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# Role of the Ovary in Controlling Luteinizing Hormone, Follicle Stimulating Hormone, and Prolactin Secretion During and After Lactation in Pigs[1]

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## ABSTRACT

Concentrations of LH, FSH, prolactin, estrogen, and progesterone were measured in blood samples collected at various intervals during and immediately following lactation to determine the role of the ovary in controlling gonadotropin and prolactin secretion and initiation of estrous cycles in the pig. Ten primiparous sows were ovariectomized (ovex, n = 5) or left intact (n = 5) 2 to 4 days after farrowing. Serum progesterone and estrogens in samples collected daily during lactation were not different between intact and ovex sows. Serum FSH increased immediately after ovariectomy and remained elevated during lactation, but LH concentrations were similar between ovex and intact sows. Both LH and FSH were higher during the last 2 weeks of lactation than during the first 3 weeks.

On Days 10 and 20 of lactation, all sows received (i.v.) two challenges of gonadotropin releasing hormone (GnRH; 400 μg each) at 135 min intervals. Peak change (maximum value minus baseline) in FSH after GnRH was higher in ovex than in intact sows on both Days 10 and 20. Peak change in FSH in ovex sows was higher on Day 20 than on Day 10, but peak change in FSH in intact sows was similar between Days 10 and 20. Peak change in LH after GnRH was similar between ovex and intact sows. Peak change in LH after the second GnRH challenge on Day 10 was higher than that after each of the other three challenges.

Although serum prolactin concentrations in selected samples collected on Days 10 and 20 were not significantly different between treatment groups, intact sows had consistently higher concentrations compared with ovex sows. Separation of four sows from their litters for 4 h during the last week of lactation resulted in a decline in serum prolactin, but prolactin increased fivefold within 15 min and tenfold during the second hour after piglets were replaced. In contrast, LH did not change prior to or after piglets were allowed to nurse.

After weaning, concentrations of estrogens peaked during 24 h around onset of estrus prior to the preovulatory surge of LH and FSH. In ovex sows treated with estradiol-17β on Days 8, 9, and 10 after weaning, estrous behavior and LH and FSH responses were indistinguishable from those observed in intact sows at the postweaning estrus. Serum LH, FSH, and prolactin increased in response to estrogen, and duration of estrus was similar to that observed in intact sows.

Results from these experiments indicate that 1) LH is low during lactation in both intact and ovex sows; 2) FSH increases following ovariectomy, presumably because of removing some ovarian inhibitor of FSH secretion; 3) prolactin, but not LH, changes acutely with suckling or weaning; and 4) surges in LH, FSH, and prolactin at estrus are induced by estrogen whether of endogenous (intact sows) or exogenous (ovex sows) origin.

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## INTRODUCTION

Lactation in the domestic pig is characterized by anestrus (Burger, 1952) and by suppressed ovarian follicular development (Palmer et al., 1965), increased prolactin secretion (Bevers et al., 1978; van Landeghem and van de Wiel, 1978; Mulloy and Malven, 1979), and depressed LH secretion (Parvizi et al., 1976). Pituitary LH concentrations are also lower during lactation (Crighton and Lamming, 1969), sug-

gesting that both synthesis and release of LH are inhibited by suckling. However, pituitary FSH content is not suppressed during lactation (Crighton and Lamming, 1969). No reports are available on serum FSH concentrations during lactation or after weaning in the pig.

Previous research in pigs suggests that increases in LH and FSH secretion at the postweaning estrus (Crighton and Lamming, 1969) may be similar to increases observed at estrus in cycling female pigs (Niswender et al., 1970; Henricks et al., 1972). However, characterization of the postweaning estrus and its associated endocrine events is limited (Bevers et al., 1978; Stevenson et al., 1978; Dyck et al., 1979).

Preliminary studies established clearly that LH concentrations were low (<1 ng/ml) during lactation and did not increase until after weaning (Stevenson et al., 1978). But is was not clear whether this suppression was due to suckling per se or due to a combination of suckling- and ovarian-induced inhibition of gonadotropin secretion. This study was conducted to determine the role of the ovary in regulating gonadotropin and prolactin secretion during and after lactation. Pituitary responsiveness to exogenous gonadotropin releasing hormone (GnRH) was assessed at two periods during lactation in intact and ovariectomized (ovex) sows. We also determined whether estradiol replacement in ovex sows after weaning induced endocrine changes and estrus similar to that observed at the postweaning estrus in intact females.

## MATERIALS AND METHODS

### *Animal Handling*

Ten gilts that farrowed between Dec. 10 and Dec. 17, 1978, were randomly assigned to be ovariectomized (ovex, n = 5) or left intact (n = 5). Cannulas were inserted in the vena cava utilizing a nonsurgical technique (Ford and Maurer, 1978), and ovariectomies were performed by midventral laparotomy under general anesthesia 2 to 4 days after parturition. Sows were removed from individual farrowing crates for ∿8 h during cannulations and surgery. Otherwise, sows were maintained in farrowing crates during the entire lactation period (29 to 36 days) and were provided with feed (corn-soybean meal, vitamins and minerals; NRC, 1979) and water ad libitum. Litters were standardized to 8 to 10 piglets per sow within 48 h after farrowing and were allowed to suckle ad libitum. Body weights of sows were recorded prior to farrowing (Day 108 of gestation), at surgery, and at weekly intervals until weaning. At weaning, sows were confined in individual stalls (0.5 × 2.5 m) adjacent to boars and were checked for estrus 3 to 4 times daily using intact boars.

