and again on d 7 post ovulation. The pH microelectrode (MI-508 Esophageal pH electrode, Microelectrode Inc., Bedford, NH) was placed in a disposable insemination catheter, which was passed through the cervix and directed to the left or right of the uterine bifurcation. The electrode was then extended 1 cm beyond the tip of the catheter into the uterine lumen. The pH meter was allowed to stabilize for approximately 3 sec before the measurement was recorded. The electrode was retracted back into the catheter, and the catheter redirected into the opposite horn, where the pH measurement process was repeated.

Blood samples were harvested via jugular venipuncture into anticoagulant-coated vacuum blood collection tubes daily during estrus and every other day between d 1 and d 7 post ovulation. Plasma was stored at −20°C for analysis of progesterone concentrations by validated radioimmunoassay kit [4].

The effect of day of the estrous cycle on uterine pH, edema, serum progesterone, and their interactions were analyzed with the PROC MIXED procedure of SAS [5]. Mare was included in the statistical model as the random effect.

Results and Discussion: Mean uterine pH for ipsilateral and contralateral horns were similar throughout all observations (Figure 1). Moreover, uterine pH did not differ during estrus and was similar to d 7 post ovulation. Uterine edema scores tended to be higher ($P < .1$) during estrus and lower 7 d following ovulation. Uterine edema followed a pattern similar to what has been reported in the literature [2]. Progesterone concentrations were below 1 ng during estrus and increased ($P < .05$) to 20 ng 7 d post ovulation. Uterine pH measurements were not correlated with uterine edema scores or follicular development.

Discussion: The objective of this study was to characterize uterine pH throughout estrus in the mare. This is the first report that documents uterine pH throughout estrus and early diestrus. Uterine pH did not differ significantly throughout the estrous cycle. Numerically, uterine pH was lowest around the time of ovulation. Interestingly, this is similar the findings of Parrish [6] who demonstrated vaginal pH decreases near time of ovulation in the mare. In our study, pH measurements were recorded only once daily and found to be highly variable between mares which is similar to previous reports in cattle [3]. In contrast, Parrish [6] took multiple pH readings per day, thereby increasing the number of observations within individual mares. We plan to employ this strategy in future studies to determine whether uterine pH is indicative of uterine health. Furthermore, the effects of plane of nutrition on uterine pH and subsequent embryo survival have been well documented in cattle, and will likewise be investigated for potential effects on equine fertility.

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Oxytocin Stimulated Release of $PGF_2\alpha$ and its Inhibition by Indomethacin and Atosiban During Culture of Equine Endometrial Explants

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Introduction: Oxytocin binds to endometrial cell receptors to activate prostaglandin synthesis. Atosiban, an oxytocin receptor antagonist, blocks oxytocin binding by preventing second messenger formation [1]. Cyclooxygenase-1 and 2 (Cox-1 and 2) are responsible for converting arachidonic acid into prostaglandin H_2 which is further reduced into $PGF_2\alpha$. Indomethacin blocks Cox-1 and 2 and prevents $PGF_2\alpha$ production. The purpose of this study was to determine the effects of oxytocin stimulation over time and evaluate the effects of Atosiban and Indomethacin on $PGF_2\alpha$ production from equine endometrial explants.

Materials and Methods: In experiment one, 1 endometrial biopsy from each of 10 mares of light horse breeding was harvested on d 2 of estrus by standard procedures. Biopsies were minced into 6 equal sections and placed in 35 mm wells and covered with 3 mL of a Hams F-12/MEM media. Explants were incubated 90 min at 37°C in a humidified atmosphere with 5% CO_2. Explants were washed and each well received 3 mL of fresh serum-free media. Half the wells served as controls and half were challenged with 250 nM oxytocin. Media was collected at .5, 1, 2, 6, and 24 h and stored at −80°C until $PGF_2\alpha$ analysis. Commercial EIA kits (Cayman Chemical Co., Ann Arbor, MI) were used according to manufacturer instructions to measure $PGF_2\alpha$.

For experiment two, 2 endometrial biopsies were taken from each of 5 mares as described above. Biopsies were minced into 10 equal sections and plated into 10 single wells. Two wells served as controls and received 3 mL of serum free media, while the remaining pair received either serum free media (3 mL) containing 4 or 8 μg/ml of Indomethacin, 50 or 100 μg/ml of Atosiban. Following 30 min of culture, one well served as a control and one received 250 nM of oxytocin. Media was collected at 6 h and stored as described above.

Data were analyzed using the PROC MIXED procedure of SAS [2]. Treatments, time, inhibitor and their interactions were considered the independent variables. Mare was considered random for all experiments.

Results and Discussion: When endometrial explants were challenged with oxytocin, $PGF_2\alpha$ concentrations were higher ($P < .0001$) at each time point over the 24 h culture as compared to controls (Figure 1). In both challenged and unchallenged explants, $PGF_2\alpha$ concentrations did not increase through 2 h, and then increased ($P < .001$) at 6 and 24 h, respectively.

In experiment two, concentrations of $PGF_2\alpha$ were higher ($P < .02$) in explant cultures challenged with oxytocin as compared to controls (Figure 2). Moreover, oxytocin failed ($P < .001$) to elicit $PGF_2\alpha$ release in explants cultured with either inhibitor.

