Progesterone, LH, Estrus and Ovulation after Prostaglandin $F_{2\alpha}$ in Heifers[1] (37274)

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General relationships among hypothalamic, pituitary and ovarian hormones have been defined during the estrous cycle in several species, including the bovine. However, the rapid loss of luteal function during 2 or 3 days before estrus was not explained satisfactorily. Anderson *et al.* (1) reviewed the persuasive evidence that the uterus regulates luteal function by elaboration of a luteolysin. Prostaglandin $F_{2\alpha}$ has properties in sheep similar to those expected of uterine luteolysin; within 24 hr after infusion into the ovarian artery, serum progesterone fell to undetectable values, serum estrogen increased, an ovulatory surge of LH occurred and the ewes exhibited behavioral estrus (2–5). The objective of the present investigation was to determine whether prostaglandin $F_{2\alpha}$ was luteolytic in cattle.

Materials and Methods. Thirty mg prostaglandin $F_{2\alpha}$ Tham salt[2] ($PGF_{2\alpha}$) in 1.5 ml saline was injected im into each of five heifers during diestrus (8–14 days after estrus) and into six heifers during metestrus (3 days after estrus). In another six heifers during diestrus (10–14 days after estrus), 30 mg $PGF_{2\alpha}$ in 1.5 ml saline was deposited in the vagina adjacent to the cervix. The heifers were observed twice daily for signs of behavioral estrus, and corpus luteum diameter and ovulation were monitored by daily palpation. Heifers were bled by jugular puncture at 12-hr intervals until onset of estrus and on Days 2, 4, 7, and 11 after the estrus induced by $PGF_{2\alpha}$, or at 12-hr intervals for 5 days if estrus was not induced by $PGF_{2\alpha}$.

Luteinizing hormone (LH) was determined by double antibody radioimmunoassay[3] (6). Progesterone was determined by radioimmunoassay similar to those for testosterone (7) and for progesterone (8). Aliquants (100 μl) of each unknown were placed in three 15 $\times$ 85 mm disposable culture tubes and about 3000 dpm ^{3}H-progesterone (80–100 Ci/mmole) was added to one of the tubes to estimate procedural losses. Each tube was vortex-mixed with 2 ml benzene–hexane (1:2) for 30 sec, then stored at —20° for at least 1 hr to freeze the aqueous phase. The organic solvent from the tube with ^{3}H-progesterone was decanted into a scintillation vial, and the solvent in the other two extraction tubes was decanted into 10 $\times$ 75 mm disposable culture tubes for radioimmunoassay as follows. The organic solvent was evaporated and 200 μl antibody[4] (diluted 1:4500 in 1:400 normal rabbit serum in 0.1 *M* phosphate-buffered saline, pH 7.1) was added. Two sets of standard tubes containing 0, 25, 50, 100, 200, 500, 1000, 1500, and 2000 pg progesterone were included in each assay and treated similarly to the unknowns. After addition of antibody, each tube was vortexed

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

[3] Luteinizing hormone (B5) standard was supplied by the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, MD. Dr. L. E. Reichert (Emory Univ.) generously supplied highly purified LH (LER-1072-2) for iodination in the radioimmunoassay.

[4] The rabbit antiprogesterone prepared against 6 β-succinylprogesterone conjugated to bovine serum albumin was supplied by Dr. G. D. Niswender, Colorado State Univ.

10 sec and incubated 30 min at room temperature. Then about 24,000 cpm ^{3}H-progesterone (1,2,6,7,^{3}H-progesterone, 80–100 Ci/mmole diluted in 200 μl 0.1% Knox gelatin in 0.1 *M* phosphate-buffered saline, pH 7.1) was added to each tube, and the tubes were vortexed 10 sec and incubated at 4° for 4–20 hr. To separate free from antibody-bound progesterone, 0.5 ml of dextran-coated charcoal (0.5 g neutral Norit and 1 g Dextran T-70 in 100 ml distilled water) was added at 4°, and each tube was vortexed for 10 sec, equilibrated at 4° for 10 min and centrifuged (2500*g*) for 10 min at 5°. Antibody bound ^{3}H-progesterone in 0.5 ml of the supernatant fluid was measured in a liquid scintillation spectrometer. Preliminary experiments revealed little variance in procedural losses (80 ± 1% extraction efficiency, $n = 22$); hence the mass of progesterone determined in each unknown was corrected for the average loss of tracer. The sensitivity of this assay was less than 25 pg progesterone; this amount displaced 22 ± 6% of the ^{3}H-progesterone bound to antibody.

