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Responsiveness of the early corpus luteum to PGF2α and resulting progesterone, LH, and FSH interrelationships in mares

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Abstract

The effect of prostaglandin F2α (PGF) treatment during development of the corpus luteum on concentrations of plasma progesterone, LH, and FSH and the resulting temporal interrelationships among hormones were studied in 72 mares in two experiments. In experiment 1, a single treatment on Day 0 (day of ovulation) or Day 1 was associated with a significant increase in progesterone, but the increase was less ($P < 0.05$) than in nontreated mares. Eight of 12 mares treated on Day 2 or 3 (combined data) had a progesterone decrease for 1 or 2 days, followed by a gradual resurgence to concentrations similar to those in controls by Day 12. Luteolysis (progesterone decrease to < 1 ng/ml) occurred in each of 18 mares treated on Day 4, 5, or 6, except for one mare with resurgence after treatment on Day 4. In experiment 2, daily PGF treatment on Days 0, 1, and 2 suppressed the progesterone concentrations to < 2 ng/ml through Day 4, followed by an increase, indicating that the luteal cells remained viable during progesterone suppression. When PGF was given on Day 0, the increase in concentration of systemic LH on Day 1 was greater ($P < 0.01$) than the increase in controls, but the FSH concentration increase was not different from controls. Neither gonadotropin increased significantly after treatment on Day 1 or 2. Both gonadotropins increased within 24 h after PGF treatment on Day 3, 4, 5, or 6. Results indicated that PGF treatment on Day 0 or 1 had a novel retarding effect on progesterone output, treatment on Day 2 or 3 had a transient regressive effect with resurgence to control levels in most mares, and treatment on Day 4, 5, or 6 had a luteolytic effect in almost all mares. The gonadotropin results on Days 0 to 6 are compatible with reported days of change in pituitary content, indicating a direct effect of PGF at the hypothalamo-pituitary area.

Keywords: corpus luteum, gonadotropins, mares, PGF2α, progesterone.

Introduction

Secretion of prostaglandin-F2α (PGF) by the uterus (Ginther, 1992), augmented by intraluteal PGF production (Beg *et al*., 2005), terminates the luteal phase in nonpregnant mares as in many other species (Arosh *et al*., 2004). Exogenous natural PGF and PGF analogs are used extensively as a luteolysin in reproductive management programs in horses (Meyers, 1997) and other farm animals (Wenzel, 1997). The luteolytic efficacy of exogenous natural PGF and its analogs is best when a mature corpus luteum is present, but limited when the corpus luteum is in early development in horses (Ginther, 1992), cattle (Inskeep, 1973), and sheep (Rubianes *et al*., 2003). A single treatment in mares was ineffective when given on Day 1 or 2 (ovulation = Day 0); on Day 3, luteolysis was induced in some mares (60%) but not in others (Allen and Rowson, 1973; Oxender *et al*., 1975; Douglas and Ginther, 1975). These early studies on capacity of the equine corpus luteum to respond to PGF when given during luteal development were based on the stimulation of estrus and ovulation. Recent studies on the effect of a single injection of PGF on concentrations of circulating progesterone involved treatment on two days of diestrus. Treatment on Days 8 or 13 resulted in a progesterone decrease below pretreatment concentrations within 40 to 50 min (Utt et al., 2006). Treatment on Days 3 or 10 decreased the progesterone concentrations to near 2 ng/ml in 1 day (Bergfelt et al., 2006). Treatment on Day 3 was effective in shortening the interovulatory interval, but in 12 of 16 mares the immediate decrease in progesterone was followed by a transient resurgence or increase in concentrations, as opposed to a continuous decrease to base-line levels. Studies on the causes of the refractoriness of the early corpus luteum to PGF in mares could be more systematically planned if detailed information were available on the effect of PGF on progesterone production at various days post-ovulation, beginning on Day 0.

An analog of PGF (cloprostenol) is being used post-breeding in mares as a myometrial stimulant to clear the uterus of intraluminal fluid and inflammatory products (Combs *et al*., 1996). Treatment on either Day 0 or 1 did not alter progesterone concentrations significantly, but mares treated on Day 2 had the lowest mean circulating concentrations on Days 3 to 7 (Nie *et al*., 2003b). In another study, treatment on Day 2 resulted in a progesterone decrease on Day 5, the day of the first post-treatment examination (Troedsson *et al*., 2001); thereafter, concentrations resurged to control

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levels by the end of the luteal phase. Daily treatment on Days 0 and 1 (Nie *et al*., 2003a) or on Days 0, 1, and 2 (Troedsson *et al*., 2001) resulted in lower progesterone concentrations on Day 2, followed by a similar resurgence in concentrations. Based on an abstract, treatment on Days 0 and 1 was associated with suppression of progesterone production until Day 3, followed by resurgence (Brendemuehl, 2002). Although there have been variable results with this PGF analog, a consistent finding was a mean decrease in progesterone concentration for treatment on Day 2, followed by resurgence, so that the concentrations returned to control levels by the end of the luteal phase.

