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Estrus, Ovulation, Progesterone and Luteinizing Hormone after Prostaglandin $F_{2\alpha}$ in Mares[1] (37765)

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Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is luteolytic in several laboratory animals (1), sheep (2), and cattle (3, 4). In cows and sheep, uterine control of luteal function depends upon close proximity of these two organs (5). The apposition of the uterine vein to the ovarian artery in sheep (6) and cows (7) may account for the observation that ^3H-$PGF_{2\alpha}$ was transferred directly from the vein to the artery (8). Since uterine control of luteal function in mares is mediated systemically (9) and the ovarian artery and uterine vein are not in intimate contact (6), this experiment was designed to determine luteal function after intrauterine or systemic administration of $PGF_{2\alpha}$ in mares.

Materials and Methods. Six grade mares (300–500 kg; 3–20 yr) were tested by daily exposure to a stallion to detect estrus. Follicular growth and ovulation were monitored by palpation daily during estrus and every third day during the interestrual period. Onset of estrus, ovulation, and end of estrus were estimated at 12 hr previous to time first detected. Depending on the stage of the estrous cycle, the mares were bled by jugular puncture 1–4 times daily. At 7–9 days after ovulation during a control cycle, 10 mg $PGF_{2\alpha}$ (Tham salt)[2] in 1.0 ml 0.85% saline was infused into the uterus. After the estrus which followed $PGF_{2\alpha}$, the mares were allowed a second control cycle. Seven to nine days after ovulation during the second control estrus, each mare was given sc 15 mg $PGF_{2\alpha}$ in 1.0 ml saline.

Progesterone was quantified by radioimmunoassay (3). To validate this assay for equine plasma, 22 samples of blood plasma representing all stages of the estrous cycle were extracted with benzene:hexane (1:2) and chromatographed on LH-20 Sephadex columns with chloroform:ethanol (96:4) to isolate progestogens from estrogens and glucocorticoids (10). The progestogens were then rechromatographed on another LH-20 Sephadex column with heptane:chloroform:ethanol:water (200:200:1:saturated) solvent to isolate progesterone from other progestogens (11). Progesterone eluted (fraction 8) well in advance of 17_α-hydroxyprogesterone (fraction 12) and 20_α-hydroxy-pregn-4-en-3-one (fraction 20) in the second solvent system.

LH was determined with a double-antibody radioimmunoassay similar to those described (12, 13). In specific, (a) purified ovine LH[3] was iodinated (^{125}I), (b) rabbit antiovine LH[4] was diluted 1:20,000 in 0.01 *M* phosphate-buffered (pH 7.0) saline with 1:400 normal rabbit serum, (c) equine LH[3] was the standard, and (d) 0.1% Knox gelatin in 0.01 *M* phosphate-buffered (pH 7.0)

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[2] $PGF_{2\alpha}$ Tham salt was supplied by Dr. J. E. Pike of The Upjohn Company.

[3] Highly purified ovine LH (LER-1056 C2) for iodination and purified equine LH (LER 1138-1; potency = 0.27 U NIH LH-S1/mg) were generously supplied by Dr. L. E. Reichart (Emory Univ.).

[4] Rabbit antiovine LH (GDN No. 15) was supplied by Dr. G. D. Niswender, Colorado State Univ.

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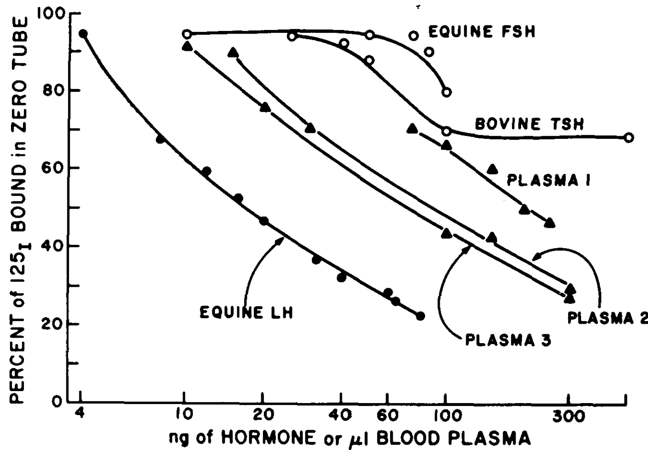

