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Gonadotropin Releasing Hormone Induced Luteinizing Hormone Release after Prostaglandin $F_{2\alpha}$ in Heifers (39148)[1]

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The effectiveness of gonadotropin releasing hormone (GnRH) in increasing serum LH in sheep and cattle is influenced by the gonadal hormone environment (1). Thus, Zolman *et al.* (2) reported that the magnitude of increase in serum LH induced by GnRH was positively correlated with serum estradiol and estrone concentrations. In addition, the quantity of LH released by a standard dose of GnRH was increased in ovariectomized heifers given exogenous estradiol relative to controls (3). These results suggested to us that pituitary sensitivity to GnRH may be increased by the abrupt changes in serum progesterone and estrogen that precede the preovulatory surge of LH in cattle (4). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes regression of the corpus luteum in cattle (5, 6), which results in a marked reduction in serum progesterone, then an increase in serum estrogens. It was our objective to determine whether the magnitude of LH release by GnRH changed with the changing steroid environment caused by $PGF_{2\alpha}$ induced luteal regression. A secondary objective was to estimate time of estrus, endogenous LH release, and ovulation in these heifers.

Materials and methods. Animals. Twenty Holstein heifers, ranging in age from 10 to 14 months, were selected for treatment on the basis of having a palpable corpus luteum. All heifers were between Days 7 and 10 of an estrous cycle, with day of estrus taken as Day 0. Ten days prior to beginning this experiment, 17 of these heifers had been treated with 30 mg of $PGF_{2\alpha}$ to synchronize estrus.[4] All heifers received 30 mg of $PGF_{2\alpha}$ intramuscularly at time 0, then each of five heifers received either: (1) no GnRH;[5] (2) GnRH at 10 hr; (3) GnRH at 30 hr; or (4) GnRH at 50 hr after $PGF_{2\alpha}$. The GnRH (40 μg) was administered intramuscularly.

Blood sampling. Relative to $PGF_{2\alpha}$ treatment at time 0, jugular blood was collected from each heifer via cannulae at 12 hr before and immediately before $PGF_{2\alpha}$ then at 8-hr intervals until 48 hr after $PGF_{2\alpha}$. Frequency of blood sampling was increased to every 3 hr beginning at 50 hr and was continued until 101 hr in order to augment chances of detecting spontaneously occurring LH peaks, i.e., those not associated with GnRH treatments. Jugular blood was also collected at 10 min before and at 5, 10, 15, 25, 30, 45, 60, 90, 120, 180, and 240 min after each GnRH treatment. Steroid hormones were measured in serum samples collected at 8-hr intervals from $PGF_{2\alpha}$ treatment at time 0 to 48 hr and in samples taken at 10 min before GnRH. Beginning 8 hr after $PGF_{2\alpha}$, ovaries of all heifers were palpated per rectum at 8-hr intervals until 120 hr in an attempt to estimate time of ovulation. In addition, ovaries of each heifer were palpated and a coccygeal venous or arterial blood sample collected at 10 or 11 days following $PGF_{2\alpha}$ treatment.

Hormone assay. Serum LH was quantified in all serum samples by double antibody radioimmunoassay. Methods for ra-

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[4] $PGF_{2\alpha}$ Tham (Trimethamine) Salt (WM-475.6) was kindly provided by Dr. J. W. Lauderdale, Upjohn Co., Kalamazoo, Michigan 49001.

[5] GnRH was kindly supplied by Dr. R. Rippel, Abbott Laboratories, North Chicago, Illinois 60064.

dioimmunoassay of LH were as previously described from our laboratory (7) except the antiserum was raised against NIH-LH-B8.[6] The antiserum used is characterized in Fig. 1. The useful range of assay sensitivity for measuring bovine LH was between 0.1 and 4 ng. A dose response curve for pooled bovine sera was paralled ($P < 0.01$) to the LH standard curve and known quantities of NIH-LH-B8 added to bovine sera were quantitatively recovered (94%). Bovine growth hormone (NIH-GH-B17), follicle stimulating hormone (NIH-FSH-B1), and prolactin (NIH-PRL-B3) caused negligible reductions in binding of labeled LH. NIH-TSH-B17 was as effective as LH in displacing ^{125}I-labeled LH yet highly purified bovine TSH[7] displaced less than 0.2% of the label. Serum progesterone (5) and estradiol (6) were measured by radioimmunoassay. Data were analyzed by split plot analysis of variance (8) and selected contrasts were compared using Scheffe's procedure (9).