A transient increase in LH and FSH in mares has been reported to follow treatment with PGF or an analog on Days 5, 6, or 7 (Noden *et al*., 1978), 8 (Nett *et al*., 1979, Ginther *et al.*, 2006), or 9 or 10 (Roser *et al*., 1982). These studies were done during high progesterone production; the PGF effect on gonadotropins during early luteal development or before Day 5 has not been reported. However, a PGF analog given i.m. or i.v. during anestrus preceding the first ovulation of the year resulted in increases in both gonadotropins within 2-10 min in samples obtained from intracavernous-sinus and jugular vein (Jöchle *et al*., 1987). In local samples from the cannula, GnRH was elevated but only after LH and FSH had reached maximum concentrations. Thus, the PGF analog had a direct stimulatory effect on gonadotropins at the hypothalamo-pituitary level.

The objective of the present experiments was to compare the effects of exogenous natural PGF when given on various days during early development of the corpus luteum (Days 0 to 6) on systemic concentrations of progesterone and gonadotropins.

Materials and Methods

Animals

Animals were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research. Nonlactating large pony mares of mixed breeds, 4 to 16 years of age, and weighing 320 to 470 kg were used in the Northern Hemisphere (43° N). Feed consisted of alfalfa/grass hay with access to water and trace-mineralized salt. Body condition for all mares was high throughout the experiments. Mares with docile temperament and no apparent abnormalities of the reproductive tract were selected, as determined by ultrasound examinations (Ginther, 1995). Mares which developed codominant follicles (≥ 30 mm) or hemorrhagic anovulatory follicles (Ginther, 1995) during the ovulatory period preceding the experimental luteal phase were not used. An early onset of the ovulatory season was induced by a lighting program (Ginther, 1992) on December 1st, so that the ovulatory season began in February and March, rather than in April and May. Thereafter, mares were kept under natural light. Experiment 1 began in early November and Experiment 2 began in May of the following year. Follicle development and day of ovulation (Day 0) were monitored daily by transrectal ultrasonography. The PGF-treated mares were given a single i.m. injection of 5 mg per mare of dinoprost tromethamine (1 ml Lutalyse; Pfizer Animal Health, Kalamazoo, MI, USA) on an indicated day.

A non-response to PGF treatment in individuals was defined by no decrease in progesterone concentrations within 2 days after treatment. Luteolysis was defined as a progesterone decrease to < 1 ng/ml. Progesterone resurgence after PGF treatment was indicated by an initial decrease in progesterone concentrations followed by an increase.

Experiment 1

The experiment extended from Day 0 to Day 12. A control group and seven PGF-treated groups were used (n = 6 mares/group). The PGF was given on Days 0 (group PGF-0), 1, 2, 3, 4, 5, or 6. A blood sample was collected from a jugular vein every day from Days 0 to 12 in all groups; on the day of treatment, the sample was collected immediately before treatment. The mares in the PGF groups on the days before treatment were designated nontreated mares as contrasted to the six mares of the control group; however, results from days of nontreatment were considered as control data. End points were systemic concentrations of progesterone, LH, and FSH from Day 0 to termination of the experiment on Day 12.

Experiment 2

The experiment extended for the length of the interovulatory interval. A control group and three PGF-treated groups were used (n = 6 mares/group). The PGF was given on Day 0 (group PGF-0), Days 0 and 1 (group PGF-0,1), and Days 0, 1, and 2 (group PGF-0,1,2). A blood sample was collected daily from a jugular vein in each mare on Days 0 to 6 and on Days 8, 10, 12, 14, and 16. Ovarian monitoring by transrectal ultrasonography was done every 2 or 4 days and daily when a 25 mm follicle was present. End points were progesterone, LH, and FSH concentrations; number of mares with a secondary major follicular anovulatory wave during early diestrus; and length of the interovulatory interval. A secondary major anovulatory follicular wave was defined by a wave with a largest follicle that reached 25 mm after Day 0, grew to ≥ 30 mm, and regressed before or during early development of the ovulatory wave.

Blood samples and hormone assays

Blood samples were collected into heparinized tubes and centrifuged (1500 x g for 20 min) and the

plasma was decanted and stored (-20°C) until assay. Plasma samples were assayed for progesterone concentrations, using a solid-phase radioimmunoassay kit containing antibody-coated tubes and ^{125}I-labeled progesterone (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA), as described and validated in our laboratory for mare plasma (Ginther *et al.*, 2005c). The intra- and inter-assay CVs and mean sensitivity in experiment 1 were 4.4%, 0.4%, and 0.05 ng/ml, respectively. The intra-assay CV and sensitivity for progesterone assay in experiment 2 were 8.1% and 0.03 ng/ml, respectively. Plasma LH and FSH concentrations were determined by radioimmunoassays as validated and described for mares in our laboratory (Donadeu and Ginther, 2002). For LH, the intra- and inter-assay CVs and mean sensitivity in experiment 1 were 6.8%, 9.3%, and 0.2 ng/ml, respectively. The intra-assay CV and sensitivity for LH in experiment 2 were 5.1% and 0.2 ng/ml, respectively. For FSH, the intra- and inter-assay CVs and mean sensitivity, respectively, were 6.0%, 5.6% and 1.3 ng/ml for experiment 1. For experiment 2, the intra-assay CV and sensitivity were 6.0% and 2.5 ng/ml, respectively.

