

## EQUINE SESSION

### Low volume uterine lavage: advantages for use in problem mares

Christina Divine, Christian Bisiau, Julie Storme, Melissa Prell, Jennifer Morrissey, Jennifer Hatzel, Patrick McCue

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

Infectious endometritis is a leading cause of subfertility in broodmares. The standard sample collection technique for diagnosis of bacterial and fungal endometritis is a guarded swab for uterine culture and a guarded swab or brush for uterine cytology. However, these methods only sample a very small area of the uterus and may not always yield microbial growth in mares with infectious endometritis. Low volume lavage (LVL) is an alternate diagnostic technique that samples the entire uterine lumen. Aim of this study was to compare the results of microbial culture and cytologic evaluation from samples collected using a guarded swab and brush versus samples subsequently collected using a LAL technique in mares suspected with infectious endometritis. Reproductive records from 27 mares managed at Colorado State University were analyzed retrospectively. Transrectal ultrasonography examination and a standard uterine swab/brush were performed prior to LVL procedure. Low volume lavage was performed using 250 ml of sterile lactated Ringer's solution and the effluent fluid was then transferred into a pair of 50 ml conical tubes for centrifugation. Microbial culture was performed on swabs and LVL pellets using Spectrum™ 4-Part (Colorado) microbial agar plates (Vetlab Supply, Palmetto Bay, FL). Glass slides for cytologic evaluation were stained with a modified Wright-Giemsa stain (MWI Animal Health™, Boise, ID) and evaluated under 400 and 1000 x microscopy. Percentages of mares with microbial growth of a uterine pathogen and presence of white blood cells on cytologic evaluation were compared by Chi Square analysis; data were considered different at  $p < 0.05$ . Initial examination results determined that all mares were in estrus at reproductive evaluation based on the presence of mild to moderate uterine edema and a dominant follicle. An increased volume ( $> 2$  cm depth) of uterine fluid was noted in 13 of the 27 mares (48.1%) and increased echogenicity of uterine fluid was noted in 12 of the 27 mares (44.4%) on initial transrectal ultrasonography examination. Cytologic evaluation of the guarded brush sample revealed the presence of white blood cells and/or the presence

of increased background debris in 12 of the 27 (44.4%) mares. Microbial growth of 1 or more pathogenic bacteria was detected in the culture of guarded swabs collected from 4 of 27 mares (14.8%). Overall, 21 of 27 mares (77.8%), had 1 or more factors that were suggestive of endometritis. Culture from LVL pellets yielded growth of pathogenic bacterial in 15 of the 27 samples (55.6%) that was higher ( $p = 0.002$ ) than guarded uterine swabs. Cytology samples from LVL pellets had white blood cells in 24 out of the 27 mares (88.9%) that was higher ( $p = 0.005$ ) than guarded uterine brushes. In summary, the LVL technique is a valuable procedure in the detection of uterine inflammation and microbial infection in problem mares.

**Keywords:** Mare, uterus, low volume lavage, problem mare, endometritis

### Use of progesterone and estradiol-17<sub>β</sub> prior to transvaginal aspiration of oocytes

Peyton Draheim,<sup>a</sup> Cory Anderson,<sup>b</sup> Paul Dyce,<sup>c</sup> Candace Lyman<sup>a</sup>

<sup>a</sup>College of Veterinary Medicine, Auburn University, Auburn, AL;

<sup>b</sup>Countryside Veterinary Clinic, St. Anthony, ID; <sup>c</sup>Department of Animal Sciences, Auburn University, Auburn, AL

This pilot study assessed the impact of progesterone and estradiol-17<sub>β</sub> treatment on ovarian follicle number. Objective was to determine whether 150 mg/ml progesterone + 5 mg/ml estradiol 17<sub>β</sub> (BioRelease P&E; BET Pharmacy, Lexington, KY) treatment can impact mare ovarian follicle numbers to increase the odds of having more ovarian follicles on a predicted day. To increase foal production, transvaginal aspiration (TVA) of mare oocytes is followed by in vitro maturation, fertilization (via intracytoplasmic sperm injection), and embryo culture. In efforts to aspirate several oocytes during 1 TVA, common practice is to repeat ultrasonographic examinations of the ovaries on multiple sequential days and schedule the procedure on the day when the mare is expected to have her 'peak' number of suitable follicles. Such a high maintenance management routine increases mare owner costs on a per foal basis. It also allows only ~ 1 - 2 days' advance notice to the practitioner performing the TVA procedure, due to the variability in follicular growth dynamics. Currently, there are no accepted treatment regimens for advanced scheduling/planning of oocyte collection via TVA in the mare. A validated estrous cycle management protocol would allow practitioners and mare owners to

plan multiple days in advance and also increasing the number of follicles that could be aspirated. Furthermore, it would be highly beneficial to the equine industry and scheduling TVA procedures. We hypothesized that a single injection of P&E result in synchronization and optimization of the number of follicles to occur at least 9 - 10 days after treatment. Eight mares underwent initial ovarian follicles counting (i.e. follicular mapping) via transrectal ultrasonography and were given 10 ml of BioRelease P&E intramuscularly on day 0; transrectal palpation and ultrasonographic examination, and follicular mapping was repeated for each mare 3 times (i.e., on days 3 and 6, in addition to days 9, 10, or 11, based on mare availability). Serum was collected on the days of ultrasonographic examinations and frozen for determining concentrations of anti-Mullerian hormone (AMH), luteinizing hormone (LH), and follicle stimulating hormone (FSH). Data on follicle count data from this study were combined with previously obtained pilot data, for a total of 24 mares. Data on follicle count were analyzed by unpaired two-tailed Student's *t*-test and AMH concentrations by a multiple comparison test. Follicle counts increased ( $p = 0.024$ ) by a mean of 4.13 follicles/mare between the first and last follicular mapping sessions. AMH concentrations on day 3 ( $0.8473 \text{ ng/ml} \pm 0.1699$ , mean  $\pm$  SEM) were significantly higher compared to day 6 ( $0.7033 \text{ ng/ml} \pm 0.1737$ , mean  $\pm$  SEM).

