically treated cases. BMC Vet Res 2014;10:6.

Suspected penile spiral deviation secondary to penile denervation in a Shorthorn bull

Cody Davis, Jessica Cowley, Amy Yanke, Katelyn Waters, Jessica Klabnik

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Performing a thorough breeding soundness examination and test mating prior to surgical correction of penile or preputial abnormalities is recommended for diagnosis of comorbidities that may impact a bull's return to reproductive soundness postoperatively. A 4-year, milking Shorthorn bull, was presented for suspected spiral deviation of the penis. Owner reported successful intromission in this bull during first breeding season but intromission failure during subsequent seasons. To assess bull's reproductive soundness, test mating and breeding soundness examination were performed. During the initial test mating, bull was uninterested in mounting. Breeding soundness examination revealed a visually normal erect penis (observed during electroejaculation) with normal internal genitalia, prepuce, scrotal contents, and scrotal circumference. Progressive motility of the sperm was adequate with acceptable sperm morphology (70% normal, 11% head abnormalities and 19% midpiece abnormalities). During second test mating, bull failed to achieve intromission during 4 mountings. First mount involved a normal erect penis that spiraled upon contact with the female's perineal area. Latter 3 mountings resulted in an immediate spiral deviation. Given the delayed spiral during the first mount and consistent inability to copulate (impotentia coeundi), damage to the dorsal nerve of the penis was suspected with secondary penile deviation. A primary spiral deviation was lower on the differential list because it is expected that the penis consistently spirals during live cover in primary spiral deviation. Abnormal penile spiraling results from an abnormal apical ligament or premature high corpus cavernosum penis pressure.[1] To further evaluate the differential diagnosis of penile denervation, a nerve conduction test was performed to quantitatively determine dorsal nerve function;[2,3] no conduction velocity was measured from distal penis, and low conduction velocity was measured immediately distal to the sigmoid flexure. Lack of innervation detected from the nerve conduction test is confirmatory for the diagnosis of penile denervation.[2,3] Damage to the dorsal nerve of the penis likely occurred due to trauma during bull's initial breeding season. In this case, it is reasonable to conclude that the penile spiral deviation is observed secondary to penile denervation. Penile denervation injuries are considered permanent injuries; therefore, the bull's prognosis for natural service is poor. However, the bull could be utilized for semen collection via electroejaculation.[3] Owner opted to utilize this bull as a teaser animal. Penile denervation should be considered as a differential or concurrent diagnosis for penile spiral deviation defects.

Keywords: Penile denervation, spiral deviation, bull, nerve conduction

References

1. Hopper RM, Wolfe DF: Restorative surgery of the prepuce and penis. In: Hopper RM: editor. Bovine Reproduction. 2nd edition, Hoboken; Joe Wiley & Sons: 2021. p. 210-229.
2. Wolfe DF, Moll HD: Examination and special diagnostic procedures of the penis and prepuce. In: Wolfe DF, Moll HD: editors. Large Animal Urogenital Surgery. 2nd edition, Baltimore; Williams & Wilkins: 1999. p. 221-231.
3. Maxwell H: Inability to breed due to injury or abnormality of the external genitalia of bulls. In: Hopper RM: editor. Bovine Reproduction. 2nd edition, Hoboken; Joe Wiley & Sons: 2021. p. 155-172.

Unilateral segmental aplasia of epididymis tail in a bull

Keri Shipman, Jessica Cowley, Katelyn Waters, Lily Lewis, Jessica Klabnik

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Epididymis is the final site of sperm maturation; therefore, aplasia of the epidydimal tract could decrease reproductive efficiency despite having a normal scrotal circumference.[1] In September of 2022, an apparently healthy 2-year, Aberdeen Angus bull, was referred due to an abnormal right testis that was diagnosed during a routine bull breeding soundness examination. As per the owner, referring veterinarian noted a firmer right testis on palpation. Medical record documented the following: 'a mal-structured right testis', very good sperm motility, 87% normal sperm morphology (7% abnormal heads and 6% tail defects) and a scrotal circumference of 39 cm. Transscrotal ultrasonography revealed missing right epididymis tail. Based on bull's age and history, congenital unilateral segmental aplasia of the epididymis was suspected. Congenital anomalies occurred secondary to defects during embryonic development. Elongation and coiling of the mesonephric duct lead to epididymal development. Only this bull's left epididymis was competent to transport semen from testis to ductus deferens. Therefore, bull's ejaculate could only contain up to 50% of total possible sperm that could be produced, despite having an acceptable scrotal circumference. It was recommended to cull the bull due to poor reproductive efficiency and the possibility of transmitting an inherited autosomal recessive defect.[2] However, owner elected to breed a few cows due to bull's quality and pedigree despite the inheritable nature of this congenital defect. This case highlighted the need for thorough palpation of scrotal contents in addition to scrotal measurement.