### *Sampling Scheme*

Blood samples were collected once daily (0900 h) during lactation, except on Days 10 and 20 when samples were collected at frequent intervals before and after two challenges (400 μg each) with GnRH (Abbott Labs.). On Days 10 and 20 when GnRH was given (i.v.), blood was collected at 15 min intervals for 2 h (beginning at 0900 h) prior to the first GnRH challenge, at 0, 10, 20, 30, 45, 60, 75, 90, 105, and 120 min after the first challenge, and at similar intervals up to 150 min after the second GnRH injection. The second GnRH challenge was given at 135 min after the first challenge. After weaning, blood samples were collected at 6 h intervals (0500, 1100, 1700, and 2300 h EST) for 7 days. Blood sampling was continued on alternate days for 3 additional weeks in the intact sows.

On Days 8, 9, and 10 after weaning, ovex sows were given (i.m.) estradiol-17β in corn oil. Estradiol-17β was administered twice daily (0500 and 1700 h) and the total dose per sow was 100, 200, and 400 μg on the first, second, and third days of treatment, respectively. Increasing daily doses of estradiol-17β were given in an attempt to mimic what was postulated to occur following weaning when ovarian follicular growth and estrogen secretion are stimulated by increased gonadotropin secretion (Crighton and Lamming, 1969). Blood samples were collected at 6 h intervals during and for 5 days after treatment with estradiol-17β. Blood was kept at room temperature for 4 h and at 4°C for 24 h prior to centrifugation (2000 × g) for 30 min to separate serum. Serum was stored at −20°C until assayed.

### *Hormone Assays*

Antiprogesterone sera (NCSU 78-3-4, NCSU 78-3-6) were produced in our laboratory by immunizing rabbits against progesterone-11-oxime-bovine serum albumin. Progesterone assays were conducted as described by Louis et al. (1973). Cross reaction with other steroids, recovery of progesterone added to serum, and parallelism of serum curves with progesterone standard curves were determined. Cross reaction of other steroids with our antisera is shown in Table 1. Less than 1% cross reaction occurred with either 20α-or 20β-dihydroprogesterone and 5α-pregnanedione. The latter two progesterone metabolites are normally found in target tissues and urine, respectively, but not in peripheral plasma since porcine corpora lutea produce progesterone and 20α-dihydroprogesterone as secretion products (Hansel et al., 1973).

Displacement of bound progesterone by increasing volumes of two pools of porcine serum was parallel to the standard curve. Average recovery was 94 ± 4% when progesterone (0.1, 0.2, 0.3, 0.6, 1.0, and 3.0 ng) was added to 100 μl of porcine serum. Sensitivity of the assay was 25 pg of progesterone per assay tube compared to tubes containing no progesterone ($P<0.05$). After extraction of 50, 100, 150, and 200 μl serum (estrous sow) in duplicate, the assay measured 0.34, 0.12, 0.21, and 0.27 ng progesterone

TABLE 1. Cross reactions of NCSU 78-3 progesterone antisera.

| Steroid | Percent cross reaction[a] | |
|---|---|---|
| | NCSU 78-3-4 (1:3000)[b] | NCSU 78-3-6 (1:5000)[b] |
| Progesterone | 100.0 | 100.0 |
| 20α-Dihydroprogesterone | 1.1 | 0.2 |
| 20β-Dihydroprogesterone | <0.2 | <0.2 |
| 17α-Hydroxyprogesterone | 14.0 | 15.0 |
| 5α-Pregnanedione | 13.0 | 15.0 |
| 5β-Pregnanediol | <0.2 | <0.2 |
| Pregnenolone | 1.3 | 1.1 |
| Testosterone | <0.2 | <0.2 |
| Cortisol | <0.2 | <0.2 |
| Corticosterone | 1.8 | 1.4 |
| 11-Deoxycorticosterone | <0.2 | 0.2 |

[a]Percent expressed as activity relative to progesterone at 50% inhibition.

[b]Antisera from two bleedings of the same rabbit were diluted differently to maintain total binding between 30% and 50%.

per milliliter. After extraction of similar volumes of serum from an early diestrous sow, the assay measured 0.82, 0.94, 1.02, and 1.08 ng progesterone per milliliter. In 21 assays, 83 ± 3% of the [$^3$H]-progesterone was recovered and intraassay and interassay coefficients of variation were 12.2% and 28.6%, respectively.

Total free estrogens were measured by radioimmunoassay using an antiserum produced in sheep against estradiol-17β-17-succinate bovine serum albumin (supplied by Dr. V. L. Estergreen, Washington State University). Assay validation and procedures using this antiserum have been reported (Nett et al., 1973).

Several validation steps were repeated to ensure repeatability of the assay in our laboratory. When 25, 50, 100, and 200 pg estradiol-17β were added to 500 μl of porcine serum (ovex gilt) in quadruplicate and assayed, average recovery of added mass was 109 ± 5%. Extraction and assay of 0.5, 1.0, and 2.0 ml of porcine serum resulted in concentrations of 34 ± 5, 33 ± 2, and 34 ± 1 pg/ml, respectively. Sensitivity of the assay was 6 pg of estradiol-17β. In 17 assays, 78 ± 5% of the [$^3$H]-estradiol-17β was recovered, and intraassay and interassay coefficients of variation averaged 15.7% and 27.5%, respectively.

Serum LH was measured by radioimmunoassay using a rabbit anti-porcine LH serum (#566) previously validated (Niswender et al., 1970). Purified porcine LH (LER 786-3) was iodinated and used as the radioligand while ovine LH (NIH-S20) was used for standards. Recovery of added mass, cross reactivity, parallelism between serum curves and standard LH curves, and sensitivity of the assay (0.03 ng LH) were not different from characteristics reported previously (Niswender et al., 1970). Intraassay and interassay coefficients of variation averaged 9.0% and 11.4%, respectively.