FIG. 1. Radioimmunoassay of standard equine LH (LER-1138-1), and relative activities of FSH and TSH and dilutions of equine blood plasmas.

saline was the diluent. Ten to 100 ng of equine FSH[5] and 25-300 ng of bovine TSH[6] were tested for cross reaction in the LH assay and plasmas from mares in various stages of the estrous cycle were tested for parallelism to the standard curve. To further test for TSH cross reactivity, two mares at about 190 days of pregnancy were given (iv) 100 μg of thyrotropin-releasing hormone (TRH)[7]. Jugular plasma was taken by venipuncture hourly for 6 hr, and serum thyroxine was determined by the Tetrasorb method (14).

Results. Radioimmunoassays for progesterone and LH. Radioimmunoassay (RIA) values for chromatographically isolated progesterone were highly correlated ($r = 0.98$; $P < 0.01$) with values for the same samples determined by RIA directly on the benzene: hexane (1:2) extracts without chromatography (av 4.5 vs 4.6 ng/ml). The RIA values for LH in four dilutions of plasmas from each of three mares were parallel to the standard curve (Fig. 1). Bovine TSH and equine FSH cross reacted in the LH assay (Fig. 1) 8% and 9%, respectively. After injection of TRH, serum thyroxine more than doubled at 60 min ($P < 0.01$) and remained high for 6 hr (Fig. 2), but RIA LH was unchanged. This observation supported the views that cross reactivity with bovine TSH (Fig. 1) may be associated with LH contimination in the TSH, and that endogenous TSH probably did not interfere with the equine LH assay.

Estrus and ovulation. During the first control estrous cycle, ovulation occurred 3.8 days after the onset of estrus or 1.5 days before the end of estrus, and the interestrual interval was 14.5 days (Table I). After deposition of 10 mg $PGF_{2\alpha}$ into the uterus estrus began in 2.2 days and ovulation occurred 5.8 days after the onset of estrus, 2 days later ($P < 0.05$) than in the control estrus. However, the interval from ovulation

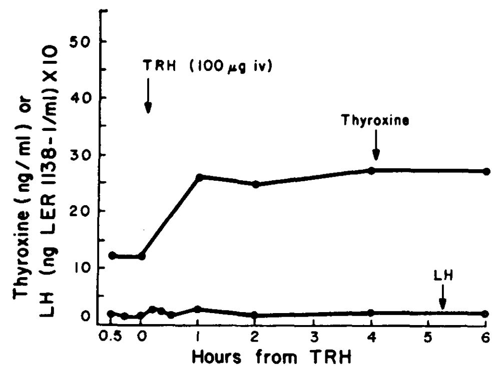

FIG. 2. Plasma LH and thyroxine after thyrotropin-releasing hormone (TRH, 100 μg iv) in two mares.

[5] Equine FSH (G-75F2-42) was supplied by Mr. L. Nuti, Univ. of Wisconsin (potency = 90.9 U NIH-FSH-1/mg).

[6] Highly purified TSH supplied by Dr. J. Pierce, UCLA.

[7]. Supplied by Dr. M. S. Anderson, Abbott Lab., N. Chicago.

TABLE I. Estrus[a] and Ovulation[a] after $PGF_{2\alpha}$ in Six Mares.

