Luteinizing Hormone Secretion in Hypophysial Stalk-Transected Pigs Given Progesterone and Pulsatile Gonadotropin-Releasing Hormone[1] (42823)

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Abstract. **This study was conducted to determine whether progesterone inhibits luteinizing hormone (LH) secretion in female pigs by a direct action on the pituitary gland. Eight ovariectomized, hypophysial stalk-transected gilts were given 1-μg pulses of gonadotropin-releasing hormone iv every 45 min from Day 0 to 12. On Days 5–12, each of four gilts received either progesterone or oil vehicle im at 12-hr intervals. Serum progesterone concentrations in steroid-treated gilts reached 70 ± 6.8 ng/ml (mean ± SE) by Day 8 and remained elevated thereafter, whereas serum progesterone concentrations in oil-treated controls were less than 1 ng/ml for the entire study. Daily serum LH concentrations were not different between gilts treated with progesterone or oil. The 1-μg pulses of gonadotropin-releasing hormone reliably evoked pulses of LH in both treatment groups. The LH pulse frequency and amplitude, assessed from samples collected every 15 min for 6 hr on Day 12, were similar for progesterone- and oil-treated gilts. These results provide evidence that progesterone does not act at the pituitary gland to alter LH secretion in pigs.** [P.S.E.B.M. 1989, Vol 190]

The inverse relationship between serum concentrations of progesterone and luteinizing hormone (LH) during the luteal phase of the porcine estrous cycle (1–3) is characteristic of a classical, negative feedback mechanism. Ovariectomy of gilts during diestrus increased plasma LH concentrations (1). Accordingly, treatment of ovariectomized sows with exogenous progesterone suppressed LH secretion (4).

Luteinizing hormone release from the anterior pituitary is pulsatile in ovariectomized (1) and intact pigs (1–3), presumably in response to episodic discharges of gonadotropin-releasing hormone (GnRH) from the hypothalamus (5). Progesterone, therefore, may decrease LH secretion in the pig by acting directly on the pituitary gland and/or inhibiting the secretion of GnRH. In an effort to determine the site of the negative feedback of progesterone in pigs, we assessed LH secretion after the administration of progesterone to hypophysial stalk-transected (HST) gilts given unvarying pulses of GnRH.

Materials and Methods

Eight domestic gilts, weighing 93 ± 10.8 kg (mean ± SE), were subjected to HST using the supraorbital approach to hypophysectomy of du Mesnil du Buisson *et al.* (6) as modified by Kraeling (7). A Teflon disc was placed between the severed ends of the stalk to prevent vascular regeneration. Approximately 2 weeks following HST surgery, gilts were ovariectomized and then fitted with indwelling jugular vein cannulas (8). The experiment began 8 days after ovariectomy.

All gilts were given 1-μg pulses of GnRH (Cystorelin; Abbott Laboratories, North Chicago, IL) every 45 min from 1900 hr on Day 0 (day experiment began) to 1400 hr on Day 12. The basis for selecting the dose of GnRH and the mechanics of delivery have been

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previously described (9). Briefly, GnRH was dissolved in 3.5% sodium citrate and was infused iv during a 5-min pulse by peristaltic pumps (Ismatec; Cole-Parmer, Chicago, IL) driven by a programmable timer (Chrontrol; Lindburg Enterprises, San Diego, CA). This GnRH replacement regimen reestablished normal pulsatile LH secretion in gilts treated with the hypothalamic blocker *N*-methyl-*N'*-(1-methyl-2-propenyl)1,2-hydrazinedicarbothioamide (9). Beginning at 1900 hr on Day 5, four gilts each received progesterone (1.35 mg/kg body weight; Sigma Chemical Co., St. Louis, MO) or oil vehicle im at 12-hr intervals until 0700 hr on Day 12. This steroid replacement regimen suppressed LH secretion in ovariectomized gilts by maintaining serum progesterone concentrations at levels which were similar to those occurring during the luteal phase of the estrous cycle (39.1 ± 2.6 ng/ml) (10). In that study, serum LH concentrations in progesterone- ($n = 11$) and oil-treated gilts ($n = 10$), following 10 days of treatment, were 0.3 ± 0.1 and 1.0 ± 0.1 ng/ml, respectively.