Statistical analyses

Hormone data were not normally distributed, according to Kolmogorov-Smirnov tests (Zar, 1984). Concentrations of progesterone were handled by square-root transformation and LH and FSH by log transformation. The LH concentrations on Day 0 showed differences ($P < 0.05$) between at least two groups in each experiment. It was thought that a disparity between groups on Day 0 could contribute to significant differences on later days. In this regard, significant autocorrelation in hormone concentrations among days has been shown in mares (Ginther *et al.*, 2005a). Therefore, the LH concentrations were converted to percentage increase or decrease from Day 0. The percentage approach was used when comparisons were made among groups, but comparisons within a group were made using the actual data. Sequential data were analyzed by the SAS MIXED procedure to determine the main effects of group and day and their interaction, using a repeated statement to account for the autocorrelation between measurements (version 8.2; SAS Institute Inc., Cary, NC). Unpaired Student's *t*-tests were used to locate differences between groups when an interaction of group and day was significant and paired *t*-tests were used between days within a group when the day effect was significant. One-way ANOVAs were used to compare changes in progesterone, LH, and FSH between the day of treatment and the next day (experiment 1) and length of the interovulatory interval among groups (experiment 2). Chi-square analyses were used to compare number of mares with various events. A probability of $P \leq 0.05$ indicated that a difference was significant. Data are presented as the mean ± S.E.M., unless otherwise indicated.

Results

Experiment 1

Each main effect (group, day) and the interaction were significant ($P < 0.0001$) for progesterone concentrations (Fig. 1). Concentrations increased between the day of treatment and the next day in groups PGF-0 ($P < 0.003$) and PGF-1 ($P < 0.002$), but the increase was less ($P < 0.005$) than in the nontreated mares. For both groups, concentrations were lower ($P < 0.05$) than in the nontreated mares, but only for 2 days after treatment. Concentrations of progesterone decreased by the day after treatment in four of six mares in group PGF-2 and in each mare in groups PGF-3, -4, -5, and -6. The percentage decrease (group effect, $P < 0.0002$) on the first day for the five PGF groups is shown (Table 1); mean and percentage decrease were greater for group PGF-5 than for groups PGF-2 and -3. Concentrations in the controls decreased ($P < 0.02$) between Day 6 (10.1 ± 1.2 ng/ml) and Day 12 (7.3 ± 1.1 ng/ml).

The number of mares in each PGF group with no response (no progesterone decrease), early luteolysis (progesterone decreased to < 1 ng/ml by Day 12), and progesterone resurgence (decrease followed by an increase) and day of luteolysis are shown (Table 2). Mares with progesterone resurgence in groups PGF-2, -3, and -4 combined had progesterone concentrations on Day 12 of 6.8 ± 0.7 ng/ml ($n = 9$), which were not different from those in control mares (7.3 ± 1.1 ng/ml; $n = 6$). The intermediate mean on Days 3 to 6 in group PGF-2 and on Days 4 to 8 in group PGF-3 (Fig. 1) resulted from four mares with resurgence in each group. The remaining two mares did not respond (group PGF-2) or responded with early luteolysis (group PGF-3). When the four mares with resurgence in each of groups PGF-2 and PGF-3 were compared to the controls, progesterone concentrations showed an interaction between group and day ($P < 0.0001$; Fig. 2). Concentrations were lower ($P < 0.05$) in each PGF group than in the controls on Days 4 to 7 and were similar between the PGF groups on Days 4 to 12. Main effects and the interaction were not significant for percentage change in LH concentrations (Fig. 2).

The main effects of day and the interaction were significant ($P < 0.0001$) for percentage change in LH concentrations from the concentration at Day 0 and for concentrations of FSH (Fig. 1). The days when percentage change in LH and concentrations of FSH in the treated groups were significantly different from the corresponding values in the control mares are shown. On Day 0, the LH increase was greater in the treated group than in the controls, but the FSH increase was not. The difference between the day of treatment and the following day in concentrations of LH and FSH are shown (Table 3). The PGF treatment resulted in an increase within 24 h in concentrations of LH for treatment on Days 0, 3, 4, 5, and 6 and in FSH on Days 0, 2 (approached significance), 3, 4, 5 (approached significance), and 6.