Interval	Estrous cycle (days)			
	Control	Intrauterine $PGF_{2\alpha}$ (10 mg)	Control[b]	Subcutaneous $PGF_{2\alpha}$ (15 mg)
Between				
(a) $PFG_{2\alpha}$ and estrus	—	2.2 ± .3	—	2.3 ± .3
(b) $PGF_{2\alpha}$ and ovulation	—	8.0 ± .6	—	7.1 ± 1.0[b]
(c) Onset estrus and ovulation	3.8 ± .5[b]	5.8 ± .7	3.8 ± .4	4.6 ± 1.0[b]
(d) Ovulation and end estrus	1.5 ± .5	1.6 ± .2	1.8 ± .6	2.4 ± .4[b]
Duration of estrus	5.2 ± .5[b]	7.5 ± .9	5.6 ± .8	7.6 ± 1.0
Interestrual interval	14.5 ± 2.5[c]	8.8 ± .9	14.2 ± .4	9.4 ± .4[b]

[a]Estrus and ovulation were estimated at 12 hr previous to time first detected.
[b]$n = 5$.
[c]$n = 2$.

to the end of estrus (1.6 days) resembled that in the control estrus. The interestrual interval (8.8 days) was 5 days shorter ($P < 0.01$) than the previous or subsequent control interestrual intervals.

The second control estrous cycle resembled the first; estrus persisted 5.6 days and ovulation occurred 3.8 days from onset or 1.8 days prior to the end of estrus. When 15 mg $PGF_{2\alpha}$ was injected sc 7–9 days after ovulation during the second control estrus, the mares returned to estrus 2.3 days later, estrus persisted 7.6 days, and ovulation occurred 2.4 days prior to the end of estrus. Estrus persisted longer ($P < 0.01$) and the interval from onset of estrus to ovulation was longer ($P < 0.05$) after sc $PGF_{2\alpha}$ than during the previous control estrous cycle, but the interval from ovulation to the end of estrus was unchanged after $PGF_{2\alpha}$.

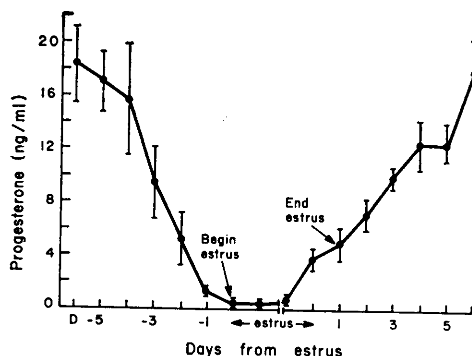

FIG. 3. Blood plasma progesterone during control estrous cycles in six mares.

Blood plasma progesterone. Blood plasma progesterone averaged 17.1 and 9.5 ng/ml at 5 and 3 days before estrus during the first control cycle; it declined to 0.6 ng/ml on the first day of estrus, then remained below 1 ng/ml until ovulation (Fig. 3). After ovulation, progesterone increased nearly linearly for 6 or 7 days. These changes in progesterone resemble those reported earlier in mares (15–17).

Plasma progesterone averaged 13.6 ng/ml at 7–9 days after ovulation when 10 mg $PGF_{2\alpha}$ was infused into the uterus. Within 12 hr, progesterone decreased to 5.8 ng/ml, and the decrease continued to 2.6 ng/ml and 0.9 ng/ml by 24 and 48 hr (Fig. 4). Since the estrus after $PGF_{2\alpha}$ persisted for a longer period, progesterone remained below 1 ng/ml for a longer period ($P < 0.01$) than during the control estrus. The concentration of progesterone in blood plasma increased after ovulation, averaging 4.1, 11.5, and 13.7 ng/ml on Days 1, 4, and 6 after estrus. During middiestrus, progesterone averaged 12.1 ng/ml; it began to decline about 4 days before the second control estrus. The changes in progesterone before, during, and after the second control estrus did not differ significantly from those during the first control estrus.

