Prostaglandin E₂ and F_{2α} production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares

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A growing equine conceptus must suppress the cyclical release of PGF_{2α} from the endometrium to effect maternal recognition of its presence in the uterus. Paradoxically, the conceptus itself secretes PGF_{2α}, together with other prostaglandins. In this study, the PGF_{2α} and PGE₂ content of, and production *in vitro* by, day 10–32 equine conceptuses were measured and the influence of pregnancy on the concentrations of these prostaglandins in the uterine lumen was examined. *In vitro*, the release of both prostaglandins per mg conceptus tissue was very high on day 10 after ovulation and lower thereafter. However, while PGF_{2α} production decreased further after day 18 of gestation, PGE₂ production remained high until day 32. Prostaglandin concentrations in yolk sac fluid were unaffected by gestational age and PGE₂ concentrations in this compartment were two to five times higher than

PGF_{2α} concentrations. PGF_{2α} concentrations reached high values in uterine flushings recovered from cyclic mares during days 14–16 after ovulation, the expected time of luteolysis, but were negligible in flushings recovered from pregnant mares at this time. Beyond day 18 of gestation, PGF_{2α} concentrations in uterine flushings were high and strikingly similar to those recorded during cyclical luteolysis. It is concluded that the equine conceptus effects maternal recognition of pregnancy primarily by inhibiting the ability of the endometrium to release PGF_{2α} during days 12–16 after ovulation. However, the conceptus appears to delay, rather than prevent, the development of the uterine PGF_{2α} release pathway and an alternative mechanism must prevent luteolysis from being triggered during days 18–32 of gestation.

Introduction

When incubated *in vitro*, peri-implantation blastocysts of many species synthesize and secrete a variety of prostaglandins that have been proposed to play important roles in blastocyst development and embryo–maternal dialogue (for a review, see Lewis, 1989). These postulated roles include blastocoel expansion and local increases in endometrial blood flow and vascular permeability that, in turn, are thought to increase the supply of histotrophe to the conceptus. In large domestic species, further interest has centred on the apparent ability of some prostaglandins, notably PGE₂, to interfere with the progression of luteolysis, a property that may be of physiological significance during maternal recognition of pregnancy (for a review, see Ottobre *et al.*, 1984). Weber *et al.* (1991a,b, 1995) reported that, from as early as day 5 after ovulation, equine embryos secrete PGE₂, which relaxes the circular smooth muscle of the isthmic region of the oviduct and enables the embryo to pass through the otherwise tightly closed ampullary–isthmic ‘sphincter’ and into the uterus. However, even after arrival in the uterus, equine embryos continue to secrete PGE₂ (Watson and Sertich, 1989; Weber

et al., 1992; Vanderwall *et al.*, 1993) and other prostaglandins, such as PGF_{2α} and PGI₂ (Watson and Sertich, 1989). Although the roles of these conceptus prostaglandins have not been established in mares, it has been proposed that they may stimulate the myometrial contractions that propel the conceptus throughout the uterine lumen during days 10–16 after ovulation (Gastal *et al.*, 1998; Stout and Allen, 2001) and thereby enable it to distribute its anti-luteolytic signal to the entire endometrium (McDowell *et al.*, 1985).

The aim of the present study was to measure the PGE₂ and PGF_{2α} content of, and secretion *in vitro* by, equine conceptuses recovered between day 10 and day 32 after ovulation, and to determine whether temporal changes in these values correlated with known physiological changes in conceptus behaviour or maternal–fetal interaction. Furthermore, the effect of the conceptus on the uterine luminal content of the two prostaglandins was determined by comparing their concentrations in uterine flushings recovered from day 14–32 pregnant versus day 14–18 cyclic mares and relating the differences to the potential effects of these hormones on luteal function and the migration and eventual fixation of the conceptus.