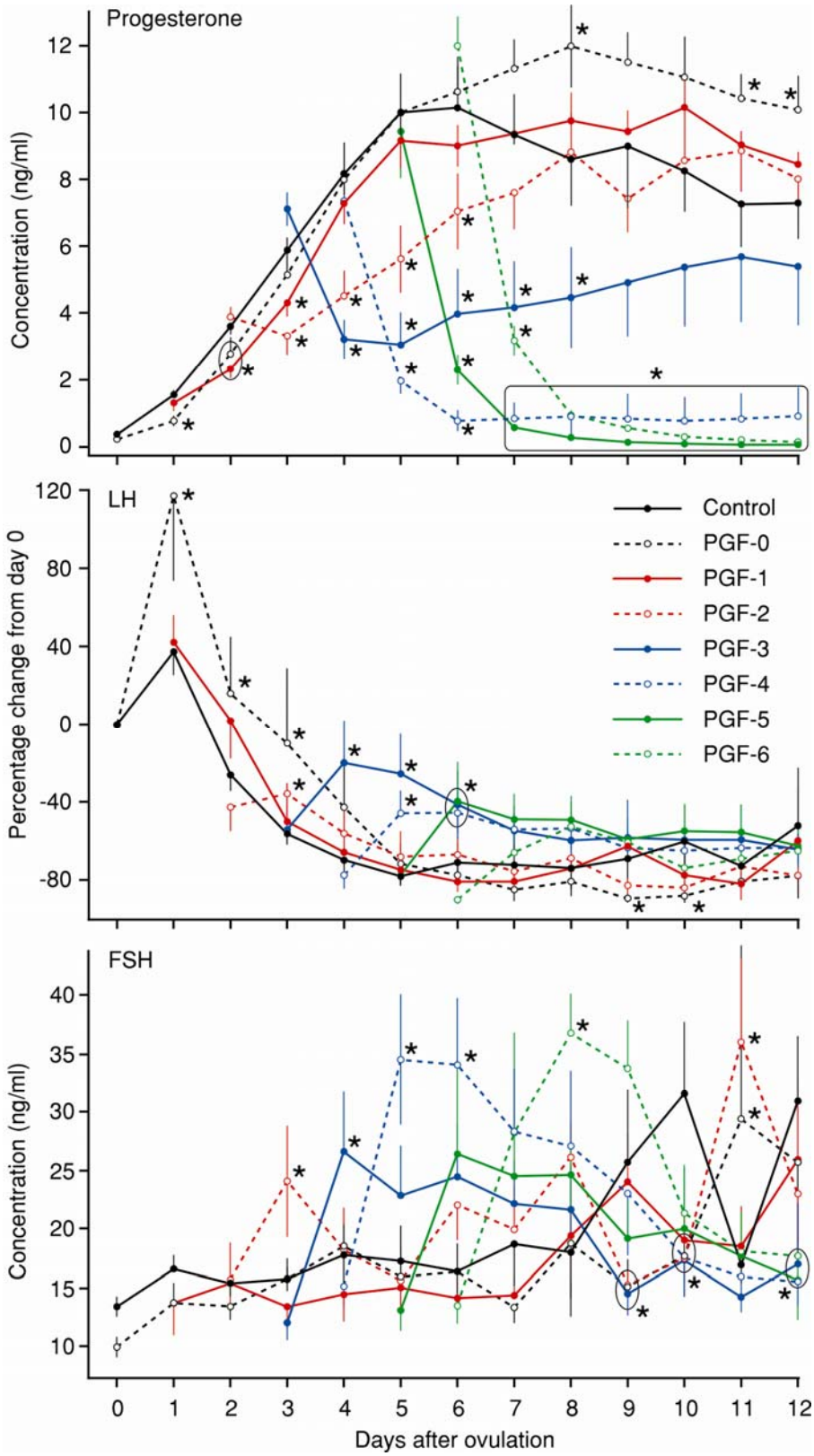

Figure 1. Mean ± S.E.M. for systemic concentrations of progesterone, percentage change in LH concentrations, and concentrations of FSH following a single treatment with PGF on the indicated day (PGF-0 = treatment on Day 0, etc.); n=6 mares/group. Asterisks indicate days on which an individual mean or enclosed means are different ($P < 0.05$) from the mean in the controls. Experiment 1.

Table 1. Effect of PGF on the decrease in progesterone concentrations for mares with a decrease between the day of treatment and the next day. Experiment 1.

End point	Day of treatment				
	2	3	4	5	6
No. with a decrease	4	6	6	6	6
Decrease (ng/ml)	1.3 ± 0.6^{a}	3.9 ± 0.5^{b}	5.4 ± 0.5^{bc}	7.1 ± 1.0^{cd}	8.8 ± 0.9^{d}
Decrease (%)	30.7 ± 10.1^{a}	55.4 ± 7.2^{b}	73.2 ± 4.6^{bc}	76.3 ± 1.4^{c}	73.2 ± 3.5^{bc}

[abcd] Within an endpoint, means with no common superscript letters are different ($P < 0.05$); n = 6 mares/group.

Table 2. Types of progesterone response after a single injection of PGF on the indicated day. Experiment 1.

	Day of treatment				
Progesterone response	2	3	4	5	6
None (No. mares)[w]	2	0	0	0	0
Luteolysis (No. mares)[x]	0	2	5	6	6
Resurgence (No. mares)[y]	4	4	1	0	0
Luteolysis (Day)[z]	- - -	5.0 ± 0.0^{a}	5.8 ± 0.2^{b}	7.0 ± 0.0^{c}	8.5 ± 0.2^{d}

[abcd] Means with different superscripts are different ($P < 0.05$); n = 6 mares/group.
[w] Concentrations similar to controls.
[x] Luteolysis = progesterone decrease to < 1 ng/ml.
[y] Decrease immediately after treatment, followed by an increase to control concentrations by Day 12.
[z] First day (mean ± S.E.M.) for luteolysis in mares with luteolysis ≤ Day 12.