We determined progesterone in aliquants of 12 cow sera by our assay and by the radioimmunoassay described by Kittok and Britt (9); results from the two methods were highly correlated ($r = 0.94$), and the means did not differ significantly (2.13 *vs* 2.17 ng/ml). However, the within-sample coefficient of variation for the 12 progesterone determinations by our method was 8%, less than the 36% for the other assay. Among various steroids tested, Niswender (10) reported that this antiserum cross reacted significantly only with 5α-pregnan-3,20-dione to the extent of 30%.

Results and Discussion. After im $PGF_{2\alpha}$ administered during diestrus, blood serum progesterone fell from 4.0 ng/ml about 60% within 12 hr and to 0.8 ng/ml at 24 hr, and remained low for 72 hr (Table I). In contrast, im $PGF_{2\alpha}$ on Day 3 of the cycle had no apparent effect on corpus luteum function because progesterone increased continuously (Table I) as expected of untreated cattle at this stage of the cycle (11). The duration of the cycle during which $PGF_{2\alpha}$ was given on Day 3 was 19 ± 1 days, near that expected in untreated heifers. Among the six heifers given $PGF_{2\alpha}$ intravaginally, one apparently failed to respond; her blood serum progesterone was 3.3 ng/ml at the time of treatment with $PGF_{2\alpha}$ and averaged 3.4 ± 0.5 ng/ml during the next 5 days. The duration of the cycle for this heifer was 19 days; hence she is omitted from further discussion. In the other five heifers treated intravaginally, progesterone dropped an average of 47% within 12 hr and continuously for 72 hr (Table I). The decline in blood progesterone after intravaginal $PGF_{2\alpha}$ appeared slightly retarded relative to that after im $PGF_{2\alpha}$ during diestrus, but the difference was not significant.

TABLE I. Blood Serum Progesterone After Intravaginal or Intramuscular $PGF_{2\alpha}$ (30 mg Tham Salt) in Heifers.

	Site of $PGF_{2\alpha}$		
	Intramuscular		
Hr after $PGF_{2\alpha}$	Diestrus[a]	Metestrus[b]	Vaginal diestrus[a,c]
		(ng/ml)	
0	4.0 ± 0.4	0.6 ± 0.1	4.6 ± 0.4
12	1.5 ± 0.2	0.6 ± 0.1	2.4 ± 0.3
24	0.8 ± 0.1	0.9 ± 0.3	1.5 ± 0.4
48	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.4
72	1.0 ± 0.2	1.3 ± 0.3	0.6 ± 0.1
120	0.5 ± 0.1	2.1 ± 0.2	0.6 ± 0.1
Day 11 of next cycle	5.0 ± 0.5	3.7 ± 0.6	3.9 ± 0.6

[a] Five heifers.
[b] Six heifers.
[c] A sixth heifer did not respond.

TABLE II. Corpus Luteum Diameter After Intravaginal or Intramuscular $PGF_{2\alpha}$ (30 mg Tham Salt) During Diestrus or Metestrus in Heifers.

Days after $PGF_{2\alpha}$	Site of $PGF_{2\alpha}$		
	Intramuscular		
	Diestrus[a]	Metestrus[b]	Vaginal diestrus[a,c]
		(cm)	
0	2.3 ± 0.1	TS	2.5 ± 0.1
1	1.8 ± 0.1	TS	2.2 ± 0.1
2	1.2 ± 0.1	1.4 ± 0.2	1.5 ± 0.2
3	0.6 ± 0.3	—	0.6 ± 0.4
5	TS	1.8 ± 0.2	TS[d]
8	TS	2.4 ± 0.1	TS

[a] Five heifers.
[b] Six heifers.
[c] A sixth heifer did not respond.
[d] Too small for precise estimation.

Corpus luteum diameter declined continuously during the 72 hr after $PGF_{2\alpha}$ during diestrus (Table II), in agreement with changes in blood progesterone. The decrease was slightly but not significantly faster after im $PGF_{2\alpha}$ than after intravaginal $PGF_{2\alpha}$. Luteal diameter was too small for accurate assessment when $PGF_{2\alpha}$ was given on Day 3 of the cycle; however, it increased to 1.4 and 1.8 cm at 2 and 5 days later, and to 2.4 cm on Day 11 of that estrous cycle (Table II).