Serum FSH was measured by a radioimmunoassay validated in our laboratory using an antiserum produced in rabbits against a highly purified porcine FSH (antiserum and purified FSH for iodination and standards were supplied by Drs. R. J. Ryan and R. J. Whitley, Mayo Clinic, Rochester, MN). Isolation and characterization of the porcine FSH (pFSH IA3-c2) have been described (Whitley et al., 1978). Porcine FSH was iodinated by the chloramine-T method (Greenwood et al., 1963).

Cross reaction with LH (LER 786-3 and NIH-S20), recovery of pFSH added to serum of castrate, intact, and hypophysectomized (hypox) pigs, and parallelism between curves and standard pFSH curves were determined. In addition, thyrotropin releasing hormone (Calbiochem, 200 μg) was administered (i.v.) to two luteal phase gilts and serum samples were collected (−60, −45, −30, −15, 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, and 240 min) to measure any cross reaction attributable to acutely high serum concentrations of TSH and prolactin (van Landeghem and van de Wiel, 1978).

Addition of 1000 ng LH (LER 786-3 and NIH-S20) resulted in less than 0.1% cross reaction in the assay. Displacement of [$^{125}$I]-FSH by increasing volumes (25 to 200 μl) of porcine sera and by increasing concentrations of NIH-FSH-P2 was parallel to the standard curve. FSH (ranging from 0.2 to 5 ng) added to hypox serum was consistently recovered from 200 μl of serum (96–108%). No significant change in FSH concentration occurred after TRH administration to gilts, indicating that high serum concentrations of prolactin and TSH did not interfere with precision of the assay. Sensitivity of the assay was 0.25 ng FSH. Intraassay and interassay coefficients of variation were 13.3% and 26.7%, respectively.

Serum prolactin was measured in the laboratory of Dr. R. R. Kraeling (USDA Research Lab, Athens, GA) by a homologous double antibody radioimmunoassay (Kraeling et al., 1979). Not all samples were assayed for FSH and prolactin because of limited amounts of antisera.

*Statistical Analyses*

Data were analyzed by split-plot analysis of variance appropriate for repeated measurements in individual animals (Gill and Hafs, 1971). Split errors for between- and within-animal variance were used to test appropriate sources of variation. Treatment differences (between-animal variance) were tested by the split error term sows within treatment. Other sources of variation such as day of lactation (within-animal variance) were tested by the split error term day × sow within treatment. Comparisons of means were conducted by orthogonal contrasts or by Scheffe's interval (Gill, 1978). Comparisons of other data with only two means were by t test (homogeneous variance) or by approximate t test when variances were heterogeneous (Sokal and Rohlf, 1969).

## RESULTS

*Sow and Litter Performance*

Ovariectomies performed between Days 2 and 4 after parturition had no effect on changes in a sow's body weight. Sows in both treatment groups (ovex and intact) each lost an average of 24.1 ± 1.1 kg of body weight between Day 108 of gestation and weaning. Piglets gained weight at similar rates during lactation, indicating that milk yields were not impaired by surgery, and consequently equivalent amounts of milk were available for ad libitum suckling by litters.

Sows became accustomed to blood sampling within 1 to 2 days and continued eating, sleeping, or nursing piglets during sampling. Cannulas remained patent in 6 of 10 sows during all experimental phases (>50 days), while 3 sows were recatheterized after weaning. When cannulas were not functional, blood was collected by puncture of the anterior vena cava.

*Hormone Profiles During Lactation*

Serum progesterone and total estrogens were elevated ($P<0.01$) in samples collected on the day of cannulation and surgery (Fig. 1A, B). Mean steroid concentrations were not different between treatment groups during lactation. Serum FSH concentrations increased after ovariectomies and were higher ($P<0.02$) during the entire lactation in ovex compared with intact sows (Fig. 1D). In both groups, FSH increased ($P<0.001$) as lactation progressed, especially during the last 2 weeks. LH concentrations were not different between groups (Fig. 1C) during lactation, but LH was higher ($P<0.02$) from Day 21 until weaning than during the first 20 days.

*Pituitary Response to GnRH*

Overall, average serum FSH concentration in ovex sows was higher ($P<0.09$) than in intact sows on both Days 10 and 20 of lactation (Fig. 2). In addition, the mean concentration of FSH in all samples from all sows was lower ($P<0.05$) on Day 10 (7.2 ± 0.2 ng/ml) than on Day 20 (9.2 ± 0.2 ng/ml). Peak change in FSH after GnRH challenge was defined as the maximum concentration after the first or second challenge minus the concentration at the time of the challenge. Peak change in FSH in ovex sows was higher than that in intact sows on both Days 10 and 20 (Table 2). Peak change in FSH in ovex sows was higher ($P<0.05$) on Day 20 than on Day 10, but peak change in FSH in intact sows was similar on Days 10 and 20 (Table 2).

Overall, average serum LH concentration was similar between ovex and intact sows on both Days 10 and 20 of lactation (Fig. 3). Mean concentration of LH in all samples from all sows was higher ($P<0.01$) on Day 10 (13.8 ± 0.5 ng/ml) than on Day 20 (8.3 ± 0.5 ng/ml). Peak change in LH after GnRH was similar between treatment groups (Table 2), but peak change in LH after the second GnRH challenge on Day 10 was higher than after the first challenge (Table 2) on Day 10 and both challenges on Day 20. On Day 20, peak change in LH was similar after the first and second challenges with GnRH (Table 2).

*Prolactin Secretion During Lactation and at Weaning*

Prolactin was measured in samples collected at hourly intervals for 8 h on Days 10 and 20 of lactation. Although data are grouped by treatment (Fig. 4), differences between treatment groups were not significant ($P<0.05$) due to large variation among sows. However, serum prolactin was consistently higher in intact compared with ovex sows on Days 10 and 20 of lactation.