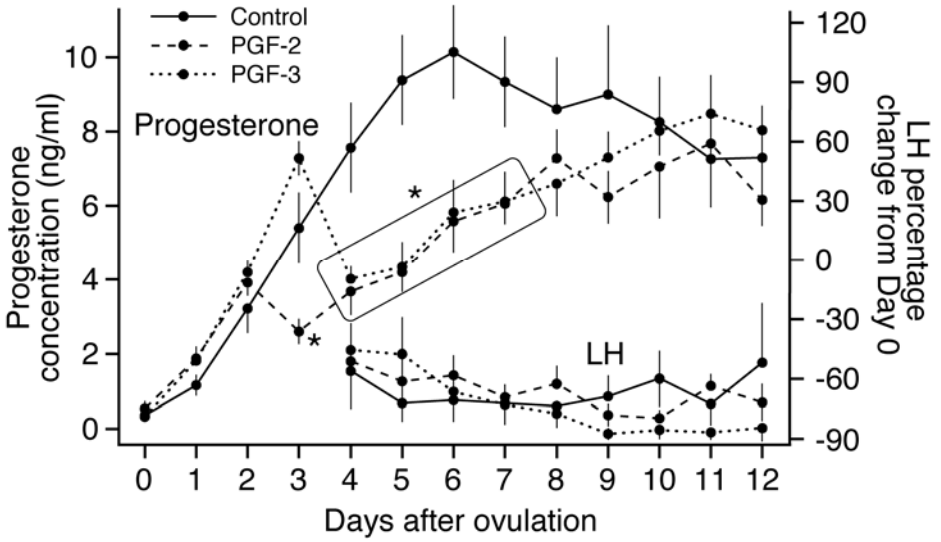

Figure 2. Mean ± S.E.M. for systemic concentrations of progesterone and percentage change in LH concentrations in six controls and in four of six mares in each PGF-treated group with resurgence of progesterone concentrations after an initial decrease. Treatment was on Day 2 (PGF-2) or Day 3 (PGF-3). Asterisks indicate days on which an individual mean or enclosed means are different ($P < 0.05$) from the mean in the controls. Experiment 1.

Experiment 2

The effects of group ($P < 0.003$), day ($P < 0.0001$), and the interaction ($P < 0.0001$) were significant for progesterone concentrations (Fig. 3). In group PGF-0, the concentrations were lower ($P < 0.05$) on Days 1 and 2 than in the controls, but not thereafter. Concentrations were lower ($P < 0.02$) in group PGF-0,1 on Days 2 and 3 than in the controls, but not thereafter. Compared to the controls, concentrations were lower in group PGF-0,1,2 on Days 2 through 12 ($P < 0.04$ to 0.0001) and higher ($P < 0.0001$) on Day 16. In groups PGF-0 and PGF-0,1, the concentrations increased each day in each mare until the maximum concentrations on Days 6 to 10, except in one mare on one day.

Table 3. Means ± S.E.M. for concentrations (ng/ml) of LH and FSH on the day of and the day after a single injection of PGF. Experiment 1.

Treatment day	Hormone	On day of treatment	One day after treatment	Probability
Day 0	LH	10.9 ± 1.4	22.8 ± 5.2	P < 0.03[y]
	FSH	10.0 ± 0.9	13.8 ± 1.7	P < 0.02[z]
Day 1	LH	8.2 ± 2.0	6.2 ± 2.1	NS
	FSH	13.7 ± 2.7	15.4 ± 2.4	NS
Day 2	LH	3.8 ± 1.1	4.1 ± 1.7	NS
	FSH	15.7 ± 3.1	24.1 ± 4.7	P < 0.08
Day 3	LH	4.6 ± 1.7	11.9 ± 5.1	P < 0.05
	FSH	12.1 ± 1.5	26.6 ± 5.1	P < 0.008
Day 4	LH	2.3 ± 0.7	5.6 ± 1.1	P < 0.01
	FSH	15.2 ± 1.6	34.5 ± 5.5	P < 0.01
Day 5	LH	1.0 ± 0.2	3.1 ± 1.0	P < 0.03
	FSH	13.1 ± 1.7	26.4 ± 8.2	P < 0.06
Day 6	LH	0.9 ± 0.1	3.1 ± 1.1	P < 0.04
	FSH	13.5 ± 1.5	28.3 ± 8.5	P < 0.05

[y] Increase greater (P < 0.05) than in controls.
[z] Increase similar to increase in controls.
NS = not significant; n = 6 mares/group.