Results and discussion. Serum estradiol and progesterone. One heifer, assigned to receive GnRH at 30 hr after $PGF_{2\alpha}$, did not have a functional corpus luteum at the time $PGF_{2\alpha}$ was administered. Results from this heifer were not included in summarization of the data. Serum estradiol and progesterone concentrations during 48 hr following $PGF_{2\alpha}$ are shown in Fig. 2. Serum progesterone (ng/ml) decreased ($P < 0.01$) from an overall average of 3.7 at time 0 to less than 1.0 at 24 hr after $PGF_{2\alpha}$ treatment and this decrease was not different ($P > 0.05$) among treatment groups. Decreased serum progesterone following $PGF_{2\alpha}$ treatment of cattle during diestrous has been reported by others (5, 6). Serum estradiol concentration for all heifers at time zero averaged 3.3 ± 3 pg/ml ($n = 19$). In control heifers and in heifers receiving GnRH at 50 hr after $PGF_{2\alpha}$, serum estradiol increased gradually to 8.7 ± 1.4 and 10.8 ± 0.7 pg/ml at 48 hr, respectively. Differences between these groups were not significant ($P > 0.05$). But

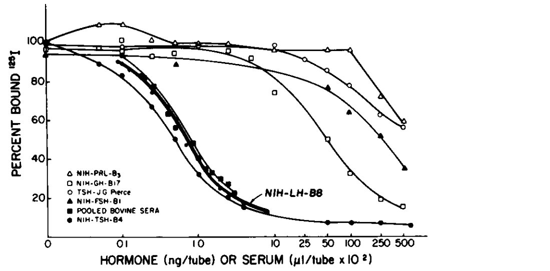

FIG. 1. Displacement of ^{125}I-labeled LH from Guinea pig anti-bovine LH sera by NIH-LH-B_8, pooled bovine sera, and other pituitary hormones indicated.

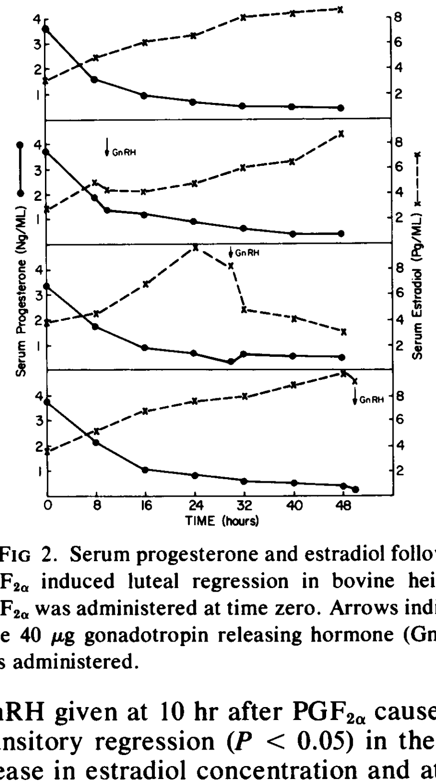

FIG 2. Serum progesterone and estradiol following $PGF_{2\alpha}$ induced luteal regression in bovine heifers. $PGF_{2\alpha}$ was administered at time zero. Arrows indicate time 40 µg gonadotropin releasing hormone (GnRH) was administered.

GnRH given at 10 hr after $PGF_{2\alpha}$ caused a transitory regression ($P < 0.05$) in the increase in estradiol concentration and at 30 hr GnRH caused a reduction ($P < 0.01$) in serum estradiol concentration that persisted throughout the sampling period. We previously reported that GnRH reduced serum estradiol concentrations of heifers when given on Day 20, but not 15, of an estrous cycle (2). Decreased serum estradiol after GnRH may be caused by increased serum LH concentrations resulting from GnRH treatment. Estrogen production by ovarian follicles of rats (10) and heifers (4) declines abruptly, after the preovulatory surge of LH. At the times when

[6] Pituitary hormone preparations were supplied by the Endocrinology Study Section, National Institutes of Health.

[7] Supplied by Dr. J. G. Pierce, Department of Biological Chemistry, UCLA School of Medicine, UCLA, Los Angeles, California 90024.

GnRH was given (10, 30, or 50 hr after $PGF_{2\alpha}$) serum estradiol (pg/ml) and progesterone (ng/ml) averaged 4.1 ± 0.8 and 1.4 ± 0.2, 8.0 ± 1.1 and 0.3 ± 0.1, and 9.4 ± 1.3 and 0.2 ± 0.1, respectively. Thus, gradations of estrogen–progesterone concentrations and ratios were achieved by this experimental design.