Keywords: Testis, epididymis, reproductive efficiency, congenital anomalies

References

1. James ER, Carrell DT, Aston KI, et al: The role of the epididymis and the contribution of epididymosomes to mammalian reproduction. Int J Mol Sci 2020;21:5377.
2. König H, Weber W, Kupferschmied H: Epididymal aplasia in bulls and boars. a. demonstration of 18 cases with recessive inheritance in the Simmentaler spotted breed. b. occurrence of abnormalities in one boar and 3 offsprings. Schweizer Archiv für Tierheilkunde 1972;114:73-82.

Semen collection in domestic cat: comparison of techniques

Kristyn Burton,[a] Aime Johnson[b]

[a]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[b]College of Veterinary Medicine, Auburn University, Auburn, AL, USA

Semen collection in cats in the clinic setting can be difficult. However, semen analysis is vital. Electroejaculation (EEJ) is currently the most reliable semen collection method but requires specialized equipment. Urethral catheterization is a technique that allows semen collection without special equipment - a catheter is placed in the urethra of a sedated tom and semen is collected passively in the catheter. Earlier studies used medetomidine at high doses. However, medetomidine has been replaced with dexmedetomidine in some countries. This study sought to compare the results of electroejaculation (EEJ) and urethral catheterization (UC) for semen collection in domestic cat using dexmedetomidine, a potent α2-adrenoceptor agonist (α2A) as a substitute for medetomidine at the equivalent dose used in earlier studies. Twelve domestic cats were collected thrice at weekly intervals. All cats received intramuscular ketamine (5 mg/kg) and intramuscular dexmedetomidine (30 µg/kg) for initial cleanout via EEJ, then randomly underwent either EEJ or UC 1 week apart. The EEJ was performed under the same anesthetic protocol as the initial cleanout. The UC was performed using intramuscular dexmedetomidine at a dose of 60 µg/kg. Success of collection, total sperm number, and motility characteristics were analyzed. Sperm was collected successfully from all 12 cats via EEJ and from 11/12 via UC. There were no significant differences in the percentage of total motile, progressively motile, or morphologically normal sperm between ejaculate types when averaged across all cats or individual cats. Although UC yielded a lower volume and higher concentration ejaculate, it consistently produced a lower total sperm number than ejaculates retrieved via EEJ (17.91 x 10^6 total sperm for UC versus 46.51 x 10^6 total sperm for EEJ). These results indicated that dexmedetomidine is a very effective α2A and performed satisfactorily in both procedures at the doses used in this study. It was also safe with no adverse effects on toms. EEJ remained the most reliable in terms of assessing semen quality and retrieving semen with adequate number of sperm for breeding purposes. However, UC with dexmedetomidine at this dose can be a remarkably consistent alternative that demonstrated 92% success rate.

Keywords: Cats, tom, sperm, electroejaculation, catheterization, dexmedetomidine

Effect of age on serum anti-Müllerian hormone concentrations in female alpacas

Dane Schwartz,[a] Cristian Patino,[a] Salman Waqas,[a] Eduardo Arroyo,[a] Alan Conley,[b] Ahmed Tibary[a]

[a]Department Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, USA
[b]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, USA

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of early antral follicles. Multiples studies identified that circulating AMH concentrations are reliable markers of the antral follicle population. In ruminants, serum AMH concentrations are well correlated with the response to superovulation treatment. Similar results were obtained for alpacas. In mares and women, serum AMH concentrations decline after 20 years of age indicating depletion of antral follicle reserves. We hypothesized that a similar effect of age on serum AMH concentrations exists in alpacas. Objective of this study was to compare serum AMH concentrations in 3 age groups. Twenty healthy, multiparous Huacaya female alpacas of 3 age groups were used (Group 1: 4 - 8 years, n = 6; Group 2: 9-13 years, n = 7; and Group 3: 16 - 21 years, n = 7). Blood samples were collected by jugular venipuncture for determination of serum AMH concentrations. Blood samples were allowed to clot then centrifuged to harvest serum that was stored at -20°C until analysis. Serum AMH concentrations were determined in duplicate according to the manufacturer's instructions assay (Rat and Mouse AMH ELISA AL-113, Ansh Labs, Webster, TX, USA). The monoclonal antibody pairs used in the AMH assays bind to the noncovalent AMH complex and do not detect other related members of the TGF- β superfamily. Samples from each female were included in the same analytical run. Intra-assay coefficients of variation for serum pools with high (13.5 ng/ml, n = 14), medium (6.2 ng/ml, n = 14) and low concentrations (2.3 ng/ml, n = 14) were 2.6, 5.5 and 4.1%, respectively. The lowest calibrator was 0.41 ng/ml, below which concentrations were estimated by extrapolation. Serum AMH concentrations among 3 groups were compared by ANOVA, with age as the main factor. Serum AMH concentration (mean ± SEM) was 2.61 ± 0.66, 1.22 ± 0.39, and 0.47 ± 0.23 for Group 1, Group 2, and Group 3, respectively. Serum AMH concentrations were lower ($p < 0.05$) in Group 3 females than in Groups 1 and 2. There were no significant differences between Group 1 and Group 2 in serum AMH concentrations. In conclusion, these preliminary results indicated that serum AMH concentrations decreased with advanced age and may reflect ovarian senescence in female alpacas. A study on a larger number of females of various ages is needed to confirm these results and their effects on reproductive efficiency in camelids.