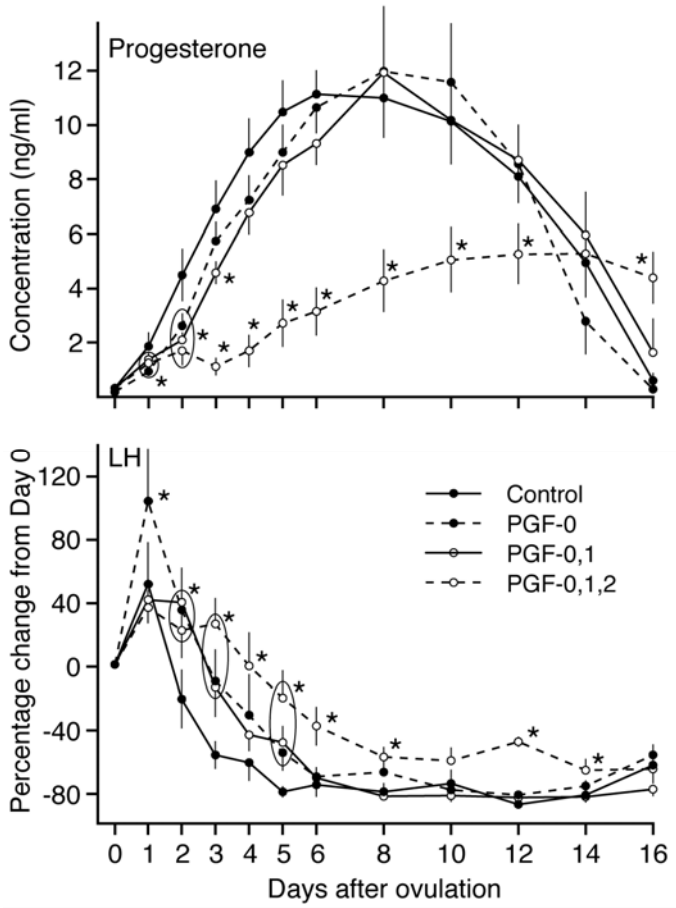

Figure 3. Mean ± S.E.M. for systemic concentrations of progesterone and percentage change in LH concentrations following treatment with PGF on Day 0 (PGF-0) or on Days 0 and 1 (PGF-0,1), or on Days 0, 1, and 2 (PGF-0,1,2); n = 6 mares/group. Asterisks indicate days on which an individual mean or enclosed means are different (P < 0.05) from the mean in the controls. Experiment 2.

Progesterone concentrations were depressed in group PGF-0,1,2 to a mean of < 2 ng/ml through Day 4 (Fig. 3). In three mares in this group, concentrations were consistently > 1 ng/ml beginning on Day 2, whereas in the remaining three mares progesterone did not consistently increase to > 1 ng/ml until Days 5, 6, or 10. In the controls, progesterone concentrations decreased ($P < 0.001$) between Days 6 and 12. Group PGF-0,1,2 differed from each of the other three groups by a longer interovulatory interval, occurrence of luteolysis before Day 16 in fewer mares, and development of a major (largest follicle > 30 mm) secondary follicular wave during early diestrus in more mares (Table 4).

The group effect ($P < 0.004$), day effect ($P < 0.0001$), and the interaction ($P < 0.001$) were significant for percentage change in LH concentrations from Day 0 (Fig. 3). Percentage change was positive and increased in all groups between Days 0 and 1 and was greater ($P < 0.009$) in group PGF-0 than in the other groups. Thereafter, the percentage decreased on the next day in the controls and group PGF-0, but the decrease was delayed for 1 day in group PGF-0,1 and for 2 days in group PGF-0,1,2. The percentage then decreased in parallel for several days so that the percentage was higher ($P < 0.05$) in group PGF-0,1,2 than in the controls for Days 2 through 8. These and other differences among groups are shown (Fig. 3). The difference among groups and the interaction of group and day were not significant for concentrations of FSH; only the day effect was significant ($P < 0.006$; data not shown).

Table 4. Effect of days of treatment with PGF on events during the interovulatory interval. Experiment 2.

	Days of treatment			
End point	None	0	0 and 1	0, 1, and 2
Interovulatory interval (days)	23.8 ± 0.5^{a}	22.0 ± 1.0^{a}	22.7 ± 1.0^{a}	26.4 ± 0.5^{b}
Luteolysis (< 1 ng/ml) by Day 16 (No. mares)	$5/6^{a}$	$6/6^{a}$	$5/6^{a}$	$0/6^{b}$
Major secondary follicular wave (No. mares)	$0/6^{a}$	$0/6^{a}$	$0/6^{a}$	$3/6^{b}$

^{ab} Values with different superscripts within an end point are different ($P < 0.05$). n = 6 mares/group.

Discussion

Progesterone increased in each nontreated mare between Days 0 and 1 (n = 42), 1 and 2 (n = 36), 2 and 3 (n = 30), 3 and 4 (n = 24), and in 15 of 18 mares between Days 4 and 5. Thus, there was a strikingly consistent increase in progesterone output in individual nontreated mares on Days 0 to 4. The decrease in mean progesterone concentrations in control mares between Days 6 and 12 in both experiments confirms a recent report that progesterone slowly declines between the maximum at Day 6 and before the beginning of spontaneous luteolysis on Day 14 (Ginther *et al.*, 2005b).