Three days prior to weaning, four sows (1 intact, 3 ovex) were separated from their litters for 4 h to determine whether temporary removal of the suckling stimulus would affect prolactin and LH secretion. Blood samples were collected at 15 min intervals for 3 h beginning 1 h prior to piglet replacement. Serum prolactin averaged 8 ± 1 ng/ml (Fig. 5A) during the 1 h period prior to piglet replacement and represented a 90% decrease in prolactin secretion

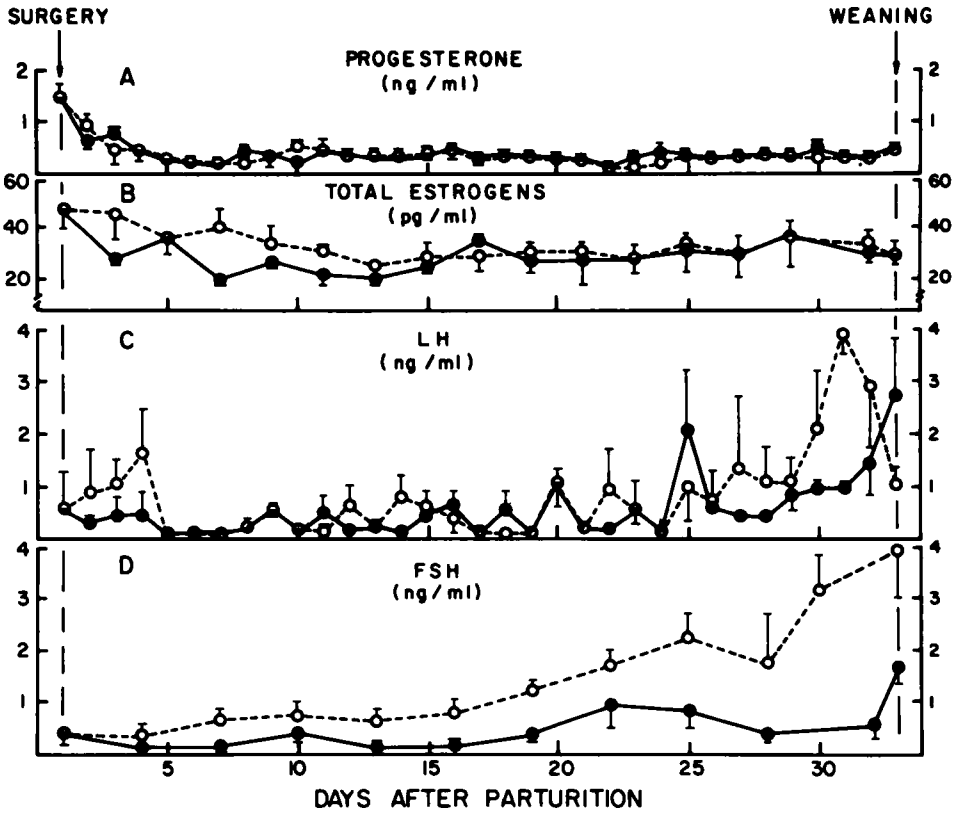


FIG. 1. Concentration (± SEM) of hormones in serum samples collected daily during lactation from five intact (—●—) and five ovariectomized (—○—) sows. Ovariectomies were at 2 to 4 days after parturition, and lactations averaged 33 ± 3 days. Data were plotted from the day of weaning for standardization.

from concentrations observed on Day 20 of lactation (Fig. 4). Following replacement of piglets, prolactin increased ($P<0.01$) fivefold within 15 min and tenfold after 2 h. In contrast, LH concentrations (Fig. 5B) did not change before or after piglet replacement.

Serum prolactin averaged 41.4 ± 0.9 ng/ml 24 h before weaning, declined ($P<0.001$) to 6.7 ± 0.5 ng/ml within 2 h after weaning, and remained less than 5 ng/ml during the following 30 h (Fig. 6). Prolactin response to weaning did not differ between treatment groups.

*Postweaning Responses in Intact Sows*

Four of five intact sows began estrus between 94 and 106 h after weaning; one sow did not show estrus during the remainder of the study. Hormone concentrations around estrus were centered on the onset of estrus for illustration (Fig. 7). Estrogen concentrations during the first 48 h after weaning (Fig. 7A) did not differ from preweaning levels, but declined ($P<0.01$) to a nadir during 54 to 18 h prior to onset of estrus. Estrogens then increased ($P<0.01$) to greater than 20 pg/ml during 24 h around onset of estrus, subsequently declined to less than 10 pg/ml for 12 h, and averaged about 15 pg/ml during the next 36 h.

Serum gonadotropin concentrations remained relatively constant during the first 3 days after weaning (Fig. 7B, C), but increased at estrus. Serum FSH increased to greater than 8 ng/ml in one sow 1 day prior to estrus, and then FSH tended to increase ($P = 0.16$) during estrus in all sows (Fig. 7B). Serum FSH did not increase synchronously with the preovulatory LH surge (Fig. 7C). Serum LH peaked ($P<0.01$) at the onset of estrus, about 12 h after the peak of estrogens. On the average, peak levels of LH occurred at the onset of estrus, but temporal relationships between increased estrogens, increased gonadotropins and the onset of estrus were variable among sows. Ovulation and