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Materials and Methods

Prostaglandin production by the conceptus

Prostaglandin concentrations in the uterine lumen. At selected stages between day 14 and day 32 after ovulation, the endometrium of 28 pregnant Thoroughbred mares was irrigated with 20 ml sterile phosphate buffered saline (PBS) that was introduced and recovered via a polytetrafluoroethylene (PTFE) cannula (Altec, Hants) passed through the working channel of a strobed-light videoendoscope (Model EC-3400F; Pentax UK Ltd, Slough). In each case, the position of the conceptus in the uterus was first identified by transrectal ultrasonography and only the 'non-gravid' horn was irrigated, thereby minimizing the risk of bathing or rupturing the conceptus. The endometrium of one uterine horn of 21 cyclic mares was similarly irrigated on day 14, 16 or 18 after ovulation. The flushings recovered were centrifuged at 1000 *g* for 10 min to pellet any particulate matter and the supernatants were decanted and stored at -70°C until assays for PGE_2 and $\text{PGF}_{2\alpha}$ were performed.

Conceptus content and secretion of prostaglandins. After completion of the endometrial flushing procedure, the conceptus in all 28 mares was approached with the tip of the videoendoscope and a new, sharpened PTFE cannula was used to puncture the conceptus and aspirate yolk sac fluid. For the day 28–32 conceptuses, yolk sac and allantoic fluids were collected separately by aspirating the allantois *in situ* and the yolk sac after the partly collapsed conceptus had been recovered by uterine lavage. Conceptus fluids were stored at -70°C until assays for PGE_2 and $\text{PGF}_{2\alpha}$ were performed.

The conceptus membranes from the 28 pregnant mares used previously and from a further 15 pregnant mares between day 10 and day 18 after ovulation were recovered by non-surgical uterine lavage using large volumes of sterile PBS introduced and recovered through a flexible polyvinylchloride tube with an internal diameter of 19 mm (Altec) passed through the cervix, as described by Meadows *et al.* (1995). Recovered conceptuses were washed twice in serum-free M199 culture medium containing Earle's salts and supplemented with 0.4 mol sodium bicarbonate l^{-1} , 50 mg bovine insulin ml^{-1} , 50 μg ascorbic acid ml^{-1} (all from Sigma Chemical Co., Poole), 30 μg crystalline penicillin ml^{-1} (Brittania Pharmaceuticals Ltd, Redhill), 30 μg streptomycin sulphate ml^{-1} (Evans Medical Ltd, Leatherhead) and 2.5 μg amphotericin B ml^{-1} (ICN Biomedicals Ltd, High Wycombe). Thereafter, day 10–25 conceptus membranes were incubated whole, but without their capsule, in 5 ml aliquots of the same medium at 37°C in 5% CO_2 in air. Day 28–32 conceptuses were first dissected into their constituent membranes, namely yolk sac, allantochorion and chorion using a dissecting microscope. The chorionic girdle was removed for a different set of experiments and the other three membranes were incubated separately in 5 ml aliquots of culture medium.

Conceptuses of all ages were cultured for 1 h, after which the medium was recovered and stored at -70°C until assays for PGE_2 and $\text{PGF}_{2\alpha}$ were performed. In addition, the membranes were desiccated in a 40°C drying oven and weighed. A further three day 14 conceptuses were incubated in culture medium to which the cyclo-oxygenase inhibitor flunixin meglumine had been added at 0.02 mg ml^{-1} to determine whether the prostaglandins measured in the conceptus-conditioned media had been synthesized *de novo* during culture, or were released from pre-existing stores.

Measurement of prostaglandin concentrations

Radioimmunoassay for $\text{PGF}_{2\alpha}$. Concentrations of $\text{PGF}_{2\alpha}$ in 1:4 dilutions of conceptus-conditioned culture media, uterine flushings and in yolk sac and allantoic fluids were measured using the radioimmunoassay described by Sheldrick *et al.* (1993). The assay used a monoclonal anti- $\text{PGF}_{2\alpha}$ serum raised in a rabbit (PG002; Steranti Research Ltd, St Albans) that the manufacturers reported to display crossreactivity of 38% with $\text{PGF}_{1\alpha}$, 0.8% with $\text{PGF}_{1\beta}$, 0.2% with PGE_1 and PGE_2 , and $< 0.1\%$ with other prostaglandins and $\text{PGF}_{2\alpha}$ metabolites. The assay sensitivity was 0.32 pg ml^{-1} and intra- and interassay coefficients of variation were 5.6 and 7.1%, respectively.

