Proceedings of the 60th Annual Convention of the American Association of Equine Practitioners - AAEP –

December 6-10, 2014
Salt Lake City, UT, USA

Next Meeting:

Dec. 3-7, 2016 - Orlando, FL, USA

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Review of Techniques for Prediction of Ovulation in the Mare

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A combination of growth pattern and size of the dominant follicle, increase in follicular wall thickness, development of an irregular shape, softening of the follicle, and a decrease in endometrial edema is suggestive of impending ovulation. Authors’ address: Colorado State University, Department of Clinical Sciences, Fort Collins, CO 80523; e-mail: Patrick.McCue@ColoState.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
A decision on when to breed a mare by live cover or artificial insemination is usually dependent on an accurate prediction of impending ovulation. Prediction of the potential interval to ovulation is accomplished in clinical practice by interpretation and integration of multiple factors on an individual mare (Table 1). The goal of this paper is to review the parameters that can be used in clinical practice to predict impending ovulation in the mare.

2. Reproductive History
An individual mare will often ovulate a follicle of approximately the same diameter each cycle.1 Consequently data from previous cycles can often be used to predict follicle size at ovulation of subsequent cycles. Unfortunately, some mares will ovulate a dominant follicle during one estrous cycle that is of a very different size, either markedly smaller or larger, than follicles of other cycles. Prediction of ovulation is difficult in these mares and more than one breeding may be required so the cycle is not missed.

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3. Growth Pattern of Follicle
A dominant follicle will typically increase in diameter by 2.7 to 3 mm per day during estrus, reach a
maximum diameter, and subsequently remain the same size for approximately 2 days prior to ovulation.\textsuperscript{2-4} The diameter of the follicle may decrease by 2 to 3 mm in the 12 hours preceding ovulation.\textsuperscript{3,5} The growth pattern of the dominant follicle is disrupted if a mare receives human chorionic gonadotropin (hCG) or deslorelin acetate to induce ovulation.\textsuperscript{4} The ovulation induction agent is generally administered when the mare is in behavioral estrus or has endometrial edema visible on ultrasound and the dominant follicle first reaches a size that is appropriate (often \textcirca{35 mm in diameter for light horse breeds}). Ovulation will typically occur 36 to 40 hours following administration of hCG or deslorelin, respectively. The induced follicle often ovulates before it attains its maximum potential diameter.\textsuperscript{1,6}

4. Diameter of Follicle
The potential diameter of the preovulatory follicle prior to ovulation can often be predicted based on mare size and breed. In addition, mares typically ovulate follicles that are similar in size in consecutive cycles.\textsuperscript{1} In general, mares of smaller light horse breeds will ovulate a follicle that is smaller in diameter than mares of larger breeds. Friesian mares are notorious for developing very large follicles (ie, 50 mm or greater) that remain present for several days prior to ovulation (Stout, personal communication). It has been noted that the diameter of a preovulatory follicle is 5 to 8 mm larger early in the ovulatory season than later in the season and is also greater in single-ovulating mares than mares with unilateral or bilateral double ovulations.\textsuperscript{7} It has been suggested that the diameter of the dominant follicle may be the most reliable single criterion in the prediction of ovulation.\textsuperscript{3,7} but follicle diameter alone is not useful in predicting ovulation in the 48 hours preceding spontaneous ovulation.\textsuperscript{8}

5. Shape of Follicle
A developing dominant follicle is typically spherical in shape during most of the estrous period. It has been reported that 84% to 89% of follicles change shape from spherical to nonspherical (pear-shaped or conical) prior to ovulation.\textsuperscript{2-4} A “cone” or “point” or “stigma” may develop as the follicle tunnels toward the ovulation fossa in the hours preceding ovulation. The follicle may become more flattened or irregular in the 3 hours prior to ovulation.