Keywords: Ovary, aging, camelid, ovarian reserve, fertility

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

Molecular mechanisms underlying endometrial cup immunotolerance: expression of Fas/FasL and Tnfr2 genes

Kassandra Crissman,[a] Jenny Sones,[a] Viviane Gomes[a,b]

[a]*School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA*
[b]*College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA*

Endometrial cups are trophoblast-derived structures that have an important role in equine pregnancy maintenance. However, mechanisms underlying maternal immunotolerance to these semiallogenic structures is incompletely understood. A precisely coordinated cellular response has been previously described in the maternal endometrium-cup interface. Namely, marked recruitment of maternal mononuclear leukocytes to the adjacent endometrial stroma occurs early in the endometrial cup development. Composed primarily of maternal CD4+ and CD8+ T lymphocytes, this leukocyte population is locally regulated by trophoblast and regulatory T cell-derived signaling molecules, preventing cytotoxicity and endometrial cup destruction. Interestingly, trophoblast cell secretion of fas ligand (FasL), a type II transmembrane protein of the tumor necrosis factor (Tnf) family, and indoleamine 2,3 disoxygenase 1 (Ido1) has been reported in other species and may provide modulation of the cell mediated immune response. Additionally, Tnf receptor (Tnfr2) has been associated with Fas/FasL-induced CD8+ T cell death. It is hypothesized that Fas/FasL, Ido1, Tnf-α, and Tnfr2 have an immunomodulatory role during endometrial cup development, leading to lymphocyte apoptosis and prevention of cytotoxicity. Endometrial cup samples were dissected from the maternal endometrium of pony mares carrying viable pregnancies during early development, between 42 to 47 days after ovulation, and regression, between 96 to 120 days after ovulation (n = 5 per group). Gene expression of Fas, FasL, Ido1, Tnf-α and Tnfr2 was assessed via RT-PCR using validated primers. Gel electrophoresis was then performed to verify amplification of the expected targets. Relative mRNA quantification was calculated using the delta-delta CT method and normalized based on the expression of equine Glyceraldehyde-3-Phosphate Dehydrogenase. Data distribution was assessed by Shapiro Wilk tests and comparisons were determined using unpaired Student's t-tests. Significance was set at $p < 0.05$ (GraphPad 9.0). When comparing developing to regressing endometrial cups, FasL gene expression was 2.7-fold higher ($p = 0.034$) whereas there were no differences on Fas levels. Gene expression of Tnfr2 was 4-fold higher ($p = 0.013$) in developing endometrial cups versus demise. Expression of Ido1 and Tnf-α were similar between timepoints. Therefore, further investigation of Fas/FasL-Tnfr2 signaling in the equine endometrial cup-maternal interface is warranted.

Keywords: Mare, endometrial cups, immunotolerance, lymphocytes, apoptosis

STUDENT POSTER

Ovarian hematoma in a Tennessee Walking Horse/ Friesian cross foal

Andrew Kirk, Robyn Wilborn, Lindsey Boone, Kara Lascola, Anne Wooldridge, Rachel Pfeifle, Patricia Egli