Radioimmunoassay for PGE_2 . Owing to the instability of native PGE_2 , before assay the PGE_2 in the samples was converted into its more stable methyl-oxime derivative by overnight incubation with a methyl-oximating reagent (0.125 mol methoxylamine hydrochloride l^{-1} and 1 mol sodium acetate l^{-1} in a 10% (v/v) solution of ethanol in distilled water; pH 5.6–5.8). Concentrations of the PGE_2 -MOX were measured using the radioimmunoassay described by Kelly *et al.* (1986). Before assay, conditioned culture media and yolk sac and allantoic fluids were diluted with PBS buffer at a ratio of 1:1 to 1:20. The assay used an anti- PGE_2 -MOX antiserum (a gift from R. W. Kelly, Edinburgh), reported by Kelly *et al.* (1986) to show cross-reactivity of 53% with the methyl-oxime of PGE_1 , 31% with the methyl-oximes of PGE_3 and 20-methyl PGE_2 , and 17.8% with the methyl-oxime of 19-hydroxy PGE_1 . Cross-reactivity with non-oximated prostaglandins was negligible. The assay had a sensitivity of 20 pg ml^{-1} and intra- and interassay coefficients of variation were 12.3 and 13.3%, respectively.

Statistical analysis

The effect of conceptus age on the release of PGE_2 and $\text{PGF}_{2\alpha}$ into culture medium was examined by ANOVA. Similarly, the rates at which prostaglandins were secreted by the separated yolk sac, chorion and allantochorion of day 28–32 conceptuses were compared using ANOVA. Differences between production by the membranes were examined further using Student's *t* test. Each comparison was repeated after correcting hormone production for the dry weight of conceptus membrane incubated to yield

tissue-specific release rates. In addition, to determine whether prostaglandins were synthesized *de novo* during culture, their release by day 14 conceptuses cultured with or without the inclusion of flunixin meglumine in the culture medium was compared using Student's *t* test. Two-way ANOVAs were used to determine whether pregnancy status (pregnant versus cyclic) or stage after ovulation had any effect on prostaglandin concentrations in uterine flushings and the effect of gestational age was examined further using Student's *t* tests. Finally, one-way ANOVAs were used to determine whether the stage of gestation affected prostaglandin concentrations in yolk sac or allantoic fluids.

Results

Concentrations of prostaglandins in the uterine lumen

The concentrations of $\text{PGF}_{2\alpha}$ in uterine flushings recovered by endoscopy from cyclic mares were variable but included relatively high values ($> 2 \text{ pg ml}^{-1}$) on days 14 and 16 after ovulation, when luteolysis should have been in progress, and consistently low values on day 18, when luteolysis should have been complete (Fig. 1a). As expected, $\text{PGF}_{2\alpha}$ was undetectable in flushings from the non-gravid horn of pregnant mares before day 18 after ovulation but, after this time, variable but high concentrations of $\text{PGF}_{2\alpha}$ were measured ($> 2 \text{ pg ml}^{-1}$; Fig. 1b), similar to those found during days 14–16 of the oestrous cycle. In pregnant mares, PGE_2 concentrations in washings from the non-pregnant uterine horn tended to increase from day 14 to day 20 after ovulation and then to decrease (Fig. 1d). However, in general, PGE_2 concentrations in uterine flushings were lower and less variable than $\text{PGF}_{2\alpha}$ concentrations and were not affected significantly by pregnancy status or time after ovulation (Fig. 1c,d), although the combination of these two factors had a significant effect ($P < 0.05$).