6. Changes in the Follicular Wall
A developing dominant follicle has a relatively thin follicular wall during early to mid-estrus. Follicular wall thickness increases progressively as the interval to ovulation decreases.\textsuperscript{4} The echogenicity is potentially due to an increase in the number of granulosa cells, accumulation of mucosubstances between cells\textsuperscript{5} or preovulatory luteinization of granulosa cells. Unfortunately, the thickening generally occurs too early to be useful in predicting ovulation.\textsuperscript{10} An increase in the echogenicity of the follicular wall\textsuperscript{5} and an increase in prominence of an anechoic layer beneath the granulosa\textsuperscript{10} have been noted as ovulation approaches. The anechoic layer has been suggested to be due to hypervascularity and edema of the theca interna. A rent or tear in the follicular wall was observed in 77% of mares prior to ovulation.\textsuperscript{5} The duration of ovulation, defined as a rapid decrease in follicular size, has been reported to occur in an average of 42 seconds (range 5-90 seconds).\textsuperscript{5} Serrations of the inner surface and/or outer periphery of the granulosa cell layer, as indicated by an irregular or notched appearance, was noted in 37% of mares 24 hours prior to ovulation and 59% of mares within 12 hours of ovulation.\textsuperscript{11}

7. Tone of Follicle
A developing dominant follicle has a firm tone during the early and middle part of the normal growth phase. The dominant follicle may become noticeably softer as detected by manual palpation within the 12 to 24 hour period prior to ovulation.\textsuperscript{3,12,13} In one study, a perceptible softening of the follicle was noted in 28% of mares prior to ovulation, and some follicles softened and then became turgid again prior to ovulation.\textsuperscript{14} In another study, it was reported that 90% of follicles were softer at 12 hours before ovulation than they had been at 72 hours prior to ovulation.\textsuperscript{14}

8. Vascularity with Doppler Ultrasound
Examination of the dominant follicle in color Doppler-mode revealed that the percentage of the follicle with color display and the prominence of the signals increased between 36 and 12 hours prior to ovulation but decreased during the last 4 hours prior to ovulation.\textsuperscript{11} In addition, a loss of color Doppler signals was evident at the apex of follicles that had lost their spherical shape.

9. Evaluation of Follicular Fluid
Echogenic particles within the follicular fluid, presumed to be clusters of granulosa cells, have been noted in 50% to 54% of equine preovulatory follicles\textsuperscript{5,15} but is not common enough or consistent enough to be predictive of impending ovulation.\textsuperscript{10}

10. Endocrine Markers
Measurement of estrogen concentration, either estradiol 17β, total estrogens, or conjugated estrogens, have been used in an attempt to predict ovulation in mares.\textsuperscript{3,16,17} In general, estrogen levels increase in relation to the increase in follicle diameter during estrus, peak approximately 2 days prior to ovulation, and decline near the day of ovulation.\textsuperscript{15,19} Unfortunately, the pattern of estrogen secretion is not consistent to be a useful diagnostic marker for prediction of ovulation. Luteinizing hormone concentrations increase gradually during estrus in the mare and reach peak levels near the day of ovulation.\textsuperscript{19,20} The presence of luteinizing hormone (LH)
was recently evaluated as a potential predictor of ovulation in mares using a commercial LH kit.\textsuperscript{21} LH was first detectable beginning 2.5 ± 1.2 days prior to ovulation and continued to be detectable in all mares out to 2 days after ovulation. Consequently, a single positive test was not a reliable indicator of impending ovulation.

11. Uterine Edema Pattern

Endometrial edema develops in response to the presence of estradiol and the absence of progesterone. In most mares, a predictable pattern of edema development and regression occurs during a heat period,\textsuperscript{22,23} which is generally consistent from cycle to cycle in a given mare.\textsuperscript{1} The amount of edema peaks approximately 2 days prior to ovulation. Ovulation typically occurs when edema is in a stage of decline or is absent.

12. Degree of Cervical Relaxation

The physical characteristics of the cervix change throughout the estrous cycle based on the relative presence of estrogen and progesterone. A mare in heat, in the presence of estradiol 17β and an absence of progesterone will have a cervix that is relaxed and draped onto the floor of the vagina, pink in color, and moist. In contrast, a mare in diestrus will have a cervix that is high on the cranial vaginal wall, closed tight, dry, and pale in color. These characteristics can be visualized on speculum examination and tone can be detected by manual palpation per rectum. The greatest degree of change in cervical color and tone occur near ovulation but are not accurate enough alone to indicate time of ovulation.\textsuperscript{1,4}

13. Periovulatory Ovarian Pain on Palpation

It has been suggested that some mares exhibit ovarian pain associated with follicular development.\textsuperscript{10,24} It is more common that mares exhibit discomfort when the site of a fresh ovulation is palpated per rectum. Consequently, expression of pain or discomfort on transrectal palpation of the ovaries cannot reliably be used as a predictor of impending ovulation.

