effects of frozen semen and bacteria on uterine inflammation in resistant or susceptible mares. The objective of this study was to compare the uterine inflammatory response (total and percent neutrophils) to frozen semen, bacteria, or both at 72 h post-treatment in young, reproductively sound mares. Thirteen mares (mean age 3 years, range 2–6 years), were randomly assigned to three unbalanced treatment groups ($n = 20$ cycles). A rest cycle was given between each treatment. The treatments were: 1×10^9 frozen–thawed spermatozoa (FS) in lactose EDTA extender ($n = 8$), 5×10^6 frozen–thawed *Streptococcus equi* subspecies *zooepidemicus* (FB) ($n = 6$), and the aforementioned doses of frozen thawed semen and bacteria together (FSB) ($n = 8$). Treatment samples were thawed, and extended to make 10 mL with Kenney extender (EZ Mixin—Basic®, ARS, Chino, CA). Estrus mares were monitored daily with rectal palpation/ultrasound, administered 2000 IU hCG IM when a 35 mm follicle was detected, and treated on the day of ovulation (ov). A single 72 h post-ov low volume uterine lavage sample was collected for culture and cytology by inserting a catheter into the uterus, infusing 60 mL PBS, massaging the uterus per rectum, and then collecting the fluid back into sterile tubes. The fluid volume recovered was recorded and centrifuged at $350 \times g$ for 15 min, the supernatant removed, the pellet resuspended in 1 mL, and the cell concentration determined using a hemocytometer. The cell concentration was used to determine the total cells in the original 60 mL of PBS. The pellet suspension was used to make cytology slides which were stained with a rapid Wright Giemsa stain (Protocol, Fischer Diagnostics, Middletown, VA). The percent neutrophils was determined from differential counts of 300 cells, at 1000 times by two investigators blinded to sample identity. Total numbers of neutrophils were determined by multiplying the percent neutrophils by the total cell count of a sample. Kruskal–Wallis one-way ANOVA analysis was performed using Statistix version 8.0 software (Analytical Software, Tallahassee, FL). Data below are listed as median (first, third quartile). There were no differences in neutrophil percent: FS 4% (3, 10%); FB 11% (3, 24%); FSB 5% (2, 12%) or total neutrophil number (10^6) FS 1.78 (0.02, 7.8); FB 1.56 (0.22, 5.27); or FSB 0.36 (0.15, 2.73) between groups. Based on previously published reports this population of young mares would be considered ''resistant,'' with <5% neutrophils at 72 h post-treatment with *Streptococcus equi* subspecies *zooepidemicus*. In conclusion, there were no significant differences induced by the three treatments at 72 h, which indicated that there were no additive or synergistic effects of intrauterine challenge with bacteria and frozen semen together. Future studies of ''susceptible'' mares that are unable to clear bacteria by 72 h post-infusion, should be performed to determine if the interactions between frozen semen and bacteria are different in these mares compared to resistant mares.

Keywords: Mare; Uterus; Semen; Inflammation; Bacteria

EVALUATION OF THREE FIXATIVES FOR REPRODUCTIVE BIOPSIES IN THE MARE AND STALLION

S.M. Birch[1], T. Snider[2], M. Payton[3], G.R. Holyoak[1]

[1]*Department of Veterinary Clinical Sciences, Center of Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA*; [2]*Department of Pathobiology, Center of Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA*; [3]*Department of Statistics, Oklahoma State University, Stillwater, OK, USA*

Bouin's and 10% buffered neutral formalin have typically been used as fixatives of choice when evaluating endometrial and testicular biopsies in the veterinary specialty of theriogenology. However, formalin has been associated with creating artifacts and Bouin's contains picric acid which poses both a health and disposal hazard. Additionally, Bouin's requires several additional washes and steps in biopsied tissue preparation. This study was done to assess histomorphology with three different fixatives. A board-certified pathologist (TS) examined endometrial biopsies from ten mares and testicular biopsies from four stallions. All tissue samples were obtained from abattoir specimens immediately after death, therefore eliminating potential autolysis while facilitating precise duplication of biopsy site and adequate size for complete histomorphologic examination. The biopsies were divided equally and fixed in each of three fixatives—10% buffered neutral formalin (F), Bouin's (B) and modified Davidson's solution (MD). In a blinded study, the pathologist graded the endometrial biopsies for overall clarity of morphological detail, creation of artifacts, and preservation of nuclear chromatin in luminal epithelium and glands, stratum compactum and spongiosum, vessels and overall staining. The testicular biopsies were graded for overall clarity of morphological detail, capsule integrity, creation of artifacts and preservation of nuclear chromatin in Leydig cells, seminiferous tubules, epididymis and overall staining. The results of this study for fixation of endometrial biopsies revealed no significant ($p < 0.05$) difference (the GLM procedure for least squares means) between the three fixatives in overall