An increase in progesterone concentration in experiment 1 occurred between Days 0 and 1 and between Days 1 and 2 in each of the 48 mares, whether or not they were treated with PGF. However, the mean 1 day increase after treatment on Day 0 (group PGF-0) or Day 1 (group PGF-1) was less than for the nontreated mares. That is, the corpus luteum was not entirely refractory or resistant to the negative effect of an injection of PGF, even on the day of first formation or ovulation. The effect on progesterone was detectable for 2 days after treatment in both groups, but by Day 4 recovery was complete in that there was no longer a difference from controls. The slower progesterone increase was confirmed in experiment 2 in groups PGF-0 and PGF-0,1 and by the response to treatment on Days 0 and 1 in group PGF-0,1,2. An increase in progesterone concentrations, but at a reduced rate, following PGF treatment on Day 0 or 1 is apparently a novel finding for any species.

A progesterone decrease, on the day after treatment, occurred in most (67%) mares in group PGF-2 and in each mare in group PGF-3. The mean progesterone decrease by the day after treatment was followed by a similar pattern of resurgence in four of six mares in each of the two groups. Progesterone concentrations were lower than in controls beginning at the day after treatment. Thus, the transient retarding effect on progesterone concentrations in mares treated with PGF on Day 0 or Day 1 or on Days 0 and 1 was replaced by transient regression with resurgence in most mares when PGF was given on Days 2 or 3. The two mares per group that did not have resurgence showed no response to PGF (group PGF-2) or had complete luteolysis (group PGF-3). The mean progesterone decrease, followed by resurgence for the mares given PGF on Day 2 is consistent with means for a previous study (Troedsson *et al.*, 2001), although the variation in response among individuals was not reported. The percentage of mares (67%) with resurgence versus a continuous decrease in progesterone concentrations when treated with PGF on Day 3 agrees with the results (75%) of a recent report (Bergfelt *et al.*, 2006). In groups PGF-4, -5, and -6, a decrease in progesterone occurred in each of the 18 mares by the day after treatment. In each mare, the decrease continued with luteolysis occurring, on average, 2 days after treatment, except for one mare with resurgence in group PGF-4. The results for treatment on Days 5 and 6 agree with

numerous studies on the luteolytic effectiveness of exogenous PGF after the corpus luteum is mature (see Introduction). The present results also indicated that the progesterone decrease on the day after treatment on Days 4, 5, or 6 was consistent for each of the 18 mares.

Studies in cattle indicated that lack of responsiveness to PGF by the early corpus luteum was not attributable to a deficiency in PGF receptors (Wiltbank *et al*., 1995) and may result from specific changes in gene expression that prevents intraluteal PGF production (Tsai and Wiltbank, 1998). In this regard, expression of mRNA for cytokines that are associated with luteolysis increased when PGF was given during mid-cycle but not when given early in the cycle (Levy *et al*., 2000; Neuvians *et al*., 2004). Other studies have related the decreased luteal responsiveness to PGF to immaturity of the luteal vasculature; a proteinaceous vasoconstrictor is produced by endothelial cells in response to PGF (Girsh *et al*., 1996). These studies in cattle on the mechanism of refractoriness of the early corpus luteum to PGF have not been conclusive and similar studies have not been done in mares. The results of the present studies may provide a basis for extending this research area to horses. Consideration can be given to the changing progesterone response to exogenous PGF over Days 0 to 6, as indicated by a retarding but not a regressing effect for treatment on Day 0 or 1, a transient regressing effect followed by resurgence for most mares treated on Day 2 or 3, and complete luteolysis for almost all mares treated on Day 4, 5, or 6.

The mean progesterone concentrations in group PGF-0,1,2 of experiment 2 did not reach control levels until the day of a decrease in concentrations in the controls. However, in four of the six individuals the progesterone profile was similar to the profile in the mares with resurgence in groups PGF-2 and -3. That is, concentrations decreased on the first day after the last treatment and then resurged to the level in controls. In the remaining two mares, resurgence was delayed in that concentrations remained at < l ng/ml until Days 6 and 10 and then increased but did not reach control levels. Thus, the progesterone maintenance at < 1 ng/ml did not involve luteolysis, but rather interference with the development of progesterone secretion capabilities; the cells remained viable.

The higher mean progesterone concentration at Day 16 in group PGF-0,1,2 than for the other three groups reflected a consistently higher concentration in each individual than for any mare in the other three groups, with one exception. This near consistent result likely was a consequence of the delay in the post-ovulatory progesterone increase. A previous study has shown that exposure to progesterone for at least 14 days is needed to prime the endometrium for secretion of endogenous PGF (Zavy *et al*., 1984). In this regard, the 3-day increase in length of the interovulatory interval in this group, compared to the controls, corresponds to the 3-day delay in the increase in progesterone concentrations. Group PGF-0,1,2 is also the only group that had mares with a secondary major wave during early diestrus. This likely resulted from the reduced progesterone concentrations over Days 0 to 12 and the associated higher LH concentrations. The positive role of LH in growth of large follicles has been reviewed (Ginther *et al*., 2004).