14. Number of Days in Estrus

The average length of the equine estrous cycle was reported to be 20.6 days,\textsuperscript{1,4} and the average duration of estrus was 5.7 days. It was further noted that 46%, 32%, and 12% of mares ovulated within 24 hours, 48 hours, or 72 hours, respectively, prior to the end of estrus, while 10% of mares were out of estrus before ovulation occurred.

15. Interval from Prostaglandin Administration

The average interval from prostaglandin administration to the next spontaneous ovulation is approximately 7 to 12 days.\textsuperscript{2,25} Assessment of follicle diameter at the time of prostaglandin administration can be used to estimate the interval to subsequent ovulation. The interval from PGF administration to subsequent spontaneous ovulation is inversely proportional to the diameter of the largest follicle at the time of treatment. In general, mares with small follicles at the time of prostaglandin administration take longer to develop a dominant follicle and ovulate than mares with a larger follicle (Table 2).

Administration of prostaglandin to a mare with a large diestrous follicle (≥35 mm) is associated with one of 3 outcomes: (1) ovulation within 48 hours in the absence of uterine edema (14.5%), (2) ovulation after 48 hours accompanied by uterine edema (75.4%), or (3) regression without ovulation followed by emergence of a new follicular wave (10.1%) (unpublished data).

16. Interval from hCG or Deslorelin Administration

Administration of an ovulation inducing agent is common in broodmare practice. Typically a follicle that has reached a minimum critical size that is characteristic for the type or breed of mare (ie, ≥35 mm in diameter for most light horse breeds) will respond to administration of hCG or deslorelin acetate and ovulate within 24 to 48 hours if the mare had been in heat for 2 to 3 days.\textsuperscript{26} If ovulation occurs prior to 24 hours, it is likely that the follicle was already primed to ovulate even before the agent was administered. hCG and deslorelin acetate will usually induce ovulation in approximately 36 and 40 hours, respectively. If used appropriately, it is generally expected that 85% to 95% of mares should ovulate between 24 and 48 hours after administration of hCG or deslorelin acetate.

17. Other Comments

It has been suggested that a majority of mares ovulate at night.\textsuperscript{27} However, several studies have indicated that ovulations are equally distributed throughout the day in mares.\textsuperscript{1,3,28}

18. Summary

No single morphologic criterion is consistently useful to predict interval to ovulation or impending ovulation. In clinical practice, a combination of growth pattern and size of the dominant follicle, in conjunction with an increase in follicular wall thickness, development of an irregular shape, softening of
the follicle, and a decrease in endometrial edema is suggestive of impending ovulation. Administration of hCG or deslorelin acetate at the appropriate time of the estrous cycle can greatly enhance prediction of ovulation.

Acknowledgments

Conflict of Interest
The Authors declare no conflicts of interest.

References


Relationship Between Ultrasonographic Findings at the Time of Breeding and Pregnancy Outcome

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Grade 3 and 4 uterine edema at ovulation did not adversely affect fertility of mares. Authors’ addresses: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546 (Squires, Ball, Troedsson); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Bradecamp, Schnobrich, Riddle); e-mail: edward.squires@uky.edu. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction
It is a routine practice to examine the mare’s uterus and ovaries during estrus in order to assess follicular development of the ovaries and edema in the uterus as an aid in scheduling breeding. Several reports have described an increase in uterine edema in response to the secretion of estrogen from the pre-ovulatory follicle.1–3 The peak in circulating estrogen occurs about 24 h prior to ovulation. Thus, the degree of uterine edema increases as the mare develops a pre-ovulatory follicle and approaches ovulation. Near the time of ovulation, the amount of edema decreases and when the mare is in diestrus, edema in the uterus should be minimal.4 Based on ultrasound examination of the uterus, the uterine edema is usually graded on a scale of 0 to 4 or 0 to 5: 0 = no edema, 3 = maximum normal edema, and greater than or equal to 3.5 to 4 = excessive edema. Assessing the degree of edema has been used to determine the appropriate time to administer human chorionic gonadotropin (HCG) or gonadotropin-releasing hormone (GnRH);4 whether the mare is in estrus; if the follicle in the transitional mare is competent;5 diagnosis of inflammation of the uterus; and if the corpus luteum is regressing.