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

On June 12, 2022, a 5-day, filly was presented at the equine emergency service for lethargy and for an apparent dehydration secondary to decreased milk production by the dam. On initial physical examination, filly appeared quiet, alert, and responsive; a strong latch and suckle ability of the foal was noted. Mucous membranes were pink, but slightly dry, with all other vitals in appropriate parameters. Patent urachus was noted on physical examination. A minimum database panel revealed a mild, mature neutrophilia, hyponatremia, and a marked metabolic acidosis, having bicarbonate measured at 12.3 mmol/l. A subsequent Nova panel was elected and performed, confirming the hyponatremia and metabolic acidosis (bicarbonate 11.6 mmol/l, pH 7.2). Filly was sedated for thoracic and abdominal ultrasonography Thoracic ultrasonography had no significant findings. However, a large mass was discovered in the cranioventral abdomen adjacent to the liver that was best viewed from the right side. Patent urachus was examined via abdominal ultrasonography, but the umbilical artery and vein were noted as normal. No other abnormal findings were observed. Hospitalization with supportive care was elected. Repeat abdominal ultrasonography was performed the next day by a board-certified radiologist, along with abdominal computed tomography. Etiologies of the mass and metabolic acidosis were not determined, but the structure of the mass suggested resection was warranted. Exploratory laparotomy, along with surgical correction of the patent urachus was elected and performed on June 16, 2022. Surgical visualization revealed a large, cystic right ovary with abnormal attachment of the ovarian pedicle. Remainder of the reproductive organs appeared normal, but complete ovariohysterectomy was performed. Patent urachus was resected at spay. Potassium penicillin and amikacin (broad spectrum antibiotics), along with flunixin meglumine (nonsteroidal antiinflammatory) were prescribed for 3 days postoperatively. Tissues were sent to histopathology for a confirmatory diagnosis. Results showed a thin capsule over the mass, along with a layer of smooth muscle resembling ovarian tissue. The center was filled with edematous connective tissue and a dense central region of uniform cells. However, abundant hemorrhage, edema, and fibrin accumulations completely prevented any diagnostic description of cellular features. Multifocal, variably dilated vessels were noted to have fibrin accumulations, described as fibrin thrombi. The center of the mass contained several cavitations filled with blood (hematoma). The histopathological diagnosis was limited in detail due to the obstructed view of the cells, but a broad diagnosis of hematoma with multifocal vascular thrombosis was made. No abnormalities were observed on the left ovary or uterus. Postoperatively, the filly recovered exceptionally. During postoperative monitoring, physical examination parameters appeared normal, and the foal continued to gain weight and remain hydrated. Foal continued to thrive after a 3-day course of antibiotics and nonsteroidal antiinflammatory medications. On June 22, 2022, the foal was discharged.

Keywords: Hematoma, multifocal vascular thrombosis, filly, urachus

Pyometra and dilated cardiomyopathy phenotype in a Great Dane female dog

Dane Schwartz,[a] Lynne Nelson,[a] Cynthia Carlson,[a] Michela Ciccarelli[a,b]

[a]*College of Veterinary Medicine, Washington State University, Pullman, WA, USA*
[b]*School of Molecular Bioscience, Washington State University, Pullman, WA, USA*

A 5-year, nulliparous Great Dane female dog, was presented in October 2022 for suspected pyometra (bloody mucopurulent vaginal discharge, anorexia, and anxious behavior). One month before presentation, she was diagnosed with dilated cardiomyopathy (DCM) via echocardiography. Her DCM was managed with oral pimobendan, given twice daily, and a cardiac prescription diet. On physical examination, dog appeared bright, alert, and responsive and had normal heart and respiratory rates with normal mucous membranes. She had elevated temperature (104.1°F) with bloody mucoid vaginal discharge and soft enlarged nodular mammary glands that expressed milk. Vaginal cytology had red blood cells with a few neutrophils and blood progesterone concentrations were 0.447 ng/ml. Transabdominal ultrasonography findings were suggestive of cystic endometrial hyperplasia. Ultrasonography of the mammary glands revealed signs of galactostasis. Hematology had marked leukocytosis (marked neutrophilia, with monocytosis and eosinophilia), and nonregenerative anemia. Marked hyperglobulinemia was identified in blood chemistry. Ovariohysterectomy was recommended as soon as possible as the diagnostic findings were suggestive of CEH/ pyometra syndrome. Clavamox and carprofen were prescribed until further evaluation by the small animal surgery service. Ovariohysterectomy was performed with no complications. Uterine cultures isolated 2 distinct morphologic types of Escherichia coli. Revaluation with cardiology service in December 2022 determined that the dog had a resolution of DCM with normal heart size and function on echocardiogram. The prior systolic dysfunction was considered most likely due to an inflammatory condition causing myocardial

Citation line: Clinical Theriogenology 2023, 15, 10001, http://dx.doi.org/10.58292/CT.v15.10001

suppression by systemic cytokines. DCM phenotype is mostly caused by nutritional deficiency, hereditary (primary), secondary to severe hypothyroidism, or systemic inflammation. A full thyroid panel had normal values. The potential cause of CEH, in this case, was repeated irregular estrous cycles without pregnancy. Secondary (chronic) infection led to a DCM-like condition that was reversed with therapy for pyometra.