The peak of the preovulatory LH surge occurred on the day after ovulation as expected (Ginther, 1992). The peak on Day 1 was greater in mares treated with PGF on Day 0 than in nontreated mares. The greater peak on Day 1 in the treated mares does not seem attributable to reduced concentrations of progesterone, considering that the progesterone concentrations were only slightly affected. After the Day 1 peak, LH concentrations decreased in parallel in the nontreated and Day 0 treated mares until reaching similar concentrations on Day 5 (experiment 1) or 6 (experiment 2). In groups PGF-0,1 and PGF-0,1,2, a decrease in LH did not occur until the day after the last treatment. In group PGF-0,1,2, progesterone concentrations were suppressed to < 2 ng/ml through Day 4, and the first significant decrease in LH occurred between Days 3 and 5. Although these data are consistent with a recent report (Ginther *et al*., 2005b) that the decrease in LH after the peak of the ovulatory LH surge results from the increasing concentrations of progesterone, a negative effect of progesterone on LH (Gastal *et al*., 1999) was obscured by an apparent direct effect of the PGF on LH. Study of the effect of altered progesterone concentrations on LH during diestrus was not attempted, owing to likely confounding between a potential negative effect of progesterone on LH versus a direct stimulatory effect of PGF on LH at the pituitary level.

An increase in LH concentrations following PGF treatment was detected for all groups, except for treatment on Days 1 and 2, when pituitary LH reserves likely were undergoing depletion. The increase in FSH concentrations in the PGF-0 group is not attributable to treatment; a similar increase occurred in controls. The lack of a treatment effect on FSH in groups PGF-0 and PGF-1 in experiment 1 is consistent with lack of a treatment effect in experiment 2. Differences in pituitary content may account for the lack of an LH and FSH response during the post-ovulatory period. Concentrations of LH and FSH in the equine pituitary are high and low, respectively, during estrus (Silva *et al*., 1986). After the post-ovulatory period (Days 0 to 2), PGF stimulated within 24 h an increase in FSH, as well as LH, concentrations, consistent with a report that the LH and FSH responses to GnRH treatment in mares were greater on Days 4 and 7 than on Day 1 (Johnson *et al*., 2002). In this regard, a direct stimulatory effect of a PGF analog on the pituitary release of LH and FSH has been reported for mares with base-line concentrations of progesterone (Jöchle *et al*., 1987; see Introduction). In the apparent absence of reports documenting an effect

of progesterone on FSH (Ginther, 1992; Gastal *et al*., 1999; 2000), a direct effect of PGF on FSH through the pituitary seems likely in the present studies and increases the likelihood that the effect of PGF on LH within 24 h was also directly at the hypothalamo-pituitary level. It is therefore concluded that the effects of PGF on LH and FSH were exerted directly at the hypothalamo-pituitary area.

During luteolysis at the end of diestrus, LH increases gradually after endogenous PGF reduces the progesterone concentrations to < 2 ng/ml (Ginther *et al*., 2005b). In experiment 1, however, exogenous PGF given on Days 4, 5 or 6 caused an LH increase within 24 h, followed by a gradual decrease over 3 or 4 days. Similar abrupt and transient LH increases during the time of luteolysis have not been reported in nontreated mares. Given that such transient surges could be masked when mean concentrations are used, we examined the profiles in individuals from several past experiments and found no indication of a similar phenomena. Thus, the LH response to exogenous PGF did not seem to simulate the LH response to endogenous PGF, even though the progesterone response was simulated.

A mean FSH surge in controls encompassed Days 8 to 11 in agreement with the relationships between an FSH surge and the initiation of the ovulatory follicular wave (for review see Ginther *et al*., 2004). The apparent discharge of some pituitary FSH content in association with PGF treatment on various days may account for the irregularities in surges toward the end of the experimental period.

In conclusion, based on systemic concentrations of progesterone, responsiveness of the early corpus luteum to a single treatment with PGF increased progressively with time. Increasing responsiveness was shown by a retarding but not regressing effect for treatment on Day 0 or 1, a transient regressing effect followed by resurgence for most mares treated on Day 2 or 3, and complete luteolysis for almost all mares treated on Day 4, 5, or 6. Daily treatment on Days 0, 1, and 2 suppressed the progesterone concentrations through Day 3; concentrations increased thereafter but did not reach control concentrations until Day 14. However, concentrations were higher on Day 16, the interovulatory interval was longer, and the number of mares with a major secondary follicular wave during early diestrus was greater. These results were attributed to low initial progesterone concentrations for several days and thereby a delay in a progesterone priming effect for spontaneous luteolysis. Concentrations of LH increased by the day after PGF treatment on Day 0, 3, 4, 5, or 6 and remained elevated above control concentrations for a few days. The lack of a significant increase in LH concentrations after treatment on Day 1 or Day 2 seemed related to the rapidly declining concentrations after the Day 1 peak of the ovulatory surge. FSH concentrations did not increase following PGF treatment on Day 0, 1, or 2 but did on each day thereafter. The LH and FSH responses to PGF on various days are consistent with reported days of high or low pituitary concentrations and maximal or minimal responses to GnRH.

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