Blood was sampled daily at 1200 hr from Day 0 to 13. The samples on Days 1–12 were collected immediately following a GnRH pulse. Additionally, on Day 12, blood samples were collected every 15 min from 0700 to 1300 hr and then single samples were collected at 1500 and 1900 hr. Serum concentrations of LH (8) and progesterone (11) were measured by specific radioimmunoassays. The sensitivities of the assays were 0.1 ng of LH and 0.01 ng of progesterone/tube. The serum volumes per tube for the LH and progesterone radioimmunoassays were 0.3 ml and 0.025 ml, respectively. The intraassay and interassay coefficients of variation for the LH assay were 5.4 and 6.1%, respectively. The intraassay coefficient of variation for the single progesterone assay was 12.2%.

Serum LH concentrations were subjected to split-plot in time analysis of variance using the general linear model procedure of the Statistical Analysis System (12). Serum LH pulse frequency and amplitude in progesterone- and oil-treated gilts on Day 12 were compared using Student's *t* test. A LH pulse was defined as an increment greater than twice the intraassay coefficient of variation that occurred within 15 min of the previous nadir (9). Pulse frequency was expressed as the number of pulses observed per 6 hr. Pulse amplitude equalled the difference between the pulse peak and the preceding nadir (9). Basal LH concentrations were calculated from the samples that did not comprise a pulse.

Results

Examination of the hypothalamo-hypophysial region of the HST gilts at necropsy showed completeness of the stalk transection and proper positioning of the Teflon barrier. Absence of detectable LH secretion on Days 0 and 13 provided endocrinologic evidence that hypothalamic inputs were not reaching the pituitary gland.

Serum progesterone concentrations in steroid-treated gilts were 0.6 ± 0.1 ng/ml before giving progesterone on Day 5, reached 70 ± 6.8 ng/ml on Day 8, and remained elevated to Day 12 (Fig. 1). The time course of serum progesterone concentrations after a single im progesterone injection on Day 12 is also depicted in the inset of Figure 1. Oil-treated gilts had serum progesterone concentrations of less than 1 ng/ml for the entire study.

Initiating pulsatile delivery of exogenous GnRH increased ($P < 0.0001$) serum LH concentrations from below detection on Day 0 to 0.6 ± 0.1 ng/ml on Day 1. Daily serum LH concentrations were similar ($P >$

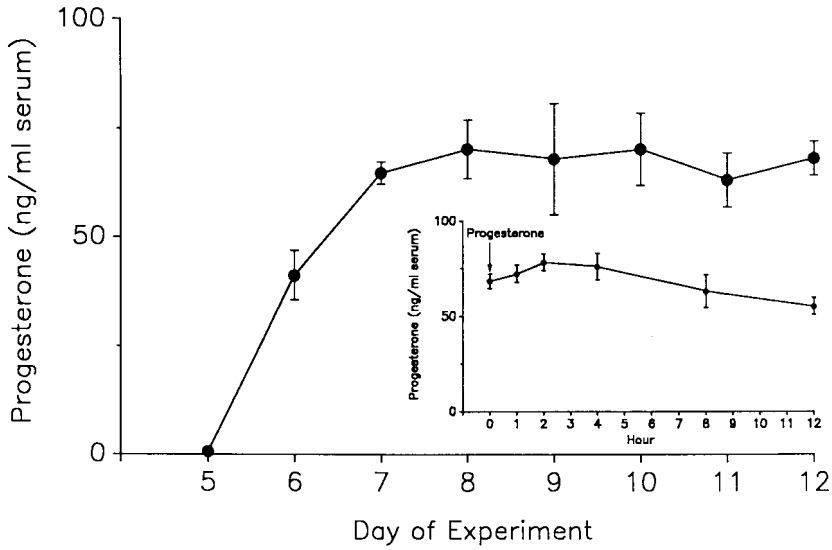

Figure 1. Serum progesterone concentrations at 1200 hr in ovariectomized, HST gilts receiving im progesterone (1.35 mg/kg body weight) at 0700 and 1900 hr on Days 5–12 (Day 0 = day experiment began, i.e., day GnRH treatment initiated). Inset is a depiction of the time course of serum progesterone concentrations following an injection at 0700 hr on Day 12. Values are mean ± SE ($n = 4$ gilts).