staining, luminal epithelium gland integrity, stratum compactum integrity or artificial vacuolation/separation or vascular histomorphology. However, MD was significantly better than the other two fixatives in reducing the formation of cytologic artifacts in both the luminal glandular epithelium and in the cells composing the stratum compactum. Furthermore, both MD and B were superior to F in improving tissue staining contrast, whereas MD and F were superior to B in preserving luminal epithelium gland chromatin structure. The results of this study for the fixation of testicular biopsies revealed no significant difference in any of the specific categories except overall fixation clarity/quality ($p < 0.05$). However, there were clear trends indicating that MD may be better than the other two fixatives in reducing the formation of cytologic artifacts in Leydig and seminiferous cell populations and in reducing sloughing of seminiferous tubule cells. Whereas MD and F were slightly better than B in reducing artifact formation in epididymal cells and F trended better in preserving epididymal epithelial cell nuclear chromatin. In conclusion, there was no one fixative significantly superior in every category of our histomorphologic evaluation. However, MD was superior to both B and F in several categories involving endometrial biopsies and in overall testicular fixation. Additionally, MD trended superior to both other fixatives in several other categories of testicular biopsy fixation. These trends may achieve greater statistical significance as we increase the number of biopsies. These findings are important to theriogenologists because of the routine use of Bouin's fixative and its associated health and disposal hazards. A safer and perhaps superior alternative is available.

Keywords: Endometrium; Testicular; Biopsy; Fixatives; Histopathology

INDUCTION OF PARTURITION AND THE NEONATAL ACUTE PHASE RESPONSE

Vivienne E. Duggan, G. Reed Holyoak, Charles G. MacAllister, Anthony W. Confer

Department of Veterinary Clinical Sciences, Center of Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA

Traditionally it has been considered that there are few clinical indications for induction of parturition in the mare as it has been associated with increased incidence of peri-partum complications such as dystocia and with unfavorable outcome in foals.

This study aimed to investigate the incidence of complications during foaling with induction of parturition in full term mares with a low dose oxytocin protocol and to assess whether the induction of parturition caused an acute phase response in neonatal foals exceeding that of the normal birth process.

Parturition was induced in 11 of 26 mares at full term gestation with low doses of oxytocin (0.5 ml). Calcium concentrations of the mammary secretions were used to predict full term gestation. Peri-partum complications in mares and their neonatal foals were recorded. Serum was collected from 18 of the neonatal foals (11 of which were from mares in which parturition was induced) from zero to 72 h post-partum. A commercially available ELISA (Tridelta Ltd., Maynooth, Co Kildare, Ireland) was used to measure the concentration of an acute phase protein, serum amyloid A (SAA), in serum samples from the neonatal foals. Foals were retrospectively divided into two groups, normal and abnormal, to eliminate the influence of increased endogenous SAA production secondary to inflammatory stimuli other than the birth process.

Complications that occurred with increased frequency in mares that were induced to foal included mild dystocia requiring minor assistance ($n = 2$) and placenta previ ($n = 1$) and those that occurred in mares that foaled naturally included retained fetal membranes necessitating therapeutic intervention ($n = 2$). Complications in foals from mares in which parturition was induced included slowness to nurse ($n = 1$) and abnormal neutrophil counts ($n = 3$). Similar complications were recorded in foals from mares which foaled naturally, i.e. one foal was slow to nurse and three had abnormal neutrophil counts. In the whole group, foals from mares in which parturition was induced ($n = 11$) had lower SAA concentrations ($p < 0.05$) at 12 and 24 h than foals from mares that foaled naturally ($n = 7$). When the analysis was restricted to completely normal foals, seven of which were from mares which were induced and two of which were from a natural foaling, the results were the same.

The findings of our study do not support previous allegations that induction of parturition in mares leads to unfavorable outcome in the neonatal foal. This induction protocol provides a manageable environment in which parturition is observed and complications can be rapidly recognized and dealt with.

Keywords: Induction of parturition; Acute phase response; Neonatal foal