All five heifers given $PGF_{2\alpha}$ im on Day 11 of the estrous cycle exhibited estrus beginning about 3 days later (Table III), and all five ovulated about 1 day after onset of estrus. After intravaginal $PGF_{2\alpha}$ on Day 11, the onset of estrus was retarded ($p \cong 0.08$) and more variable relative to that after im $PGF_{2\alpha}$. Similarly, the interval from $PGF_{2\alpha}$ to LH peak was longer ($p < 0.01$) after intravaginal than after im $PGF_{2\alpha}$, but the comparable difference in intervals from administration of $PGF_{2\alpha}$ to ovulation was not significant. Blood serum LH remained near values typical of diestrus until 12 hr before estrus in heifers given $PGF_{2\alpha}$ on Day 11 (Table IV). Then we observed elevated LH representing portions of the LH surge which normally persists about 6–8 hr near the onset of estrus in cows (12, 13). The LH surge was missed in one heifer given $PGF_{2\alpha}$ im on Day 11, probably due to the 12-hr bleeding intervals.

We conclude that 30 mg $PGF_{2\alpha}$ administered im is luteolytic during diestrus in heifers, but we found no luteolysis or luteostasis on Day 3 after estrus. In a preliminary report, Rowson *et al.* (14) concluded that $PGF_{2\alpha}$ administered into the uterus was not an effective luteolysin from Days 1–4 of the cycle. The declines in luteal diameter and blood serum progesterone, and the intervals to onset of estrus and LH surge after im $PGF_{2\alpha}$ on Day 11 resembled those which normally begin about 3 days before estrus in heifers (13). Perhaps the intravaginal $PGF_{2\alpha}$ was not absorbed as rapidly or as completely as im $PGF_{2\alpha}$ because luteolysis was delayed about 1 day and considerably more variable than after im $PGF_{2\alpha}$, and intravaginal $PGF_{2\alpha}$

TABLE III. Intervals to Onset of Estrus, Peak LH and Ovulation After Intravaginal or Intramuscular $PGF_{2\alpha}$ (30 mg Tham Salt) During Diestrus in Heifers.

Interval from $PGF_{2\alpha}$ to	Site of $PGF_{2\alpha}$	
	Intramuscular[a]	Vaginal[a,b]
	(hours)	
Onset estrus	74 ± 3	117 ± 18
Peak LH	64 ± 4[c]	128 ± 19
Ovulation	104 ± 6	138 ± 20

[a] Five heifers.
[b] A sixth heifer did not respond.
[c] Four heifers.

TABLE IV. Blood Serum LH After Intravaginal or Intramuscular $PGF_{2\alpha}$ (30 mg Tham Salt) During Diestrus in Heifers.

Time after $PGF_{2\alpha}$	Site of $PGF_{2\alpha}$	
	Intramuscular[a]	Vaginal[a,b]
	(ng/ml)	
From $PGF_{2\alpha}$ to LH surge	0.6 ± 0.1	0.7 ± 0.1
At LH peak	4.1 ± 1.2[c]	5.8 ± 2.0
Subsequent diestrus	0.5 ± 0.1	0.7 ± 0.1

[a] Five heifers.
[b] A sixth heifer did not respond.
[c] Four heifers.

apparently was not luteolytic in one heifer.

Summary. $PGF_{2\alpha}$ (30 mg Tham salt) was injected im into five heifers during diestrus (Days 9 to 13 of the estrous cycle), im into six heifers during metestrus (Day 3), and intravaginally into six heifers during diestrus. After im $PGF_{2\alpha}$ during diestrus, (a) luteal diameter decreased from 2.3 ± 0.1 cm to 1.8 ± 0.1 cm at 24 hr and to 0.6 ± 0.3 cm at 72 hr, (b) blood serum progesterone fell from 4.0 ± 0.4 ng/ml to 1.5 ± 0.2 ng/ml at 12 hr and to 1.0 ± 0.2 ng/ml at 72 hr, and (c) estrus began at 74 ± 3 hr and ovulation occurred at 104 ± 6 hr. After intravaginal $PGF_{2\alpha}$ during diestrus, one heifer failed to respond; the other five responded similarly to those given im $PGF_{2\alpha}$ during diestrus except that luteolysis was more variable and delayed about 1 or 2 days compared to that after im $PGF_{2\alpha}$. $PGF_{2\alpha}$ (im) was neither luteolytic nor luteostatic in metestrus. We conclude that 30 mg $PGF_{2\alpha}$ is luteolytic during diestrus in heifers, but im administration is more effective than intravaginal administration.

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