Discussion: Equine endometrial explants responded to oxytocin during culture and is similar to previous reports [3,4]. The oxytocin-stimulated release of $PGF_2\alpha$ reported here involves oxytocin binding to a receptor that initiates the prostaglandin synthesis cascade as demonstrated by the block of $PGF_2\alpha$ release in the presence of Atosiban. This inhibition is similar to that seen *in vitro* studies using oxytocin-stimulated myometrial strips from non-pregnant [1] and pregnant women [1,5].

Indomethacin equally blocked $PGF_2\alpha$ secretion in oxytocin-stimulated equine endometrial explants. This inhibition is similar to what King and Evans [3] found in equine endometrial explants. This further confirms $PGF_2\alpha$ is being produced through the prostaglandin synthesis cascade and not through an alternative pathway.

Conclusion: In conclusion, these data confirm that equine endometrial explants can be stimulated with oxytocin to secrete $PGF_2\alpha$; this release can be inhibited both through an oxytocin receptor antagonist and a Cox inhibitor. These data elucidate further the inflammatory response and provide an *in vitro* system to evaluate factors that can modulate the uterine inflammatory response.

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Comparison of deslorelin and histrelin for induction of ovulation in mares

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Introduction: In breeding management of the horse, the use of an ovulation inducing agent facilitates ovulation in a predictable timeframe [1,2]. The gonadotropin releasing hormone (GnRH) agonist deslorelin is an ovulation induction agent frequently used in equine breeding management. In addition to deslorelin, there are numerous synthetic GnRH agonists, including buserelin and histrelin that have been created by altering the 10-amino-acid composition of native GnRH. Deslorelin, buserelin and histrelin are 9-amino-acid peptides with an alkylamide-terminus and amino acid substitutions at the sixth amino acid position which results in increased potency over native GnRH [3]. Lindholm and coworkers reported that the efficacy of buserelin at inducing ovulation in the mare was not significantly different than deslorelin and that low doses (1.0 mg and 0.5 mg) were equally effective as the traditional dose (1.5 mg) [4]. The goal of this study was to evaluate the efficacy of two doses of histrelin at inducing ovulation in the estrous mare.

Materials and Methods: Light horse mares (n = 67), ranging in age from eight to twelve years, in estrus with a follicle ≥34 mm in diameter and ultrasonographically visible uterine edema were randomly assigned to receive 1.5 deslorelin (Francks Pharmacy, Ocala, FL) or 1.0 mg or 0.5 mg of histrelin (BioRelease Technologies, Lexington, KY) intramuscularly. Administrators of the histrelin were blinded to the dosage at the time of administration. All mares were examined once daily by transrectal ultrasonography after GnRH agonist administration to determine the interval to ovulation and the percentage of mares that ovulated within 48 hours after administration. In regards to this study, ultrasound examinations were discontinued after ovulation was confirmed.

Follicle size and edema score at the time of treatment, and the interval between treatment and ovulation were compared by one-way ANOVA (SAS). Percentages of mares that ovulated within 48 h after 1.5 mg deslorelin, 1.0 mg histrelin, or 0.5 mg histrelin were compared by chi-square analysis. Data are presented as the mean ± standard deviation (SD).

Results and Discussion: There were no differences in either the intervals from GnRH agonist treatment to ovulation or the percentages of mares that ovulated within 48 h between treatment groups ($P > .05$) (Table 1). There was a significant difference in edema score at time of GnRH agonist ($P = .002$), but not in the size of the largest follicle present at time of dose administration. Four mares that received histrelin had double ovulations. No side effects were noted for any of the drugs during the course of study.

The use of an ovulation induction agent is a crucial part of reproductive management for mares bred in a live cover program or when using cooled-transported or cryopreserved semen. Administration of these drugs at the optimal time of the estrous cycle allows for a timed insemination relative to expected ovulation. Human chorionic gonadotropin (hCG) has also been widely used to induce ovulation in the mare. Efficacy between hCG and deslorelin is similar in a single cycle, [5] although a reduction in efficacy has been reported following repeated use of hCG in a given season [6]. This study was designed and completed prior to the recent availability of a FDA approved deslorelin product for the induction of ovulation in mares (SucroMate™; Thorn Bioscience, Louisville, KY).

Conclusion: In conclusion, doses of 1.5 deslorelin, 1.0 and .5 mg of histrelin were equally effective in inducing mares to ovulate within 48 h of administration.

Table 1
Interval to ovulation and percentage of mares that ovulated within 48 h after administration of 1.5 mg of deslorelin or 1.0 mg or 0.5 mg of histrelin

Treatment	Dose	(n)	Interval to Ovulation (days)	Ovulation within 48 h
Deslorelin	1.5 mg	30	1.97 ± 0.5[b]	93%[b]
Histrelin	1.0 mg	19	1.63 ± 0.6[b]	95%[b]
Historelin	0.5 mg	18	1.83 ± 0.4[b]	100%[b]

[a,b] Column values with different superscripts are significantly different ($P < .05$).

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