Keywords: Cystic endometrial hyperplasia, echocardiogram, ultrasonography, ovariohysterectomy

Chronic hydrometra in a goat

Daniella Adams,[a] Andrew Muir,[a] Shannon Dehghanpir,[a] Emi Sasaki,[b] Robert Foster,[c] Nadia Richmond,[a] Naomi Falconnier,[b] Jenny Sones,[a] Clare Scully[a]

[a]Department of Veterinary Clinical Sciences
[b]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA
[c]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Hydrometra is an important cause of subfertility in goats. It is characterized by accumulation of sterile clear fluid in the uterus with a persistent corpus luteum.[1] A 2-year, Nubian doe, was presented for a 90- day pregnancy examination. Abdominal ultrasonography revealed a bifurcated fluid-filled uterus indicative of hydrometra. She was treated with 2 doses of intramuscular dinoprost tromethamine (Lutalyse®), 1 in hospital and 1 at home. Ten months later, she was presented for laparoscopic artificial insemination. No abnormalities were noticed on physical examination. Patient was anesthetized with ketamine, butorphanol, midazolam, and lidocaine locally, placed in dorsal recumbency, and clipped and prepped. Two small incisions were made in the skin and a blunted end teat cannula was introduced into abdomen for insufflation. Brown fluid filled the CO2 delivery tube. Another attempt at the other incision was performed with similar result. After this, an exploratory laparotomy and ovariohysterectomy was performed and the abdomen was flushed with sterile saline and procaine penicillin was injected into abdomen. Patient was treated with subcutaneous ceftiofur sodium and intravenous flunixin meglumine. Cytology of the uterine fluid suggested chronic hemorrhage with possible low-grade inflammation. Uterine fluid had a low number of nucleated cells, several erythrocytes, and birefringent amorphous crystals. Although no infectious organisms were lymphocytes (6%), and eosinophils(1%) were present. Macroscopic evaluation and histopathology of the uterus revealed chronic hydrometra with endometrial, myometrial, and perimetral fibrosis, myometrial hypertrophy and bilateral ovarian hypoplasia. Uterus measured 20 x 14 x 6 cm and the uterine wall was expanded up to 2 cm thick and firm with a light tan/white and glistening mucinous endometrium. Endometrium was largely replaced with concentric fibrosis that was up to 1.25 mm thick and had no surface epithelial cells. Fluid dilation of the uterus indicated an outflow obstruction that may be due to segmental aplasia of the cervix or cranial vagina. Both ovaries lacked follicles, there were 1 primordial and 1 secondary follicle on the left ovary and several tertiary follicles in the right ovary. Patient was discharged with meloxicam and naxcel along with stall rest. She reportedly recovered without complication. This case was unique in its presentation of chronic hydrometra without a corpus luteum. It also highlighted the impact on doe fertility in cases of chronic hydrometra and the good prognosis of life with treatment. Serial monitoring of hydrometra is recommended after initial treatment.

Keywords: Chronic hydrometra, fibrosis, corpus luteum

References

1. Kornalijnslijper, JE Bevers MM, van Oord HA et al: Induction of hydrometra in goats by means of active immunization against prostaglandin F2α. Anim Reprod Sci 1997;46:109-122.

Gonads to no 'nads: ovariohysterectomy after chronic pyometra in a mare

Ellianna Blair, Ahmed Mosallam, Jessica Klabnik, Lindsey Boone, Candace Lyman

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn AL, USA

A 10-year, Tennessee Walking Horse mare, was previously presented to her primary practitioner for multiple episodes of white vulvar discharge and perceived hind end discomfort. Examinations frequently revealed pooling of uterine fluid and uterine lavage therapy was provided on numerous occasions. After 2 years of recurring episodes, the mare was referred to university teaching hospital where intermittent reproductive examinations were performed during the following 18 months. Examination findings included anywhere from no uterine fluid or vulvar discharge to pyometra with 5 cm of uterine fluid pooling with white or serosanguinous vulvar discharge. Scant growth of Escherichia coli, susceptible to all antibiotics tested, was obtained but only in enhancement broth. Eventually, the mare was presented with intensely foul-smelling vulvar discharge and a uterine culture swab had heavy growth of Escherichia coli, *Klebsiella pneumoniae*, and *Streptococcus equi* subsp. *zooepidemicus.* Hysteroscopy revealed vaginal and endometrial adhesions pocketing fluid within the uterus, preventing fluid evacuation. The owner inquired about cervical wedge resection: however, as no cervical pathology existed and the cervix was capable of relaxation, this was not determined to be a curative solution. Ovariohysterectomy was suggested, many months later the owner elected to pursue surgery. Preparation for ovariohysterectomy included uterine lavages for 2 days to remove hyperechoic fluid to minimize the risk of abdominal contamination during uterine removal. Gentamicin and lidocaine were infused into uterine lumen for relaxation. A standing laparoscopic approach for ovarian pedicle transection using cautery was planned; thereafter, prolapsing the uterus through the cervix was attempted as an alternative to laparotomy under general anesthesia. In standing stocks, the sedated mare's ovarian pedicles and broad ligament were visualized with laparoscopy equipment, injected with lidocaine, then cauterized and transected with no complications. Unfortunately, the attempt to manually prolapse the uterus into the vagina and through the vulva was unsuccessful, as the cervix did not dilate wide enough to allow introduction of a hand into uterus while the ovarian pedicle transection was occurring. At that point, the laparoscopic portals were closed and the mare was anesthetized for placement in dorsal recumbency. A caudal ventral midline incision allowed exteriorization of the ovaries and uterus. Soon after, substantial amount of abdominal bleeding was noticed but the origin could not be located; uterine transection and