The decline in progesterone after subcutaneous $PGF_{2\alpha}$ (15 mg) did not differ significantly from that after intrauterine $PGF_{2\alpha}$, and estrus began when progesterone fell below 1 ng/ml. On the average, the decline in

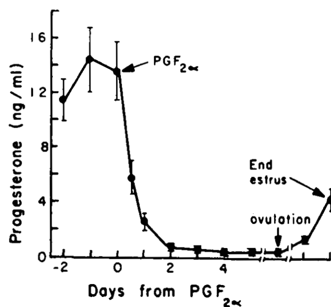

FIG. 4. Blood plasma progesterone after intrauterine $PGF_{2\alpha}$ (10 mg) in six mares.

progesterone for 3 days after $PGF_{2\alpha}$ treatment was more rapid than that during the 3 days before estrus during the control cycles (Figs. 3 and 4), but this difference only approached significance ($P \cong 0.06$).

Blood plasma LH. Daily averages for LH during middiestrus ranged from 53 ± 14 to 70 ± 11 ng/ml (baseline) in the first control cycle (Fig. 5); LH increased to 236 ± 75 ng/ml the day prior to estrus, and to a broad peak of about 800–1000 ng/ml which persisted for 2 days after ovulation. LH gradually returned toward baseline, averaging 123 ± 35 ng/ml when PGF_{2a} (10 mg) was deposited in the uterus at 7–9 days after ovulation. On the first day of the estrus after PGF_{2a}, LH averaged 317 ± 64 ng/ml; it increased to a broad peak of about 900–1100 ng/ml during the 2 days after ovulation (Fig. 6). Then LH declined continuously for 8 days and averaged 69 ± 8 ng/ml for 5 days during middiestrus. The pattern of changes in LH around the second control estrus did not differ from that during the first control estrus; LH increased from a baseline of 150 ± 57 ng/ml on the day prior to estrus to 1237 ± 345 ng/ml by 60 hr after ovulation and then declined until PGF_{2a} was given subcutaneously. Analysis of variance of LH data from five mares on 5 days (the day before estrus, the first day of estrus, the day of ovulation, the first day after estrus, and Day 8 after ovulation) indicated there was no difference ($P > 0.50$) in LH values between the two control cycles and the cycle which began after intrauterine PGF_{2a}.

Discussion. The major conclusion from this research is that PGF_{2a} caused dramatic luteolysis in mares, whether injected subcutaneously or into the uterus. All mares were in estrus within 4 days after treatment, in agreement with observations in ponies (18).

The estrous cycle which began after PGF_2 was similar in every measured criterion to control cycles, except that the duration of estrus and the interval from onset of estrus to ovulation were longer after PGF_{2a} treatment than during control estrus. Our data provide no explanation for the prolonged estrus after PGF_{2a}. That the second control estrous cycle resembled the first in every measured criterion indicates no residual effect of intrauterine PGF_{2a} on the subsequent control cycle. In other words, timing of corpus luteum growth and regression, followed by follicular growth and ovulation during the second control estrous cycle were reset with the ovulation longer after PGF_{2a} treatment as would have occurred during a normal estrus.

The broad elevation of LH we observed resembled those reported by Whitmore *et al.* (19) and Pattison *et al.* (20). In all three studies, blood LH increased beginning near the onset of estrus and continuing until after ovulation; the subsequent decrease appeared nearly symmetrical to the increase. However, we report higher plasma LH than others reported (19, 20). A major portion of this difference may be due to different reagents; Whitmore *et al.* (19) used an anti-PMS first antibody and an equine LH standard of approximately 10-fold higher potency than that used in our study. Pattison *et al.* (20) used the same antiovine LH[4] used in the present study, but they used ovine LH (LER-

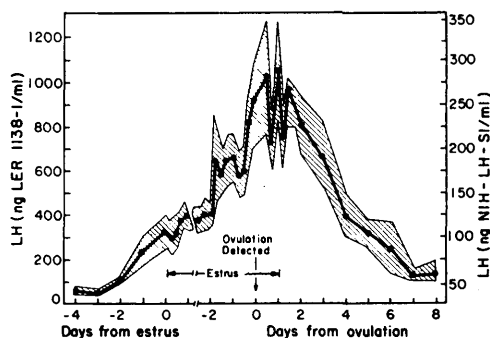

FIG. 5. Blood plasma LH during control estrous cycles in six mares.