Conceptus content and secretion of prostaglandins

When incubated *in vitro*, day 10–32 conceptus membranes released nanogram quantities of PGE_2 and $\text{PGF}_{2\alpha}$ into the culture medium. Addition of the cyclo-oxygenase inhibitor, flunixin meglumine, to the culture medium in which day 14 conceptuses were incubated resulted in a significant reduction in the concentrations of PGE_2 (2.6 ± 0.4 versus $9.4 \pm 1.9 \text{ ng ml}^{-1}$; $P \leq 0.01$) but not $\text{PGF}_{2\alpha}$ (6.5 ± 1.0 versus $7.0 \pm 1.1 \text{ ng ml}^{-1}$). The amount of $\text{PGF}_{2\alpha}$ released by the conceptuses increased significantly as a function of increasing gestational age ($P < 0.0005$). However, the age-related increase in $\text{PGF}_{2\alpha}$ production occurred primarily between day 10 and day 18 of gestation when conceptus size also increased rapidly. Indeed, when values were corrected for the dry weight of membrane incubated, an alternative effect of gestational age was apparent ($P \leq 0.0001$; Fig. 2a) in which tissue-specific concentrations of $\text{PGF}_{2\alpha}$ production were very high on day 10 after ovulation, intermediate on days 14–18 and even lower between day 20 and day 25 ($P \leq 0.01$). As with

Fig. 1. Mean $\pm$ SEM concentrations of (a,b) $\text{PGF}_{2\alpha}$ and (c,d) PGE_2 measured in uterine washings recovered from day 14–18 cyclic mares (a,c: $n = 6-8$ at each stage) and day 12–32 pregnant mares (b,d: $n = 3-5$ at each stage). The high concentrations of $\text{PGF}_{2\alpha}$ measured during days 20–25 of gestation closely resembled those found during the luteolytic phase of the oestrous cycle (days 14–16) and were significantly higher than the values measured earlier (days 12–18) or later (days 30–32) in gestation ($P < 0.05$). In cyclic mares, the high inter-sample variation meant that differences between the days of the oestrous cycle were not significant.

$\text{PGF}_{2\alpha}$, PGE_2 release increased significantly with advancing gestational age ($P \leq 0.001$) and correcting for the dry weight of membrane incubated again revealed high rates of

Fig. 2. Mean $\pm$ SEM amounts of (a) PGF_{2α} and (b) PGE₂ secreted by day 10–25 equine conceptuses ($n = 3–9$ at each stage) incubated *in vitro* for 1 h, as a function of the amount of tissue incubated. Secretion of both prostaglandins was affected significantly by gestational age ($P \leq 0.0001$ for PGF_{2α}; $P < 0.02$ for PGE₂) and was very high on day 10 after ovulation. Thereafter, PGE₂ secretion was relatively constant and did not differ significantly between day 14 and day 25, whereas PGF_{2α} secretion decreased further after day 18 of gestation such that tissue-specific release rates on days 14–18 were significantly higher than those on days 20–25 ($P \leq 0.01$).

production on day 10 after ovulation and lower rates thereafter, although the difference was less marked than for PGF_{2α}. Furthermore, although tissue-specific PGF_{2α} production decreased further after day 18, PGE₂ release per unit tissue remained relatively high, such that PGE₂ was the dominant prostaglandin secreted by older conceptuses; this finding was even more pronounced in day 28–32 conceptuses (Fig. 3a,b). For both prostaglandins, production by the separated membranes of day 28–32 conceptuses differed significantly with the type of membrane incubated ($P < 0.02$ for PGF_{2α}, Fig. 3a; $P \leq 0.0001$ for PGE₂, Fig. 3b). In each case, production by bilaminar choriovitellus (yolk sac and chorion) was greater than production by trilaminar allanto-chorion (chorion, mesoderm and allantois), that, in turn, was greater than that by trilaminar choriovitellus (chorion, mesoderm and yolk sac).

The concentrations of PGF_{2α} and PGE₂ measured in yolk

Fig. 3. Mean $\pm$ SEM amounts of (a) PGF_{2α} and (b) PGE₂ secreted by the separated membranes of day 28–32 equine conceptuses ($n = 6$) incubated *in vitro* for 1 h, as a function of the amount of tissue incubated. The secretion rate of prostaglandins differed markedly depending on the type of membrane cultured (bilaminar choriovitelline > allantochorionic > trilaminar choriovitelline; $P < 0.05$).

sac fluid were remarkably constant throughout the period studied and, at all times, PGE₂ was present at about two to five times the concentration of PGF_{2α} (Fig. 4 a,b). PGE₂ was also present at higher concentrations than PGF_{2α} in allantoic fluid (Fig. 4c,d), although the concentrations of both prostaglandins in this latter compartment were much lower than in the adjacent yolk sac ($P \leq 0.0005$ for PGF_{2α}; $P \leq 0.0001$ for PGE₂).