Clinically there are mares that, for unknown reasons, continue to have a high edema score even at ovulation or after ovulation. Some have suggested that maximum or excessive edema at ovulation may be due to subclinical endometritis, excessive estrogen secretion, or failure of progesterone to increase.3,5 Regardless, the practitioners must decide whether to ignore the presence of excess edema or treat the uterus. Apparently, there have been no studies to determine if high edema scores near ovulation adversely affect fertility or the incidence of early embryonic loss. This study tested the hypothesis that maximum or excessive uterine edema near ovulation lowered pregnancy rates and increased early embryonic loss in a large group of Thoroughbred mares.

2. Materials and Methods
Nine hundred and twenty Thoroughbred mares were examined with ultrasound for a total of 1127
cycless during the 2013 breeding season on day 2 (day prior to breeding and day ovulating inducing agent was usually given) and day 0 in relation to ovulation (day of OV = 0). Uterine edema was given a score of 0 to 4 with 0 = no edema, 3 = maximum, and 4 = excessive edema. Uterine fluid was also graded based on the vertical measurement of intrauterine fluid in cm. Other data included antibiotics (Y/N), uterine lavage (Y/N), and pregnancy at 14 and 40 to 50 days. Mares were classified in 3 age groups: young (2–9 years), middle-aged (10–16 years), and old (>16 years) and 3 reproductive statuses at the time of breeding: foaling, maiden or barren. Since there were only 4 mare cycles with grade 4 edema at the time of ovulation, the edema scores of 3 and 4 were combined for analyses.

Risk factors for pregnancy status were identified in univariable analysis with an $X^2$ test to compare categorical data between groups. Multivariable logistic regression with the dependent variable of pregnancy rate/cycle was used to develop a model that included independent variables with a $P < 0.20$ in a stepwise backward method using SAS ProcJMP; a priori values of $P < 0.05$ were considered to be significant in the final model.

### 3. Results

The parameters that had significant effects in the logistic model on pregnancy rates per cycle and early embryonic losses were age ($P < 0.03$), reproductive status ($P < 0.01$), uterine lavage ($P < 0.04$), and age × status $P < 0.001$). The presence of uterine fluid greater than 1 cm tended ($P < 0.09$) to affect pregnancy rate/cycle. Edema scores of 3 and 4 prior to ovulation and at ovulation did not significantly affect the pregnancy rate or embryonic loss. Whether mares were given antibiotics also did not affect the outcome parameters (pregnancy rate/cycle or embryonic loss). Based on the $X^2$ analysis, the pregnancy rate per cycle was lower for old mares (28/74, 38%) versus middle-aged mares (237/379, 63%) or young mares (480/673, 71%). Barren mares had greater $P < 0.05$ early embryonic losses between 14 to 50 days (291/217, 13%) than those for foaling (63/727, 8.7%) and maiden (10/182, 5.5%), but pregnancy rates were similar ($P > 0.05$) among mares of various reproductive statuses (66%, 144/217, 65%, 472/727, or 71%, 129/182 for barren, foaling, or maiden mares, respectively). When age was examined as a continuous variable and not categorical, there was an age × status interaction. Mares over 11 years old and barren had a lower pregnancy rate than those barren and less than 11 years.

The presence of greater than 1 cm of uterine fluid at the time of ovulation increased ($P < 0.02$) embryonic loss (19/113, 17%) compared to those mares with <1 cm of uterine fluid (60/668, 8%) but the pregnancy rates were similar (58 vs 67%). This effect was primarily due to the presence of fluid in older mares (age × fluid). Based on the $X^2$ analysis, the presence of grade 3 or 4 edema at ovulation did not ($P = 0.94$) affect the pregnancy rate or embryonic loss. However, those mares that were lavaged had a greater embryonic loss (50/384, 13%) than those not lavaged (52/741, 7%).