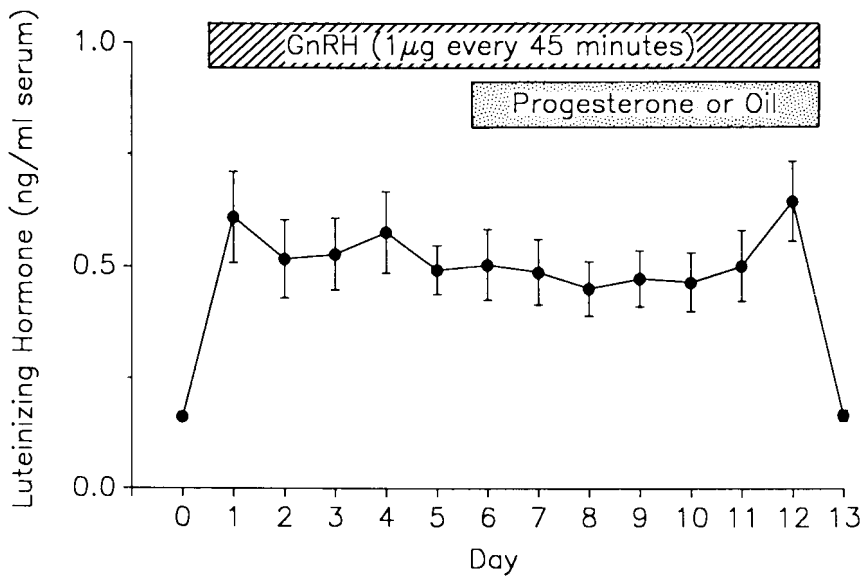

Figure 2. Combined daily serum LH concentrations for progesterone- and oil-treated ovariectomized, HST gilts receiving pulses of GnRH. Values are mean ± SE (n = 8 gilts).

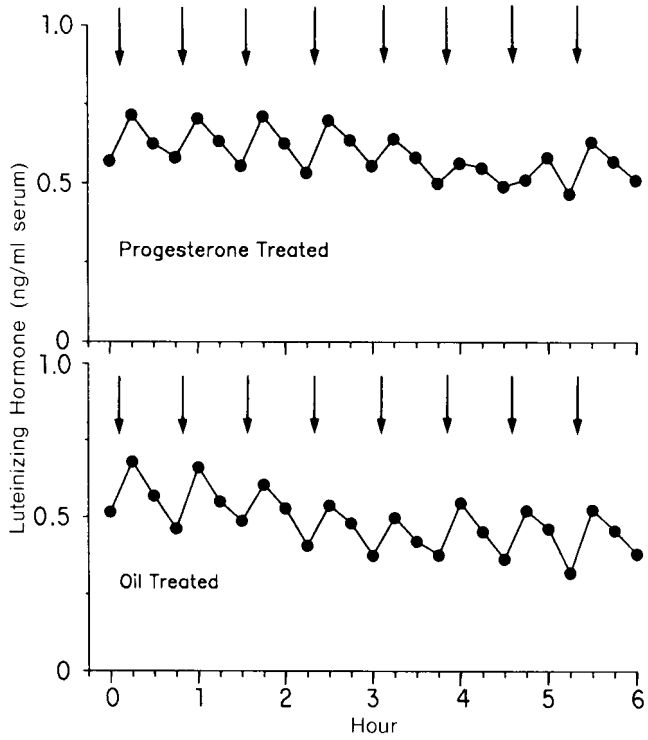

Figure 3. Serum LH concentrations on Day 12 for representative, ovariectomized, HST gilts receiving 1-μg pulses of GnRH and given either progesterone or oil im. Arrows indicate pulses of GnRH.