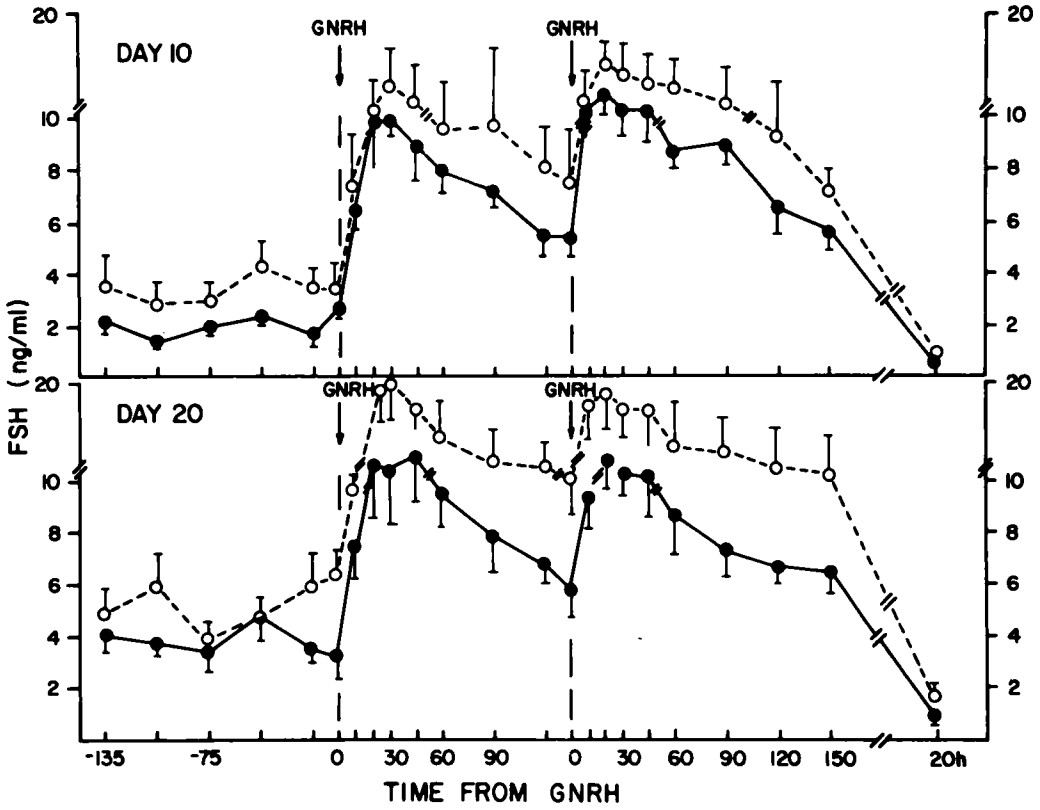


FIG. 2. Concentration (± SEM) of FSH in serum samples collected before and after GnRH challenge on Days 10 and 20 of lactation. Five intact (—•—) and five ovariectomized (—○—) sows were each given two challenges of GnRH (400 μg each) at 135 min intervals on each day.

TABLE 2. Peak change in LH and FSH concentrations (ng/ml) following GnRH challenges on Days 10 and 20 of lactation.[a]

| Treatment group[b] | Day 10 | | Day 20 | |
|---|---|---|---|---|
| | GnRH challenge | | | |
| | First | Second | First | Second |
| | FSH | | | |
| Ovex | 11.1[c•] | 12.4[c•] | 14.6[d••] | 14.1[d••] |
| Intact | 9.3[c] | 10.8[c] | 9.0[c] | 8.7[c] |
| | LH | | | |
| Ovex | 35.6[c] | 52.1[d] | 26.9[c] | 23.6[c] |
| Intact | 24.6[c] | 48.8[d] | 26.9[c] | 20.5[c] |

[a]Peak change is the maximum concentration after GnRH minus the average concentration at the time of GnRH challenge, expressed as the least-squares mean.

[b]Ovariectomized (ovex, n = 5) or left intact (n = 5) 2 to 4 days after parturition.

[c,d]Means in the same row with different superscripts are different (P<0.05).

•Different from means for intact pigs in same column (P<0.05).

••Different from means for intact pigs in same column (P<0.01).