### 4. Discussion

Edema was not a parameter in the logistic model that affected pregnancy rate or embryonic loss. Furthermore, if we examined the pregnancy rates and embryonic losses in mares with minimal edema (0 to 2) compared to those with maximal or excessive uterine edema (3–4), there was no effect on pregnancy rate or early embryonic loss so this could be a reflection of the relatively small number of cycles in which mares had excessive edema (grade 4). Thus, we concluded that the presence of edema scores of 3 and 4 prior to and after ovulation had no apparent effect on the pregnancy rate per cycle. The overall incidence of mares with grades 3 and 4 uterine edema at ovulation was 73/1126 (6.5%). These mares also had a higher incidence of uterine fluid >1 cm (18%) than those mares with edema scores of 0 to 2 (9.5%). The fact that excessive edema did not lower pregnancy rates or increase embryonic losses was unexpected. Others have suggested that excessive edema at ovulation or after ovulation may be an indication of subclinical endometritis or perhaps an endocrine imbalance. Unfortunately, we were not able to determine the cause of excessive edema around the time of ovulation under the conditions of this study. In contrast, the presence of fluid > 1 cm did affect pregnancy rate and embryonic loss. Others have reported that the fluid during estrus decreases fertility, depending upon the amount of fluid and grade of fluid. The echogenicity of uterine fluid was not available in this group of mares. Mares that had uterine lavage during estrus also had a higher embryonic loss rate. This is probably not a direct result of the lavage procedure but an indication that mares that needed a lavage were more than likely those with fluid, older mares, or those with some other reproductive problem.

It was not surprising that reproductive status and age affected pregnancy rates and the incidence of early embryonic loss. Others have reported lower fertility and higher embryonic loss for aged mares and for barren mares compared to younger maiden or foaling mares. There was, in fact, an interaction of age with reproductive status and age and the presence of uterine fluid. Mares over 11 years of age had a higher incidence of having moderate to large amounts of fluid and more barren mares were represented in the aged category than the other two age groups.

In summary, maximum or excessive uterine edema near the time of ovulation did not adversely affect pregnancy rates or the incidence of early embryonic loss.
Acknowledgments

We would like to thank Dr. Stephanie Preston of the Gluck Equine Research Center for her consultation on the statistics.

Conflict of Interest

The Authors declare no conflicts of interest.

References and Footnote


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Anti-Müllerian Hormone Predicts Follicular Reserve in Aged Mares

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Follicular reserve (measured as antral follicle count; AFC) declines with mare age. Determination of serum anti-Müllerian hormone (AMH) concentrations is highly correlated with follicular reserve in older mares, and AMH determination may be useful to assess the reproductive longevity of older mares. Authors’ addresses: Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0099 (Claes, Ball, Scoggin, Esteller-Vico, Kalmar, Squires, Troedsson); and Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616 (Conley); email: b.a.ball@uky.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
The number of follicles remaining in the ovary (follicular reserve; AFC) declines with age in mares until reproductive senescence is reached but at variable ages. Anti-Müllerian hormone is produced by small follicles, and circulating concentrations of AMH have been used to measure follicular reserve in other species. The objective of this study was to examine the relationship between AMH and AFC in mares of different ages.

2. Materials and Methods
Young (3–8 years; n = 10), middle-aged (9–18 years; n = 16), and old (>18 years; n = 19) mares were examined by transrectal ultrasonography, and all antral follicles were counted (AFC) across multiple estrous cycles. Plasma AMH concentrations were measured by ELISA across multiple estrous cycles. Data were analyzed by nonparametric correlation.