0.1) for both treatment groups before and during progesterone or oil administration (Fig. 2).

The 1-μg pulses of GnRH reliably evoked pulses of LH. Profiles of serum LH concentrations on Day 12 from gilts representative of each treatment group are depicted in Figure 3. Basal serum LH concentrations, pulse frequency, and amplitude were similar ($P > 0.1$) for progesterone- and oil-treated gilts (Table I).

Discussion

In the pig, serum progesterone concentrations are lowest during the periovulatory period, increase steadily until Day 7 of the estrous cycle (onset of estrus = Day 0), and remain elevated until Day 13, at which time a decline, concurrent with luteal regression, is initiated (2). Previous characterizations of the hormonal milieu present during the various stages of the porcine estrous cycle have revealed that during the luteal phase, concentrations of serum progesterone frequently exceed 50 ng/ml (1, 2). Therefore the serum progesterone concentrations established in the present investigation approximated those levels normally occurring during the luteal phase.

Exogenously administered progesterone did not alter LH secretion in response to exogenous pulses of GnRH. These data provide evidence that progesterone does not act directly on the anterior pituitary gland and are consistent with the notion that progesterone inhibits LH secretion in gilts by acting at the level of the central nervous system. These results are consonant with an earlier study (13) in which administration of a synthetic progestin blocked estrus and the preovulatory LH surge in cyclic gilts given pregnant mare serum gonadotropin. The results of the current study do not preclude, however, the possibility that progesterone may act in concert with other steroids, such as estradiol, to decrease hypophysial responsiveness to GnRH. Such a synergistic mechanism has been postulated for sheep (14).

In cattle, pulses of LH are generally secreted concomitantly with pulses of follicle-stimulating hormone, purportedly in response to discontinous stimulation by GnRH (15). There are instances, however, during the luteal phase of the bovine estrous cycle, when pulses of follicle-stimulating hormone are unaccompanied by

Table I. Basal LH Concentrations, LH Pulse Frequency, and Amplitude on Day 12 for Progesterone- and Oil-Treated Ovariectomized, HST Gilts Receiving Pulses of GnRH

Parameter	Treatment	
	Progesterone	Oil
Basal LH concentrations (ng/ml)	0.56 ± 0.11[a]	0.46 ± 0.10
Pulse frequency (pulses/6 hr)	6.8 ± 0.6	4.8 ± 2.0
Pulse amplitude (peak-nadir; ng/ml)	0.14 ± 0.01	0.20 ± 0.07

[a] Mean ± SE. Parameters did not differ ($P > 0.1$) between treatment groups.

pulses of LH (16). If one accepts the premise that GnRH is the sole hypothalamic-releasing hormone for both gonadotropins, there exists the possibility that progesterone may act on the bovine pituitary to render some pulses of GnRH ineffectual or "silent" in terms of LH secretion (16). This does not appear to be the case in pigs, since in the present investigation pulses of GnRH reliably evoked pulses of LH in the face of elevated serum progesterone concentrations.

Indeed, the ability of progesterone to alter LH secretion by a direct action on the anterior pituitary gland appears to vary among domestic animal species. Beef cows receiving twice daily progesterone injections from Days 16 to 19 postpartum had a diminished response to exogenous GnRH (17). Furthermore, the ability of GnRH to self-prime bovine pituitary cells *in vitro* and the augmentation of this response by estradiol are both inhibited by progesterone (18). Serum LH concentrations after administration of GnRH in stalk-transected ewes were unaffected by progesterone administration alone (14). Progesterone, however, decreased responsiveness of ovine pituitary cells to GnRH *in vitro* (19).

In conclusion, progesterone treatment failed to alter LH secretion in HST gilts given unvarying pulses of GnRH. We suggest that progesterone inhibits LH secretion in pigs by acting at the level of the central nervous system.

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