The mare exhibits nocturnal uterine contractions in the last 6 days of gestation. It is hypothesized that oestradiol 17β (O17β) may be associated with the nightly increase in uterine contractions. The 24-h secretion pattern of plasma O17β was measured in 3 pony mares in late gestation to identify changes in release as the mare neared parturition. Blood was collected weekly at 08:00 hours beginning on day 240 and every third day from day 330 until delivery. Serial blood samples were collected from each mare every 30-min for 24-h beginning on gestation day 310 and every sixth day thereafter until parturition. Concentrations of O17β were elevated at night with lowest concentrations occurring directly before sunset (p < 0.01). The natural log of the variance was increased at sunset (p < 0.01) and was decreased during the 6-h period immediately after sunrise. This pattern was especially evident in the 6 days that preceded parturition. The contrast between nocturnal and daytime concentrations of O17β in the last 6 days of gestation may contribute to night-time delivery in the mare.

Little is known about the mechanisms that lead to parturition in mares. Uterine myoelectrical activity increases progressively each night in the 6 days preceding parturition (Henry 2002), in a pattern similar to that of primates (Morgan et al. 1992). In the baboon, nocturnal uterine contractions appear to be initiated by a surge in maternal oestradiol concentration during the last 10–12 days of pregnancy. The surge occurs earlier each evening and the nightly increase in nocturnal contractions eventually results in labour (Wilson et al. 1991). It is not known what initiates nocturnal uterine contractions in the mare. Oestradiol 17β (O17β) has the greatest affinity for the oestrogen receptor in the mare’s uterus in late gestation. It may bind to the large number of uterine oestrogen receptors and play a role in the onset of parturition in the mare (Chavatte-Palmer et al. 2000). Our preliminary data indicate that mares exhibited pulses in plasma O17β when plasma was collected for 3 h surrounding sunrise and sunset in the 6 days before parturition. Yet, plasma progestin profiles did not fluctuate (B. Sheerin and M. LeBlanc, unpublished data). We hypothesized that O17β is released in pulses at night as the mare nears parturition and that the increase in pulses corresponds to the nightly increase in uterine myoelectrical activity reported previously. The aim of this study was to determine the pattern of plasma O17β secretion in the last 24 days of gestation and to identify if pulses increased in the 6 days that preceded parturition.

Materials and Methods
Three pregnant pony mares with known gestation lengths were maintained at the College of Veterinary Medicine, University of Florida. Mares received normal preventive health care as follows: deworming every 2–4 months, annual vaccinations against rabies and tetanus, annual Coggins test, vaccination against Eastern Equine Encephalitis and Western Equine Encephalitis three times a year, and vaccinations against viral abortion during the fifth, seventh, and ninth months of pregnancy. This project was approved by IACUC (A315). One of the mares was surgically fitted with uterine myometrial electrodes on day 279 of gestation to monitor daily uterine activity until parturition. Myoelectrical data will not be presented. Blood was collected weekly at 08:00 hours beginning on day 240 and every third day from day 330 until delivery. Serial blood samples were also collected from each mare every 30 min for 24 h from a jugular catheter beginning on gestation day 310 and every sixth day thereafter until parturition. Plasma was harvested and stored at −20°C. Plasma O17β concentrations were measured using an 125I radioimmunoassay kit (Diagnostic System Laboratories, Inc., Webster, TX, USA). Cross-reactivity with other oestrogens was as follows: 6.10% equilenin, 3.40% oestrone, <1.0% for all other oestrogens. Sensitivity of the assay was 10 pg/ml. Mass recovery was determined by adding 40, 100, 500 and 1500 pg/ml of oestradiol 17β to 100 µl of gelding plasma. Recovery of added (X) vs (Y) O17β was described by linear regression (Y = 13.30 + 0.94X; r² = 0.998). The displacement curve of a plasma sample from a pooled sample of pregnant mare plasma was parallel to the O17β standard curve. The linear regression equations for plasma and standard curve were Y = −1.168X + 0.456 and Y = −1.94X + 4.39, respectively. Intraassay and interassay coefficients of variation were 15.4 and 7.8%, respectively.