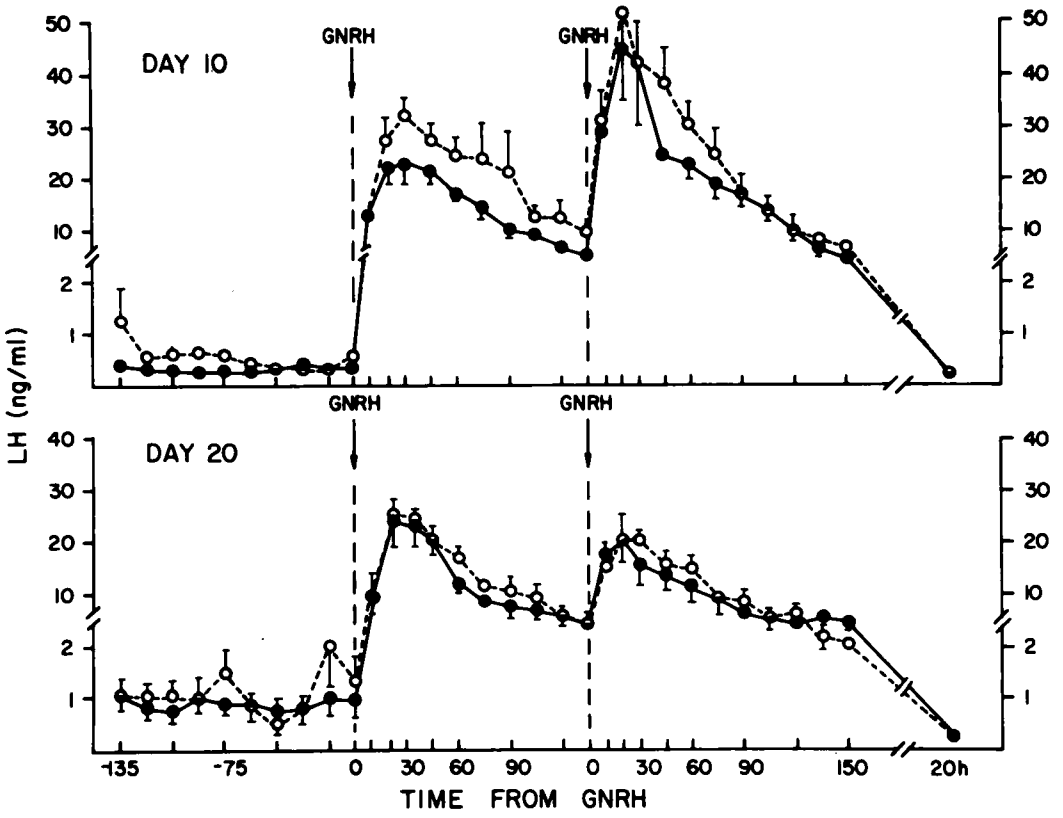


FIG. 3. Concentration (± SEM) of LH in serum samples collected before and after GnRH challenge on Days 10 and 20 of lactation. Five intact (—●—) and five ovariectomized (—○—) sows were each given two challenges of GnRH (400 μg each) at 135 min intervals on each day.

subsequent development of corpora lutea were indicated by increased ($P<0.01$) progesterone (Fig. 7A).

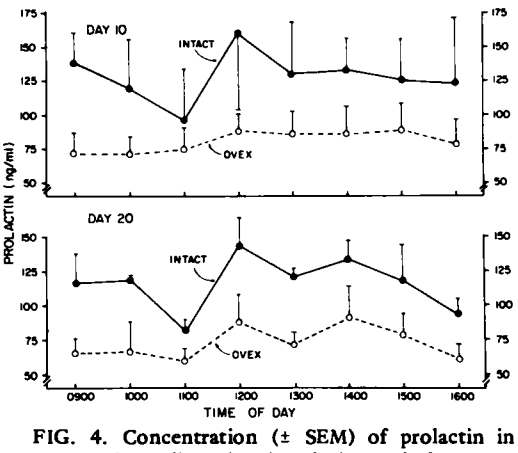


FIG. 4. Concentration (± SEM) of prolactin in serum samples collected at hourly intervals from two intact (—●—) and two ovariectomized (—○—) sows on Days 10 and 20 of lactation.

*Postweaning Responses in Estradiol-17β-Treated Ovex Sows*

Mean serum hormone responses at weaning and after estradiol replacement in ovex sows are illustrated (Fig. 8). Estrogens (Fig. 8A) fluctuated (8 to 32 pg/ml) during 7 days after weaning. However, during estradiol replacement, estrogens (37 ± 2 pg/ml) increased ($P<0.05$) only twofold relative to pretreatment concentrations. Blood was not collected until 6 h after each estradiol injection; however, total estrogens never exceeded 100 pg/ml in any individual sample. Immediately after the end of estradiol replacement, estrogens gradually declined to pretreatment levels. Progesterone levels (Fig. 8B) did not change until 2 to 5 days after estradiol treatment when they increased ($P<0.01$), but only to levels less than 0.8 ng/ml.

Serum FSH (Fig. 8C) fluctuated broadly after weaning and declined ($P<0.05$) to less than 1 ng/ml for 48 h during Days 3 and 4, when estrogen concentrations (Fig. 8A) were

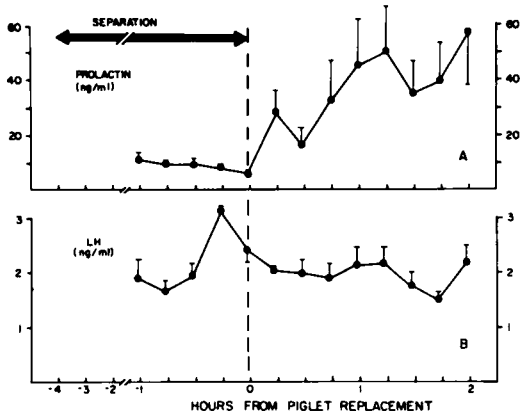


FIG. 5. Concentration (± SEM) of prolactin and LH in serum samples collected from four sows that were separated from their litters for 4 h during the last week of lactation.

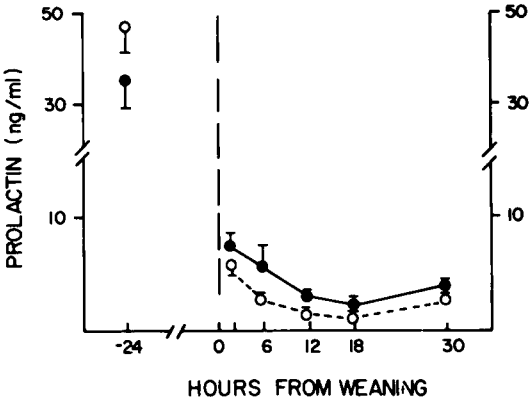


FIG. 6. Concentration (± SEM) of prolaction in serum samples collected from five intact (—●—) and five ovariectomized (—○—) sows prior to and after weaning.

also lowest. Serum FSH then increased to a plateau (2 to 4 ng/ml) during estradiol treatment. At 12 h after the last estradiol injection, FSH levels peaked ($P<0.01$) to greater than 5 ng/ml for 12 h and then decreased to basal levels.