abdominal closure were performed. Before recovery, the mare received an infusion of aminocaproic acid to minimize excessive bleeding by way of preventing blood clot breakdown. Postoperative transabdominal ultrasonography revealed a moderate hemoabdomen but the blood appeared clotted rather than fluid, suggesting active hemorrhage had stopped. Free blood within the abdomen substantially decreased over the first 12 hours postoperatively, likely due to autotransfusion. Five days of systemic procaine penicillin and gentamicin were provided before transitioning to oral trimethoprim/sulfamethoxazole and metronidazole. A tapering course of flunixin meglumine was provided for pain and inflammation. Two weeks following surgery, the referring veterinarian reported that the mare was healing well with no vulvar discharge.

Keywords: Pyometra, ovariohysterectomy, equine spay, manual uterine prolapse

Induction of abortion in a mare with hydroallantois

Kristyn Burton, Reed Holyoak, Dale Kelley

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA

Hydroallantois is a rare condition characterized by a sudden increase in allantoic fluid volume leading to marked abdominal distention. This occurs quickly over the course of several days, typically between 6 and 10 months of pregnancy. The condition is serious, as it can lead to prepubic tendon rupture - a complication that seriously impacts the reproductive future of a mare. A 14-year, American Quarter Horse mare, was presented at 301 days pregnancy with abdominal distention and a history of treatment for placentitis. Transrectal palpation and ultrasonography revealed that the uterus was above the pelvic brim, and the allantoic fluid was at a higher volume than normal. Fetus could not be visualized. Due to the duration of pregnancy remaining and concern over the mare's future fertility it was decided to terminate pregnancy. An intravenous catheter was placed and 1 liter of lactated Ringer's solution was given and heart rate monitored. A sterile 21 inch CH18 Foley catheter was inserted through the cervix into the allantois, and over a period of 2.5 hours a total volume of 45.4 liters was drained. After draining the allantoic fluid, parturition was induced by giving 60 units of oxytocin in 1 liter of 0.9% NaCl solution intravenously over 60 minutes. At 45 minutes the front limbs were visualized in the birth canal, 10 minutes later hind limbs appeared near the right shoulder, indicating that the fetus was malpositioned. Digital palpation revealed extended fetal left hind limb with right hind limb crossing over the left hindlimb. Intravenous detomidine and ketamine were given to place the mare under anesthesia in order to facilitate repositioning and extraction of the fetus. Foal was stillborn and between 4 and 6 weeks premature. Mare was given 1 liter of intravenous lactated Ringer's solution during recovery, followed by flunixin and sulfadiazine ~ 2 hours after procedure. Fetal membranes passed 3 hours after recovery. Mare recovered uneventfully and it was advised rest for at least 2 estrous cycles prior to breeding, with a breeding soundness examination.

Keywords: Hydroallantois, mare, abortion

Unilateral testicular enlargement in a Quarter Horse stallion

Lexie Oncale, Dale Kelley

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University Stillwater, OK, USA