Discussion

The release of prostaglandins by day 5–16 equine conceptuses incubated *in vitro* has been documented previously (Watson and Sertich, 1989; Weber *et al.*, 1991a, 1992; Vanderwall *et al.*, 1993); in the present study the known period of PGE₂ and PGF_{2α} secretion was extended to day 32 of gestation. In addition, it was demonstrated that the cyclooxygenase inhibitor flunixin meglumine suppressed the release of PGE₂ by cultured day 14 blastocysts, thereby confirming that PGE₂ is synthesized *de novo* by the conceptus, at least at this stage of gestation. In contrast, the

Fig. 4. Mean $\pm$ SEM concentrations of (a,c) PGE₂ and (b,d) PGF_{2α} measured (a,b) in yolk sac fluid recovered from equine conceptuses on days 12–32 of gestation ($n = 3–5$ at each stage) and (c,d) in allantoic fluid recovered between day 28 and day 32 ($n = 3–4$ at each stage). Yolk sac fluid concentrations of both prostaglandins were constant throughout the period studied and PGE₂ concentrations were consistently two to five times higher than PGF_{2α} concentrations. Concentrations of both prostaglandins were lower in the corresponding allantoic fluid samples ($P < 0.001$).

failure of the cyclo-oxygenase inhibitor to reduce PGF_{2α} secretion markedly during the 1 h incubation indicates that a large proportion of the PGF_{2α} released during this time was pre-formed. In both cases, prostaglandins secreted by the early equine conceptus appear to derive primarily from the bilaminar omphalopleure, as the bilaminar chorio-vitellus was the major source of prostaglandins during days 28–32, as it is in day 20–24 sheep conceptuses (Lewis and Waterman, 1985), and because equine conceptuses secrete prostaglandins from as early as day 5 after ovulation (Weber *et al.*, 1991a). In this context, it is not surprising that PGE₂ and PGF_{2α} were present at higher concentrations in yolk sac than allantoic fluids. More surprising was the constancy of prostaglandin concentrations in both conceptus fluids (yolk sac and allantoic) during the period of gestation studied, especially given the marked increases in the concentrations of oestrone (Zavy *et al.*, 1984), oestrone conjugates (Heap *et al.*, 1982) and oxytocin and arginine vasopressin (Waelchli *et al.*, 2000) that occur during this period. This stability of prostaglandin concentrations in conceptus fluid may reflect the influence of relatively constant (PGE₂) or decreasing (PGF_{2α}) rates of prostaglandin production, combined with the increasing blastocyst fluid volumes (until day 18) and a limited ability of the blastocysts to catabolize prostaglandins. Certainly, ovine (Lewis *et al.*, 1982) and rabbit (Jones *et al.*, 1985) blastocysts have very little ability to catabolize prostaglandins. Indeed, the latter tend to sequester prostaglandins from surrounding fluid and store them in intact form (Jones *et al.*, 1985). Similarly, Sharp *et al.* (1984) found no evidence that equine blastocysts convert PGF_{2α} to its major metabolite, 13,14-dihydro-15-keto PGF_{2α} (PGFM), either *in vivo* or *in vitro*.

The analysis of the rates of prostaglandin release as a factor of the amount of conceptus tissue incubated helped to reveal gestation-related differences in prostaglandin secretion that did not relate simply to conceptus size. The relatively high per-unit-tissue rate of PGF_{2α} production by day 10–18 equine conceptuses indicates that there may be a specific function for this hormone during the early period of gestation when the conceptus is expanding rapidly, remains mobile and must act to ensure the prolongation of luteal function. From day 18 of gestation, PGE₂ was the dominant prostaglandin produced and, therefore, it is feasible that this hormone plays a more significant role than does PGF_{2α} during the post-fixation phase of preimplantation development. The greater abundance of PGE₂ in yolk sac fluid also invites speculation as to whether this hormone is involved in luteal maintenance, as has been proposed in other large domestic animal species (for a review, see Ottobre *et al.*, 1984). However, Watson and Sertich (1989) were unable to demonstrate a luteotrophic effect of equine conceptus products on cultured luteal cells and neither Vanderwall *et al.* (1994) nor Volkmann *et al.* (1995) could consistently prolong luteal lifespan in cyclic mares by intrauterine administration of PGE₂. Thus, there is no compelling evidence to support an anti-luteolytic role for PGE₂ in horses.