Serum LH concentrations. Serum LH concentrations before and after GnRH treatment at 10, 30, and 50 hr after $PGF_{2\alpha}$ are shown in Fig. 3. Average serum LH concentrations were increased ($P < 0.01$) by GnRH in all groups. But, magnitude of increase, as measured by area under the LH response curve (ng ml^{-1} min) was less ($P < 0.01$) at 10 hr than at 30 or 50 hr after $PGF_{2\alpha}$ (38 ± 7 vs 162 ± 32 and 274 ± 132). The large standard error of the mean at 50 hr was attributable to one heifer that had an LH response of 798 ng ml^{-1} min. We previously reported that the LH response of GnRH increases with time after estradiol in ovariectomized heifers (3). Apparently, the ability of the pituitary to release LH in response to GnRH also increases with time after $PGF_{2\alpha}$ induced luteal regression, but whether increased pituitary sensitivity to GnRH is due to decrease serum progesterone, increased serum estradiol, or a combination of these factors cannot be determined from these data. We previously reported that the quantity of LH released by GnRH in heifers was positively correlated with serum estradiol and estrone but not progesterone concentrations at the time GnRH was administered (2). The fact that pituitary sensitivity to GnRH increases with time may reflect changes in the anterior pituitary that require *de novo* protein synthesis as has been described previously for rats (12).

A transient increase in average serum LH concentration was observed for all groups of heifers following $PGF_{2\alpha}$. Serum LH (ng/ml) averaged 0.9 ± 0.2 at time zero and was increased ($P < 0.01$) to 2.3 ± 0.4 at 8 hr after $PGF_{2\alpha}$ treatment. These data are in agreement with a previous report from our laboratory describing an increase in serum LH concentration at 1.5 to 6 hr after $PGF_{2\alpha}$ administration to cows (13). In addition, Carlson *et al.* (14) reported increased

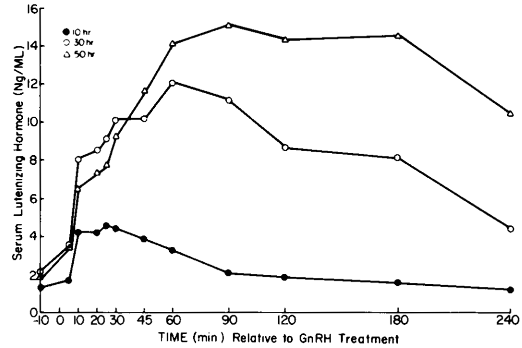

FIG. 3. Serum LH concentration prior to and following 40 μg gonadotropin releasing hormone (GnRH) treatment at 10, 30, or 50 hr after $PGF_{2\alpha}$ induced luteal regression.

serum LH concentrations in jugular blood of ewes at 30 and 60 min after an intracarotid injection of $PGF_{2\alpha}$.

Estrus, ovulation, and spontaneous LH peaks.[8] Occurrence of estrus, ovulation, and spontaneous LH peaks in these heifers are summarized in Table I. All heifers in the group not injected with GnRH and in groups receiving GnRH at 10 or 50 hr after $PGF_{2\alpha}$ exhibited behavioral estrus. In contrast, heifers treated with GnRH at 30 hr after $PGF_{2\alpha}$ did not exhibit estrus behavior. The premature decrease in estradiol production, caused by GnRH given 30 hr after $PGF_{2\alpha}$ (Fig. 2), may have restricted estradiol concentration or period of exposure below that needed for manifestation of estrus. Similar to our results, others (13) have reported that GnRH inhibited the behavioral estrus which normally occurs in heifers after removal of progesterone pessaries. In the present experiment, occurrence of estrus was delayed ($P < 0.05$) in heifers receiving GnRH at 10 hr relative to that of controls. This delay in time of estrus may have resulted from interruption of follicular steroidogenesis by GnRH at 10 hr and reflect additional time required to restore serum estrogen levels or follicular development to that comparable with controls.

Ovulation was confirmed in all control

[8] Spontaneous LH peak-defined as an increase in serum LH greater than 5 ng/ml which occurred independently of GnRH treatments.