A 16-year, American Quarter Horse stallion, was presented in June 2022 with an enlarged scrotum and poor fertility documented during the previous breeding season. On presentation, a breeding soundness examination was performed. On semen evaluation, the ejaculate appeared slightly opaque and had a total sperm number of 159 x 10^6 sperm, 50% total motility and 30% progressive motility. Sperm morphology evaluation using differential interference microscopy showed 38% normal sperm morphology, with the primary abnormality of abnormal heads (43%). Palpation of scrotum identified the right testis as noticeably large and firm, with a marked indentation on the ventral aspect. Left testis was soft on palpation, substantially smaller than the right testis, and was high in the scrotum. On ultrasonography right testis measured 9.6 x 9.4 x 13.8 cm (width x height x length) with a volume of 655 cubic cm (cc) and had a heterogenous echotexture. Left testis measured 3 x 4.2 x 6.4 cm with a volume of 81 cc and had a homogenous echotexture. No other abnormalities were noticed during palpation or ultrasonography; notably, the lymph nodes and accessory sex glands appeared normal. Based on the diagnostic findings, the stallion was presumed to have right testicular neoplasia. Major differentials included: Sertoli cell tumor, Leydig cell tumor, seminoma, and teratoma. Hemicastration was the recommended treatment to potentially preserve fertility and for definitive diagnosis of the testicular enlargement by histopathology. Stallion underwent semi-closed hemicastration of the enlarged testis. Testis was cut into 4 sections and fixed in a formaldehyde solution and submitted for histopathology. Testicular tissue had aggressive invasion into the capsular stroma with lymphatic and vascular infiltration consistent with seminoma. Semen was collected 8 months after hemicastration. Ejaculate was slightly opaque with a total sperm number of 92 x 10^6 sperm, 20% total motility and 7% progressive motility. Sperm morphology evaluation showed 43% normal sperm, with the primary abnormality being abnormal heads (26%). Although histopathology determined the tumor to be malignant, there was no evidence of metastasis on the breeding soundness examination. Stallion's low total number of sperm and low progressive motility could suggest deeper issues beyond the tumor such as testicular degeneration and intrinsic subfertility due to genetics. Most literature highlighted seminomas as one of the most common tumors of stallions. However, there is limited information and studies on the effects of testicular neoplasms on fertility. This case emphasized a need for more research to better understand the impact of testicular neoplasms on sperm development and how to best manage them to maintain the stallion's reproductive life.

Keywords: Stallion, testicular neoplasia, hemicastration

Leiomyosarcoma associated with uterine rupture in an American Quarter Horse

Matthew Rafferty,[a] Salman Waqas,[a] Denise McSweeney,[a] Steven Edmonds,[b] Nicholas Muir,[a] Michela Ciccarelli[a]

[a]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA
[b]Department of veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

A 13-year, maiden Quarter Horse mare, was referred to the university teaching hospital in November 2022 for possible surgical removal of a uterine mass. In August 2022, mare was diagnosed pregnant (~ 45 days) by the referring veterinarian but in September she was diagnosed not pregnant. In October, mare was again evaluated for a slight intermittent vaginal discharge and transrectal ultrasonography revealed a mass in the left uterine horn. On arrival, fresh blood and a large clot were noticed on trailer floor. Additionally, mare exhibited colic signs including belly kicking, rolling, tachycardia, tachypnea, and hypothermia. Mare was given a dose of intravenous flunixin meglumine and placed in a dark stall isolated from other horses to calm her before further assessment. In the stall, the mare continued to roll while expelling profuse bloody discharge and clots from the vulva. Due to poor prognosis and grave future fertility even if she recovered, owner opted for euthanasia. On necropsy, a large (14 x 12 x 8 cm) pedunculated mass extending from the uterine body to the left uterine horn was noticed. A 6-cm fullthickness uterine wall tear was identified on the greater curvature of the left uterine horn where the mass was pressuring. A large clot occupied the entire left uterine horn, and concurrent mild hemoabdomen was noticed. On histological evaluation, the mass was diagnosed as leiomyosarcoma. Tumors of the urogenital tract of the mare are rare, with most arising from the ovary. The most common uterine tumor is leiomyoma. Although there have been 2 previous reports of equine uterine leiomyosarcoma in the last 40 years,[1,2] none have been larger than 4-cm in diameter or associated with uterine rupture. This case highlighted the importance of prompt evaluation and diagnosis of reproductive issues in horses, particularly in the presence of pregnancy loss, abnormal masses, and vaginal bleeding.

Keywords: Uterine tumor, reproductive emergencies, vaginal bleeding, mare

References

1. Lofstedt RM, Spurlock G, Williams R: Leiomyosarcoma in the uterus of a mare. Compend Contin Educ Pract Vet 1987;9:93.
2. Hinojosa AM, Holyoak GR, Moll D, et al: Theriogenology question of the month. Leiomyosarcoma. J Am Vet Med Assoc 2003;223:786-788.