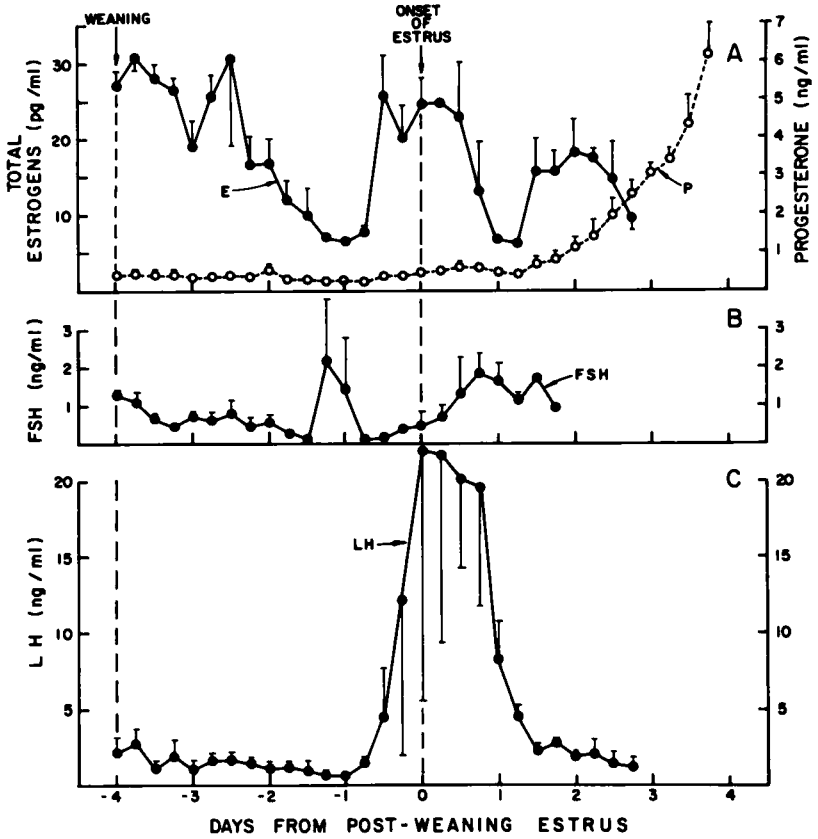


FIG. 7. Concentration (± SEM) of hormones in serum samples collected during 8 days after weaning in four intact sows. Data were centered on the onset of estrus.

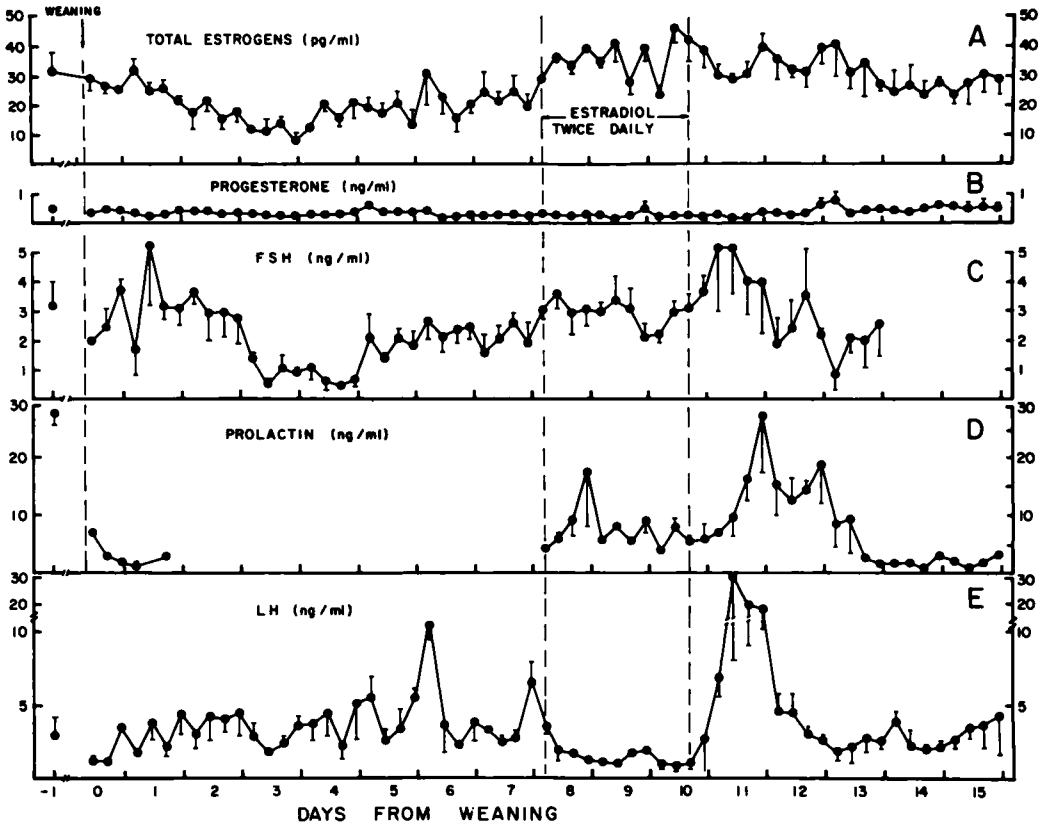


FIG. 8. Concentration (± SEM) of hormones in serum samples collected from four ovariectomized sows after weaning and prior to and after treatment with estradiol-17β. Estradiol-17β was administered twice daily (0500 and 1700 h) on Days 8, 9, and 10 after weaning. Total daily doses were 100, 200, and 400 μg on Days 8, 9, and 10, respectively.