Statistical analysis
Plasma O17β concentrations in the four collection days that preceded parturition were analysed using an ANOVA mixed procedure and the general linear model (GLM) of SAS (Statistical Analysis System 2000). Each 24-h collection period was grouped into 6-day intervals. Period 1 included data collected 19–24 days before parturition, period 2 – 13–18 days, period 3 – 7–12 days, period 4 – the last 6 days before parturition. The mixed model for analysing the 24-h sampling period was mare
(random), 24-h period, 30-min timed samples, 24-h period by 30-min timed samples and error. Difference among the 24-h sampling periods were tested by orthogonal contrasts: period 1 and period 2 vs period 3 and period 4; period 1 vs period 2; period 3 vs period 4. Additional analyses included dividing each 24-h sampling session into four 6-h periods centred on sunset to compare nocturnal and daylight variation in oestradiol concentrations. A second analytical approach examined variance and natural log of variance of four sequential plasma samples to characterize variation in O17β. The initial sequential sampling time to estimate sampling variance was the first four 30-min samples of the 24 h. The second sequential sampling period comprised 30-min samples 2–5 (deletion of first sample and inclusion of sample 5). This sequence of sample groupings was done throughout the 24-h sampling to generate 45 sequential variance estimates within each 24-h period. The mixed model for analysing the natural log of the variance for sequential sample groupings for plasma oestradiol was mare (random), 24-h period, 6-h period, sampling time, 24-h period by 6-h period, 24-h period by sampling time, 6-h period by sampling time, 6-h period by sampling time by 24-h period and error. Pdiff mean comparisons were used to examine differences among 6-h periods.

**Results**

Oestradiol 17β concentrations in plasma varied among collection times and days (p < 0.01). Elevated concentrations of O17β occurred at night with lowest concentrations occurring directly before sunset (Fig. 1; p < 0.01). Increases in the natural log of the variances were detected at sunset (p < 0.01) while decreases were...
Discussion

The mechanism that controls parturition in the mare is not known, but it may be associated with nocturnal variations of O17β. In the cow, an increase in cortisol initiates parturition by decreasing progesterone and increasing oestrogen production. Rising oestrogen results in prostaglandin release that stimulates uterine activity and leads to labour (Eissa and El-Belely 1990). In primates, the forward shift of the nocturnal O17β surge is believed to increase oxytocin release and oxytocin receptor formation leading to an increase in uterine contractions (Wilson et al. 1991). When Rhesus monkeys were infused with Δ4-androstenedione, the placental precursor of oestrogens, a premature shift occurred in the nocturnal surge of oestradiol. This result in an increase in plasma oxytocin concentrations and nocturnal uterine activity, leading to premature delivery (Guissani et al. 1996, 2000). The results of our study indicate that the mare exhibits nocturnal increases in variation of plasma O17β during late gestation. These increases in variation are most prominent at night, with the greatest difference between day and night hours during the 6 days preceding parturition. This difference corresponded to the increase in nocturnal uterine myoelectrical activity (Henry 2002).

Mare 3 exhibited a sharp decline in plasma O17β 60 days before parturition that was associated with surgical instrumentation. This mare had the lowest mean O17β concentrations during the last 24 days of gestation and experienced a dystocia on day 340 of gestation. Plasma concentrations of O17β declined steadily before delivery and the pattern of daily secretion was similar to that of the other two mares. The lowered maternal O17β concentrations are likely related to surgery. Whether the decreased O17β concentrations contributed to the dystocia is conjectural. This mare was the only instrumented mare (n = 7) that experienced a dystocia. Therefore, other factors may have been involved. In conclusion, the contrast between nocturnal and daytime concentrations of O17β in the last 6 days of gestation may contribute to night-time delivery in the mare.

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