3. Results and Discussion
AFC was lower in old compared to middle-aged or young mares. The repeatability of AFC and AMH was high within and across estrous cycles. Overall, AFC was positively associated with plasma AMH concentrations although this relationship was influenced by age. The relationship between AFC and AMH in aged mares (ρ = 0.86; P < 0.0001) and middle-aged mares (ρ = 0.60; P = 0.01) was greater than the correlation between AFC and AMH in young mares (ρ = 0.40; ns). Plasma AMH concentrations are predictive of antral follicle counts in older mares and may be useful to assess follicular reserve as well as reproductive longevity.
Acknowledgments

The authors thank Minitube of America® for providing the AMH ELISA. We also thank Dr. E. Woodward, C. Fedorka, and Dr. G. Davolli for their assistance with the clinical part of this research project. Funding for this project was provided by the Albert G. Clay Endowment of the University of Kentucky.

Conflict of Interest

Dr. Troedsson has a professional affiliation with MOFA™, Minitube of America.
Suppression of Estrogen Biosynthesis During Late Pregnancy in Mares

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Reduction in maternal estrogen concentrations by up to 90% during late gestation did not affect gestation length or neonatal viability although birth weights were reduced. These findings call into question the value of supplementing estrogens for pregnancy maintenance during late gestation. Authors' address: Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546; e-mail: b.a.ball@uky.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
Estrogen concentrations during pregnancy in the mare are markedly elevated, and some studies have suggested a potential relationship between low circulating estrogen concentrations in pregnant mares and pregnancy loss. The role of elevated estrogens in equine pregnancy is largely unknown, and the aim of this study was to evaluate the effects of an inhibitor of estrogen synthesis, letrozole, on equine pregnancy during the last trimester.

2. Materials and Methods
Mares were treated with letrozole (n = 6; 500 mg every four days) or untreated as control (n = 6) beginning at 240 days of gestation until foaling. Mares were bled weekly, and concentrations of estrogens, androgens, and progestins were analyzed by ELISA. Gestational length, neonatal viability, and foal birth weights were determined.

3. Results
Letrozole reduced peripheral estrogens during late pregnancy by approximately 90% with a concomitant increase in androgens and no change in progestins. Gestational length was unchanged, neonatal viability was normal, and birth weights of foals born to letrozole treated mares was reduced by approximately 14%.

4. Discussion
Inhibition of estrogen synthesis during late gestation did not disrupt pregnancy or adversely affect neonatal viability although birth weights of foals born to letrozole-treated mares were reduced.

Acknowledgments
The authors thank Dr. Raeside for generously providing the DHEA-S antibody and the Clay Endowment and the Mellon fellowship for support.

Conflict of Interest
The Authors declare no conflicts of interest.
Pharmacokinetics of Ceftiofur Sodium in Equine Pregnancy

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Ceftiofur sodium had poor penetration into fetal membranes and compartments after administration to normal, pregnant mares. This drug is not recommended for treating mares with placentitis. Authors’ addresses: Large Animal Clinical Sciences (Macpherson, Pozor, Benson, Runcan, Hatzel, Larson, van den Berg, Kelleman, Sanchez), and Physiological Sciences (Vickroy), College of Veterinary Medicine, University of Florida, Gainesville, FL 32610; Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Giguère); The Gluck Center, University of Kentucky, Lexington, KY 40546 (Troedsson); and Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (LeBlanc); e-mail: macphersonm@ufl.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
The pharmacokinetics and drug disposition of ceftiofur sodium, after administration to pregnant mares, was evaluated.

2. Methods
Eleven pregnant pony mares (D270–326) were administered ceftiofur sodium intramuscularly at 2.2 mg/kg (low dose; n = 6) or 4.4 mg/kg (high dose; n = 5). Plasma was obtained at T = 0, 0.5, 1, 2, 4, 8, 12, and 24 h after ceftiofur administration. Eight pony mares were re-enrolled in the study ≥ 3 days from foaling to ensure steady state concentrations of the drug at the time of foaling. Mares were administered ceftiofur sodium (4.4 mg/kg, IM) daily until foaling. Parturition was induced one hour after ceftiofur sodium administration. Allantoic and amniotic fluid, plasma (mare and foal) colostrum, and placental samples were collected at delivery and for 24 h. Samples were analyzed for desfuroylceftiofur acetamide (DCA) concentrations by high performance liquid chromatography (HPLC).