TABLE 1. Estrus, Ovulation, and Spontaneous Luteinizing Hormone (LH) Release in Heifers Treated with Gonadotropin Releasing Hormone (GnRH) following Luteolysis with Prostaglandin $F_{2\alpha}$.[a]

Item		Time[b] of GnRH injection (hr)			
		Not injected	10	30	50
Estrus	Number of heifers	5/5	5/5	0/4	5/5
	Time (hr)[b]	61 ± 4	82 ± 8[c]	—	66 ± 8
Spontaneous	Number of heifers	5/5	4/5	0/4	3/5
LH Peaks	Time (hr)[b]	64 ± 4	75 ± 6[d]	—	72 ± 12
	Magnitude (ng/ml)	21 ± 4	31 ± 12	—	15 ± 3
Ovulation[e]	Number of heifers	5/5	4/5[d]	3/4	4/5
	Time (hr)	85 ± 5	96 ± 9	63 ± 3[f]	72 ± 2[f]

[a] $PGF_{2\alpha}$ (30 mg Tham salt) and GnRH (40 μg).
[b] Relative to treatment with $PGF_{2\alpha}$ at time zero.
[c] Significantly longer than controls ($P < 0.05$).
[d] One heifer was in estrus at 101 hr after $PGF_{2\alpha}$ and would therefore have had an LH peak and ovulation beyond the experimental period.
[e] Occurring during 101 hr following $PGF_{2\alpha}$.
[f] Significantly less than the comparable mean for control heifers ($P < 0.05$).

heifers and in four out of five heifers treatment with GnRH at 10 hr after $PGF_{2\alpha}$. Ovulation in heifers in the latter group was probably not due to GnRH treatment since ovulation usually occurs 20 to 30 hr after LH release in cattle (4). Rather, there appeared to be a delay in ovulation in heifers receiving GnRH at 10 hr after $PGF_{2\alpha}$, although the mean is not significantly different from that of the controls. One heifer in each group given GnRH at 30 or 50 hr after $PGF_{2\alpha}$ failed to ovulate during 101 hr following $PGF_{2\alpha}$. Each had an ovarian follicular cyst at 10 or 11 days post-treatment. Ovulation was confirmed in all remaining heifers in both groups and the interval to ovulation for each group was less ($P < 0.05$) than the comparable value for control heifers. Ovulation in heifers treated at 30 hr after $PGF_{2\alpha}$ must have resulted from GnRH induced LH release since spontaneous surges of LH were prevented. These results suggest that GnRH given at 10 hr after $PGF_{2\alpha}$ delayed ovulation, but when given at 30 or 50 hr after $PGF_{2\alpha}$ hastened ovulation.

Spontaneous LH peaks were detected in all control heifers and four out of five heifers treated with GnRH at 10 hr after $PGF_{2\alpha}$ (Table I). In contrast, administration of GnRH completely prevented the naturally occurring spontaneous LH surge in all heifers treated at 30 hr and two out of five heifers treated at 50 hr. We previously reported that GnRH given to ovariectomized heifers at 12 hr after estradiol treatment would completely inhibit the surge of LH that normally occurs 12 to 24 hr after estradiol (3). How a GnRH-induced release of LH prevents the surge of LH release normally associated with estrus or after estradiol cannot be defined at this time. Although GnRH administered at frequent intervals to bulls (14) or ewes (15) will cause a progressive decrease in release of LH, complete suppression as seen in the present experiment was not achieved.

Summary. Twenty Holstein heifers in diestrus were given 30 mg of $PGF_{2\alpha}$ Tham salt (im). Thereafter each of five heifers received no further treatment or 40 μg of GnRH (im) at 10, 30, or 50 hr after $PGF_{2\alpha}$.

Serum progesterone decreased to less than 1 ng/ml at 24 hr after $PGF_{2\alpha}$ and remained low throughout the remainder of the sampling period. Following $PGF_{2\alpha}$, estradiol concentrations increased gradually in serum but GnRH given at 30 hr after $PGF_{2\alpha}$ abruptly decreased estradiol concentration. The magnitude of LH release induced by GnRH given at 30 or 50 hr after $PGF_{2\alpha}$ was greater than that at 10 hr. All heifers exhibited behavioral estrus except those in group GnRH-50. Spontaneous LH peaks, which usually occur after $PGF_{2\alpha}$ were observed in 5/5 (control), 4/5 (GnRH-10), 0/5 (GnRH-30), and 2/5 (GnRH-50) heifers.

We conclude that pituitary responsiveness to GnRH increases with time after $PGF_{2\alpha}$ and GnRH given at 30 hr completely inhibits and at 50 hr partially inhibits endogenous LH release.

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