The production of appreciable quantities of $\text{PGF}_{2\alpha}$ by equine conceptuses is somewhat paradoxical given that the corpus luteum of a mare remains highly susceptible to the luteolytic effects of administered $\text{PGF}_{2\alpha}$ until at least day 32 of gestation (Kooistra and Ginther, 1976). However, as neither uterine venous $\text{PGF}_{2\alpha}$ (Douglas and Ginther, 1976) nor peripheral plasma PGFM (Kindahl *et al.*, 1982) concentrations increase during this period it appears that $\text{PGF}_{2\alpha}$ secreted by the conceptus does not enter the systemic circulation in quantities likely to compromise corpus luteum function. It is more likely that conceptus prostaglandins remain within the uterine lumen where they have local actions such as stimulating the myometrial contractility that propels the conceptus around the uterus until day 16 (Gastal *et al.*, 1998; Stout and Allen, 2001). Furthermore, as the intrauterine administration of PGE_2 causes a marked increase in uterine tone and contractility (Volkman *et al.*, 1995; Gastal *et al.*, 1998), it is possible that the bias in conceptus prostaglandin secretion towards PGE_2 that occurs after day 18 is involved in the marked increase in uterine tone that is a feature of this stage of equine pregnancy (van Niekerk, 1965; Bain, 1967). It is also possible that conceptus prostaglandins play a role in the rapid expansion of the early equine blastocyst (a 10^6 -fold increase in volume between day 7 and day 16). In mice (Biggers *et al.*, 1978) and sheep (Lewis, 1986), accumulation of blastocyst fluid depends on an influx of Na^{2+} ions driven by a specific $\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$ pump that, in turn, appears to be regulated by prostaglandins (especially $\text{PGF}_{2\alpha}$; Chida *et al.*, 1986). Although accumulation of equine blastocyst fluid has also been proposed to depend on a $\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$ pump (Waelchli *et al.*, 1997), it is not known whether prostaglandins influence $\text{Na}^{+}\text{-K}^{+}\text{-ATPase}$ activity in this species. Conceptus prostaglandins have also been proposed to stimulate increases in uterine blood flow and vascular permeability that increase nutrient supply to the developing conceptus (for a review, see Lewis, 1989), while PGE_2 is an immunosuppressant that, in mice and women, is thought to contribute to maternal immune tolerance of the allogenic fetus. Whether PGE_2 has similar immunosuppressive effects during equine pregnancy is not clear, but it does not appear to be the primary immunosuppressant produced by equine trophoblast (Roth *et al.*, 1992).

In day 14–18 cyclic mares, $\text{PGF}_{2\alpha}$ concentrations in uterine flushings were variable but, nonetheless, followed the general pattern described by Zavy *et al.* (1978) in that they tended to be high on day 14 but decreased sharply thereafter, as the mares returned to oestrus. Although the absolute concentrations of $\text{PGF}_{2\alpha}$ measured in uterine flushings in the present experiment were considerably lower than those reported previously by Zavy *et al.* (1978), Berglund *et al.* (1982) and Watson and Sertich (1989), the discrepancy probably resulted from differences in the uterine flushing technique. In all the previous studies, the uterus was flushed with 60 ml PBS infused via a transcervical balloon catheter and collected 'blind' after vigorous ballotment of the uterus *per rectum*. As Berglund *et al.*