3. Results and Discussion
Mean (± SD) peak concentrations of DCA were 3.97 ± 0.50 mcg/mL (low dose) and 7.45 ± 1.05 mcg/mL (high dose). Terminal half-life was shorter after administration of the low dose (2.91 ± 0.59 h) than after the high dose (4.10 ± 0.72 h). The mean plasma concentration of DCA from mares at foaling
was 7.96 ± 1.39 mcg/mL and colostrum was 1.39 ± 0.70 mcg/mL. The DCA concentrations in allantoic
and amniotic fluid, placental tissues, and foal
plasma were nearly undetectable. These results
infer incomplete passage of DCA across fetal mem-
branes after administration of ceftiofur sodium to
normal pony mares.

Acknowledgments

Conflict of Interest

This work was supported by the Grayson-Jockey
Club Research Foundation and Zoetis Animal
Health.
Exploration of Safety of a Gastrointestinal Mucosal Cytoprotectant, Oral Misoprostol Regimen in Early-Gestation Mares

Jennifer K. Linton, VMD*; and Sue M. McDonnell, PhD, CAAB

Fourteen of 15 early-gestation pregnancies appeared unaffected by a gastrointestinal mucosal cytoprotectant oral misoprostol regimen (GMCOMR). Eight mares allowed to carry to term delivered normal foals. Authors’ address: School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348; email: jlinton@vet.upenn.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction

Misoprostol, a prostaglandin E1 analog, has both gastrointestinal cytoprotectant and abortigenic properties, with increased risk for neurologic, musculoskeletal, and cognitive defects following human fetal exposure. In previous work in mares, 11 mid-gestation pregnancies appeared unaffected by administration of a gastrointestinal mucosal cytoprotectant oral misoprostol regimen (GMCOMR).

Here we explored the safety of the same GMCOMR in early-gestation pregnancies and resulting neonates.

2. Materials and Methods

Eleven healthy pony mares and four healthy horse mares in early gestation (37–85 days) were administered a GMCOMR (3.5–5 mcg/kg, Q12, PO for 5 consecutive days). Serum samples for progesterone assay were obtained daily during treatment and for five days after treatment had ended. Pregnancy were monitored by transrectal palpation and ultrasonography. Four horse and two pony pregnancies were purposely terminated two (n = 1) to five (n = 5) weeks after treatment had ended. Eight pony pregnancies were allowed to continue to term.

3. Results

Fourteen of the 15 pregnancies appeared unaffected. One pony mare whose pregnancy appeared unaffected at four days after treatment had ended was found to be not pregnant six days later. Serum progesterone concentrations all remained within the range of normal for early gestation. The mare’s baseline progesterone concentration was lower than any other mare and significantly lower than the mean of five other pony mares whose gestation was within six days (5.48 vs. mean of 16.01 ± 4.1SD, P < 0.01). The eight mares allowed to carry to term delivered without complications. All neonatal musculoskeletal, neurologic, and cognitive measures were within normal limits.

4. Discussion

These results suggest a relatively low risk of disruption of early-gestation pregnancy or adverse effects
on foals following GMCOMR and not different ($P > 0.10$) from gender-matched control foals during the same time frame within the herd.

Acknowledgments

Funds and facilities for this project were provided by the Raymond Firestone Research Trust Grant and the Dorothy Russell Havemeyer Foundation.

Conflict of Interest

The Authors declare no conflicts of interest.

Reference

Effects of Intrauterine Infusion of a Water-Based Suspension of Enrofloxacin on Mare Endometrium

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Daily infusion of an alcohol-free water-based enrofloxacin suspension for 3 days does not appear to cause any permanent harmful changes to the endometrium and may be useful for treatment of endometritis due to enrofloxacin-sensitive bacteria. Authors’ addresses: Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580-2070 (Schnobrich, Barber, Bradecamp); and Comparative Theriogenology, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Pearson, Tibary); e-mail: mschnobrich@roodandriddle.com. *Corresponding author; †Presenting author. © 2014 AAEP.