**Keywords:** Mare, progesterone, estradiol, follicle, transvaginal aspiration, oocyte

### Prepartum amniotic rupture in a Thoroughbred mare

David Alexander, Kindra Orr, Kate Christie, Peter Morresey, Christine Bartley, Maria Schnobrich

*Rood & Riddle Equine Hospital, Lexington, KY*

Prepartum amniotic rupture has been described in humans.<sup>1</sup> In mares, there are reports of prepartum placental disruption associated with hydrosical conditions. This report describes a full thickness prepartum amniotic tear in a Thoroughbred mare, initially diagnosed by transrectal ultrasonography at 236 days prepartum. A 7-year-old Thoroughbred mare was pregnant with her first foal. Transrectal ultrasonography (15, 17, 28, 42, 60, 89, 119, 148, and 183 days) findings were normal and the mare apparently had a filly. Transrectal ultrasonography evaluation on 211 days revealed a normal amnion and a combined thickness of the uterus and placenta (CTUP) of 0.6 cm. At 236 days, transrectal ultrasonography was performed as part of routine screening and no amnion could be identified. Allantoic fluid was moderately and uniformly increased in echogenicity and the fetus was visualized with no amnion separating the fetus from the allantoic membrane. No precocious mammary gland development was noted, but substantial placental edema was observed in the chorioallantois and endometrium and CTUP was 2 cm. Serum amyloid A was slightly elevated ( $32 \mu\text{g/ml}$ ) with normal white blood cell count ( $7.6 \times 10^3$ ), and fibrinogen ( $200 \text{ mg/dl}$ ). Total estrogens were  $1,040.04 \text{ pg/ml}$  and progesterone concentrations were  $4.51 \text{ ng/ml}$ . Treatment for placentitis (once

every 24 hours for altrenogest [ $0.044 \text{ mg/kg}$ ], 57 mg of firocoxib and 500 mg of flunixin meglumine, and once every 12 hours for pentoxifylline [ $8.5 \text{ mg/kg}$ ] and doxycycline [ $10 \text{ mg/kg}$ ]) was initiated. Serial transrectal and transabdominal ultrasonography were performed and the chorioallantois continued to thicken and was edematous. Amnion was intermittently viewed in transrectal ultrasonography, but in an abnormal and wrinkled appearance. At all times, fetal heartbeat was within 80 - 100 bpm. At 313 days of pregnancy, the mare was admitted for monitoring, and altrenogest, firocoxib and pentoxifylline treatment continued. In addition, once every 24 hours, 2,660 mg of aspirin was given. At day 317, the mare foaled. No amnion was visible after observing the mare's water breaking, foaling required manual assistance, and the amnion was not noted. Fetus was born alive but euthanized due to 90° contracture of both front fetlocks. Fetus and fetal membranes were submitted for histopathology analysis and revealed an amniotic rent measuring 10 cm with rolled edges and a diffusely thickened amnion. Allantois was thickened with areas of adenomatous hyperplasia and embedded hair shafts. To our knowledge this is the first reported case of prepartum amniotic rupture diagnosed by transrectal ultrasonography in the mare

**Keywords:** Mare, amniotic, rupture, prepartum

### Reference

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### Evaluation of passively acquired rabies antibody titers and immune responses in healthy foals vaccinated against rabies at 4 or 6 months of age

Karen Wolfsdorf,<sup>a</sup> Luke Fallon,<sup>a</sup> Jeanette McCracken,<sup>a</sup> Deb Amodie,<sup>b</sup> Jacquelin Boggs,<sup>b</sup> Bobby Cowles,<sup>b</sup> Nathan Voris<sup>b</sup>

<sup>a</sup>Hagyard Equine Medical Institute, Lexington, KY; <sup>b</sup>Zoetis, Parsippany, NJ

Passively acquired antibodies provide protection against disease in foals prior to completion of a primary immunization series. Postvaccination immunologic protection against rabies is expected with titers  $\geq 0.5 \text{ IU/ml}$ , but information on the lifespan of passively acquired antibodies and influence on immune response is lacking. In the spring of 2020, the American Association of Equine Practitioners updated vaccination guideline recommendations to begin rabies vaccination at 4 to 6 months of age instead of initiating at 6 months of age. The update made initiation of foal vaccination recommendations consistent for all 5 core equine diseases (Eastern equine encephalomyelitis [EEE], Western equine encephalomyelitis [WEE], West Nile virus [WNV], tetanus, and rabies). The aim was to evaluate the rate of passively acquired rabies antibody decline and compare the immune response to vaccination when initiated at 4 or 6 months of age. Forty-nine foals, in 3 farms, born to mares vaccinated against EEE, WEE, WNV, tetanus, rabies, equine influenza virus,