(1982) found that $\text{PGF}_{2\alpha}$ concentrations in uterine fluids collected in this way were reduced substantially if, before flushing, the uterus was removed by hysterectomy or the mare was treated with the prostaglandin synthesis inhibitor flunixin meglumine, it was apparent that the bulk of the $\text{PGF}_{2\alpha}$ measured was released in response to the cervical dilation and uterine stimulation inflicted during the collection procedure. In the present experiment, uterine flushing was performed by endoscope-guided infusion and collection of 20 ml PBS with only mild uterine ballotment to aid mixing, a technique that provokes peripheral PGFM increases on very few occasions (Stout *et al.*, 2000). Thus, in most instances the technique used in the present study probably reflected unstimulated intrauterine $\text{PGF}_{2\alpha}$ concentrations, while the occasional high concentration recorded probably reflected either spontaneous releases of $\text{PGF}_{2\alpha}$ or occasional pulses stimulated by the flushing procedure. In pregnant mares, Berglund *et al.* (1982) and Watson and Sertich (1989) both reported that, on day 14 after ovulation, uterine luminal $\text{PGF}_{2\alpha}$ concentrations were significantly lower than in cyclic mares at the same stage. Therefore, these authors proposed that maternal recognition of pregnancy in mares is achieved by total inhibition of uterine $\text{PGF}_{2\alpha}$ secretion. In the present study, $\text{PGF}_{2\alpha}$ was similarly undetectable in uterine flushings recovered from the conceptus-free horn of pregnant mares before day 18 of gestation. However, the concentrations in flushings collected from the non-gravid horn at later stages increased to such an extent that both the mean and the peak concentrations recorded between day 20 and day 25 of gestation were similar to those recorded on day 14 of dioestrus in cyclic mares. Although this result contradicts the report of Zavy *et al.* (1984) that $\text{PGF}_{2\alpha}$ concentrations in the uterine lumen of pregnant mares were negligible up to and including day 20 of gestation, again the discrepancy almost certainly reflects differences in the method of collection. Zavy *et al.* (1984) flushed pregnant uteri only after removal of the uteri from mares by hysterectomy and, therefore, after their detachment from neural and vascular connections and the potential for $\text{PGF}_{2\alpha}$ release in response to cervical dilation and uterine handling. Therefore, it is concluded that the high uterine luminal $\text{PGF}_{2\alpha}$ concentrations measured between day 18 and day 30 of gestation in the present study were a consequence of the transcervical flushing procedure. If so, $\text{PGF}_{2\alpha}$ release presumably followed the pathway outlined by Sharp *et al.* (1997): namely, cervical dilation or uterine trauma induced an oxytocin release that, in turn, elicited $\text{PGF}_{2\alpha}$ release from the endometrium. In this respect, the low intrauterine concentrations of $\text{PGF}_{2\alpha}$ measured before day 18 of gestation are consistent with the finding of Goff *et al.* (1987) that the increase in oxytocin responsiveness that occurs during days 11–16 after ovulation in cyclic mares is absent during pregnancy. The high $\text{PGF}_{2\alpha}$ concentrations measured after day 18 almost certainly reflect the finding by Starbuck *et al.* (1998) that a significant peripheral PGFM response to oxytocin challenge returns by day 18 of gestation.

Therefore, it is proposed that the equine conceptus achieves luteostasis primarily by delaying the development of the endometrial prostaglandin release or oxytocin response pathways or both, thereby allowing the pregnant mare to pass through a period between day 10 and day 16 after ovulation when oxytocin may be secreted from the uterus or hypothalamus (for a review, see Stout and Allen, 1999) without triggering $\text{PGF}_{2\alpha}$ release. However, as the ability of the uterus to secrete $\text{PGF}_{2\alpha}$ in response to mechanical stimuli and oxytocin challenge appears to recover between day 18 and day 30 after ovulation, the continued avoidance of luteolysis must result from a different mechanism, for example, by an absence of any significant oxytocic stimuli. If so, the day 18–30 conceptus is in a precarious position when systemic oxytocin release could inadvertently provoke $\text{PGF}_{2\alpha}$ release leading to luteolysis and early pregnancy loss. From a practical standpoint, any profound uterine manipulation such as, for example, transrectal ‘crushing’ to reduce twin conceptuses to a singleton, might stimulate an oxytocin release that could initiate the luteolytic cascade.

In conclusion, the large amounts of PGE_2 and $\text{PGF}_{2\alpha}$ produced by preimplantation equine conceptuses are probably involved in local functions important to conceptus development, movement and nutrition. Furthermore, it is proposed that maternal recognition of pregnancy in mares may involve a conceptus-directed delay, rather than abolition, of the ability of the endometrium to secrete $\text{PGF}_{2\alpha}$ in response to a suitable challenge.

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