1. Introduction
Bacterial endometritis is a common cause of low pregnancy and foaling rates. Many isolates are sensitive to enrofloxacin only. Intrauterine infusion of a commercial enrofloxacin preparation was associated with severe hemorrhagic inflammation and fibrosis in the endometrium. This study examines the effect of an alcohol-free water suspension of enrofloxacin after intrauterine administration.

2. Materials and Methods
Eight light-breed mares were used in the experiment. Each mare was administered a water-based suspension of enrofloxacin (2.5 mg/kg in 50 mL), intrauterine, daily for 3 days during estrus. The effects of the infusion on the reproductive tract were clinically evaluated (transrectal ultrasonography, vaginoscopy) before treatment, during treatment, and 19 days after treatment. Endometrial biopsies were taken before treatment, after the 3rd treatment, and 19 days after the last infusion.

3. Results
Intrauterine infusion of a water-based enrofloxacin suspension was associated with a transient, but not statistically significant, inflammatory response. There was a significant transient increase in intrauterine fluid, and its echogenicity. Endometrial biopsy grade showed a significant increase (p < 0.05) following the 3-day course of infusion but returned to pretreatment level on the next cycle.

4. Discussion
These findings suggest that a water-based enrofloxacin suspension may be useful for treatment of bacterial endometritis sensitive to enrofloxacin,
without the deleterious effects seen with administration of the commercial product.

Acknowledgments

Conflict of Interest

The compounded formulation used in this study was donated by Rood and Riddle Pharmacy. The compounded product was used because the FDA-approved product is not appropriate for intra-uterine use. The agent for the compounding pharmacy did not review this paper and was not part of the study design or data collection or data analysis. Knowledge of the contents of the compounded formulation were made available to the primary investigators. Three authors (Schnobrich, Bradecamp, Barber) work for Rood and Riddle Equine Hospital, which is affiliated with the Rood and Riddle Veterinary Pharmacy. Dr. Lisa K. Pearson has no conflict of interest, Dr. Ahmed Tibary has no conflict of interest.

Footnote

*Compounded solution contains Xanthan gum, Polysorbate 80, sterile water, and USP grade Enrofloxacin powder.
Relationship Between Sperm Quality and the Embryo Recovery Rate of Cooled-Shipped Stallion Sperm

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This study describes sperm quality following cooled-storage and the relationship to embryo recovery rate. Cutoff points are reported for sperm quality features that identify differences between cooled-shipped samples of high and average fertility that allows the practitioner to evaluate the stallion’s potential as a cooled-shipped semen candidate. Authors’ addresses: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4475 (Love); Noble Equine Veterinary Service, Purcell, OK 73080-4712 (Noble); 2314 Stone Court, Katy, TX 77493 (Standridge); and Department of Animal Science, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX 77843-4475 (Bearden, Cavinder); email: clove@cvm.tamu.edu. *Corresponding and presenting author. © 2014 AAEP.

1. Introduction
The clinician is limited by the lack of information regarding the level of sperm quality that should be expected for adequate fertility in samples received for breeding. The objective of this study was to determine the relationship of sperm quality after cooled-storage to fertility based on embryo recovery rate.

Materials and Methods
Ejaculates (n = 488) from Quarter Horse and Paint stallions (n = 135) were collected and shipped to an embryo transfer facility for artificial insemination. Samples were evaluated for total sperm motility, morphology, viability, semen concentration and volume, and DNA quality using the Sperm Chromatin Structure Assay. Fertility was measured using embryo recovery rate.

Results
A total of 204 mares were bred 492 estrus cycles. Cutoff values that separate average and high fertility groups were identified for total sperm motility (≥ 65%), progressive sperm motility (≥ 45%), extended sperm concentration (≥ 31.8 million sperm/mL), total sperm number in the inseminate (≥ 1.14 billion sperm), viable sperm (≥ 71%), sperm with abnormal DNA (≤ 26.8%), and morphologically normal sperm (≥ 47%).

Discussion
In this study, guideline values for cooled-stored stallion sperm were identified that describe differ-
ences in fertility. These guidelines will allow the clinician to more critically interpret the results of sperm quality analysis from cooled-shipped semen.

Acknowledgments

Conflict of Interest
The Authors declare no conflicts of interest.