A case report of feed and water contamination causing poor breeding season pregnancy rate in beef heifers

Matthew Rafferty, Ramanathan Kasimanickam

Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA

Selenium is an essential trace mineral of livestock that is responsible for many functions, including antioxidant defense, immune function, thyroid hormone synthesis, muscle function, and fertility. While many regions of the United States contain an adequate amount of selenium in the soil, the pacific northwest has been historically known to be deficient in this mineral in several counties. In Grant County, Washington, soil levels of selenium are 0.12 ± 0.04 ppm, as reported by the United States Geological Survey, with concentrations in plants being roughly the same or less than that of the soil, in nonaccumulator plants. In 2014, a poor breeding season pregnancy rate (74.2% [72/92]) in a well-managed herd of Angus heifers was realized at fall pregnancy diagnosis. The ranch generally averages breeding season pregnancy rates of > 90%. Through the investigative process, blood, well water, and feed (both baled and on pasture) samples were submitted for analysis. Results from the blood mineral panel analysis revealed selenium values ranging 0.71 - 3.1 ppm (~ reference range is 0.12 - 0.3 ppm), slightly elevated zinc concentrations, and copper values at the low end of the reference range. It was noticed that the heifers had been grazing separately from the rest of the herd on a pasture with a well containing water that had extremely high levels of iron, very high levels of manganese, and extremely hard. The feed analysis report revealed selenium values of up to 30.9 ppm on a dry matter basis. No other signs of mineral imbalance at the animal level were noted. It was also made known that there was a potential or mixing of sewage water with the pasture that has been documented as a source of selenium environmental contamination. To address the investigation's findings, the herd of heifers was removed from the pasture, open heifers were culled, mineral supplementation was stopped, monthly blood mineral panel monitoring was employed, and a new well was installed. Since excess iron concentration may reduce copper absorption in cattle leading to copper deficiency causing impaired reproduction, slow-release copper bolus was given to all heifers before the following breeding season. Reproduction rates of the following 2 years were 92.6% (87/94) and 90.6% (87/96). This case highlighted the importance of prompt investigation of low pregnancy rates in well-managed herds, testing of feed and water sources for herds, and herd management to correct the issues before the next breeding season.

Keywords: Trace minerals, environmental contamination, herd management

One placenta's-itis: stable for two?

Sierra Ott, Robyn Wilborn, Peyton Draheim, Candace Lyman

Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

A 17-year, Thoroughbred mare, was presented to a veterinarian for concerns of premature udder development and streaming of milk at 300 days pregnancy. Veterinarian noted an elongated vulva and an abnormal combined thickness of uterus and placenta (CTUP) of 19 mm via transrectal ultrasonography. Treatment for ascending placentitis was initiated that included altrenogest, trimethoprim sulfa, and flunixin meglumine. Mare was referred to the university teaching hospital for evaluation. At presentation the mare was bright with a heart rate of 64 beats per minute (bpm) and a respiratory rate of 16 bpm. An external reproductive examination was performed and findings were unremarkable except for milk actively dripping from the mare's teats. Transrectal ultrasonography confirmed a thickened CTUP of 21 mm with corrugated chorioallantoic margins and suspicion of placental detachment near cervix. Fetal heart rate was not obtained, despite after many attempts at stimulation, responsive movement of the fetus was observed. Mare was hospitalized and therapy was changed to intravenous potassium penicillin, gentamicin, and flunixin meglumine; oral altrenogest was continued as before. On day 2, the udder was no longer streaming milk, and transabdominal ultrasonography revealed a fetal rib cage with no heartbeat. However, fetal movement was felt on transrectal palpation; oral pentoxifylline treatment was added for improved uterine blood flow and to further support pregnancy. Repeat transabdominal ultrasonography led to visualization of a fetal heart rate of 80 bpm in the left hemi-abdomen; a right hemi-abdominal scan revealed a rib cage with no heartbeat, indicating that the mare was experiencing a twin pregnancy with a deceased fetus. On day 307 of pregnancy the mare's intravenous catheter was removed, mare was transitioned to oral broad-spectrum antibiotics and flunixin meglumine, and altrenogest was discontinued from day 315. During next 2 weeks mare was observed in hospital. Early morning on day 325, mare entered stage II labor. A fetus presented cranially but ventral deviation of the head prevented delivery; assisted vaginal delivery and correction of the malposture resulted in quick delivery of a live filly that died shortly afterwards from respiratory complications. Following delivery of the filly, the chest of a second fetus was identified with flexed forelimbs and a neck and head turned backwards. An underdeveloped, deceased colt was delivered after manual manipulation while the mare was standing; mare remained bright and alert throughout delivery. A manual vaginal examination revealed a shallow laceration, starting 10 cm cranial to the dorsal commissure of the vulva, and was 5 cm in length; this was expected to heal without complication. Fetal membranes were passed within 3 hours after delivery and were normal. Placentitis and termination of a late term twin pregnancy can have similar clinical presentations in both stage of gestation and premature udder development – particularly following demise of 1 twin fetus. Transabdominal ultrasonography is required to determine the presence of a twin pregnancy.

Keywords: Placentitis, mare, twins, dystocia, pregnancy

INDEX