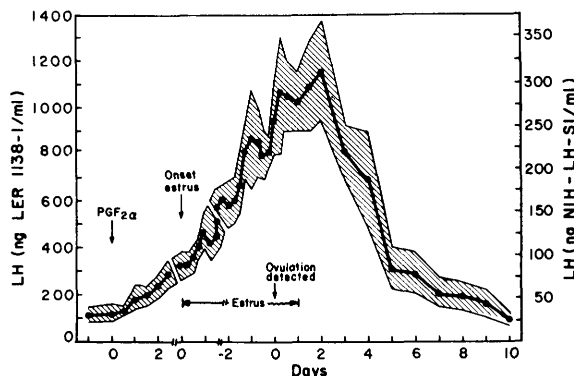

FIG. 6. Blood plasma LH after intrauterine $PGF_{2\alpha}$ (10 mg) in six mares.

1056-C2) for a standard. Sustained elevations of plasma LH throughout estrus with maximum concentrations after ovulation in mares is in contrast to the brief surge (6–8 hr) of LH prior to ovulation in most other species. The LH surge occurs coincident with onset of estrus in the cow (10, 21), during early estrus in the ewe and sow (21), and on the second day of estrus in the bitch (22). The significance of the prolonged elevation of LH during estrus in mares is not apparent to us. That it may be an artifact of cross reaction with other pituitary hormones seemed unlikely. The data in Fig. 2 discounted any significant interference of TSH in the equine LH RIA, because while plasma thyroxine doubled within 1 hr after TRH injection, LH did not change. Furthermore, in addition to a TSH release, TRH injections also released prolactin and growth hormone in several other species (23).

Comparison of the progesterone data in Figs. 3 and 4 with the LH data in Figs. 5 and 6 indicated that progesterone fell to near basal values before the LH surge began near the onset of estrus. Furthermore, the LH surge persisted until progesterone began to increase at 1 or 2 days after ovulation. These observations suggest negative control of progesterone on LH release in mares.

From a practical point of view, $PGF_{2\alpha}$ has potential for ovulation control in brood mares. Although the synchrony of ovulation after PGF_2 is not as precise as in cows (3, 4), the data in Table I indicate sufficient ovulation synchrony to warrant breeding mares without detection of estrus after $PGF_{2\alpha}$.

Summary. After a control estrous cycle, 10 mg $PGF_{2\alpha}$ was deposited in the uterus in six mares (300–500 kg; 3–20 yr) at 7–9 days after ovulation. Blood plasma progesterone fell from 13.6 to 5.8 ng/ml within 12 hr and to 0.9 ng/ml at 48 hr after $PGF_{2\alpha}$, estrus began in 2.2 days and ovulation occurred at Day 5.8 of estrus. The estrus after PGF_2 persisted longer than estrus in control cycles (7.5 vs 5.2 days; $P < 0.05$), but the interval from ovulation to the end of estrus was unchanged (1.6 vs 1.5 days). An increase in blood plasma LH began the day before estrus and continued past ovulation; LH declined beginning 2 days after ovulation and continuously for 8 days. A control estrous cycle after the $PGF_{2\alpha}$ cycle resembled in every measurement the control cycle before $PGF_{2\alpha}$, indicating no carry-over effect of $PGF_{2\alpha}$. Subcutaneous injection of 15 mg PGF_2 7–9 days after ovulation also caused luteolysis, estrus, and ovulation — resembling in every detail the comparable events after intrauterine $PGF_{2\alpha}$. We conclude that $PGF_{2\alpha}$ given subcutaneously or into the uterus is a powerful luteolysin with no carry-over effect on subsequent control estrous cycles in mares.

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