Serum LH fluctuated between 1 and 5 ng/ml for 4 days after weaning, then gradually increased and became more variable until estradiol replacement. During estradiol replacement, LH levels were less than 2 ng/ml, but at 18 h after the end of estradiol treatment, LH peaked at >10 ng/ml for 18 h, then declined to pretreatment concentrations. Duration of estrus, time from onset of estrus to onset of the LH surge, and duration and magnitude of the LH surge were indistinguishable between intact sows at the postweaning estrus and ovex sows treated with estradiol (Table 3).

During estradiol treatment, prolactin averaged 7.7 ng/ml (Fig. 8D). At 30 h after treatment, prolactin peaked ($P<0.01$) at 25 ng/ml and remained elevated (>10 ng/ml) for 30 h before declining to levels similar to those observed after weaning. Overall, serum concentrations of LH, FSH, and prolactin were observed to rise in response to the stepwise increase in estradiol treatment.

## DISCUSSION

### *Control of FSH and LH Secretion During Lactation*

These results demonstrate that LH and FSH secretion are controlled by divergent mechanisms during lactation in pigs. The ovary appears to be essential for inhibiting FSH secretion, because ovariectomy during lactation resulted in an increase in basal FSH secretion and an increase in the amount of FSH released in response to exogenous GnRH. In contrast, ovariectomy during lactation had no effect on basal or GnRH-induced secretion of LH. Similar observations have been made during lactation in rats (Smith and Neill, 1977), monkeys (Weiss et al., 1976), and humans (Rolland et al., 1975).

TABLE 3. Duration of estrus and characteristics of the associated surge of LH in intact sows after weaning and in ovariectomized sows after treatment with estradiol-17β.

| Response | Intact (n = 4) | Ovex[a] (n = 4) |
|---|---|---|
| Duration of estrus (h) | 46 ± 6[b] | 48 ± 5 |
| Interval from onset of estrus to onset of LH surge (h) | 9 ± 3 | 12 ± 2 |
| Duration of LH surge (h) | 33 ± 3 | 36 ± 4 |
| Peak LH during surge (ng/ml) | 49 ± 9 | 32 ± 17 |

[a]Ovex sows received (i.m.) 100, 200, and 400 μg estradiol-17β in corn oil on Days 8, 9, and 10 after weaning, respectively. Each daily dose was split into equal portions and administered at 0500 and 1700 h.

[b]$\bar{x}$ ± SEM.

Serum progesterone and total free estrogens did not change following ovariectomy; therefore control of FSH and LH secretion during lactation was not attributable to feedback by these ovarian steroids on gonadotropin secretion. Thus, we suggest that inhibition of FSH secretion during lactation is due to some nonsteroidal factor that was removed by ovariectomy. Ovaries from ovex sows contained numerous 1 to 5 mm Graafian follicles (11 to 27 follicles per sow), and porcine follicles of this size contain a nonsteroidal substance(s) that suppresses FSH but not LH secretion in gonadectomized rats (Marder et al., 1977; Welschen et al., 1977). This factor is probably folliculostatin, the putative inhibitor of FSH production (Campbell and Schwartz, 1979).

The low level of LH secretion during lactation was apparently due to suppression by suckling. Normally, LH rises after castration in pigs (Wilfinger et al., 1974; Parvizi et al., 1976), but that was not the case in this study. Concentrations of total free estrogens in serum were nearly as high during lactation as during the postweaning estrus. But when pigs were weaned from ovex sows, LH increased over the next 6 days and then declined during the period of estrogen treatment. After estrogen treatment, there was a synchronous surge in LH in the four ovex gilts. Thus, the hypothalamo-pituitary axis was sensitive to estrogen feedback after weaning in ovex gilts. Elsaesser and Parvizi (1980) suggested that the sensitivity of this feedback system changes during lactation because they observed an increase in LH after estrogen treatment on Day 35 of lactation but not when the same dosage of estrogen was given on Day 5.

Exactly why FSH should be controlled by some nonsteroidal factor from the ovary and LH by suckling during lactation is unknown. However, we propose that these divergent mechanisms prevent excess follicular growth and atresia that might occur if FSH alone were secreted above basal levels. The endocrine state of the lactating sow is markedly different from that observed during estrous cycles or pregnancy. During estrous cycles, LH secretion is related to cyclic variations in estrogens and progesterone and vice versa (Henricks et al., 1972). During pregnancy, LH secretion is inhibited by high levels of progesterone and estrogen from the ovary and feto-placental unit (Ash and Heap, 1975; Baldwin and Stabenfeldt, 1975). However, during lactation progesterone levels are almost nondetectable while estrogen levels are variable. This creates a situation where "normal" feedback relationships between ovarian steroids and LH may be nonexistent.

Crighton and Lamming (1969) measured LH and FSH in pituitaries from intact and ovariectomized sows. They found that LH levels were low during lactation but FSH levels were similar to those observed during estrous cycles. Furthermore, ovariectomy did not alter pituitary LH levels during lactation but ovariectomy caused an increase in pituitary LH in cyclic gilts. They concluded that suckling inhibited synthesis and release of LH while synthesis of FSH was unchanged but release of FSH was inhibited. Data from the present study on the release of LH and FSH after GnRH generally support these conclusions. In our study, average concentration of FSH in serum increased from Day 10 to Day 20 and was higher in ovex than in intact sows. Furthermore, peak FSH response after GnRH was higher in ovex than in

intact sows on both Days 10 and 20. Peak FSH response after GnRH increased from Day 10 to Day 20 in ovex but not intact sows. These results suggest that the ovaries not only inhibit rate of FSH secretion but also affect rate of FSH synthesis. In contrast, ovariectomy did not affect basal or GnRH-induced LH secretion, but the amount of LH released after the second GnRH challenge on Day 10 was greater than that released after other challenges. This may mean that pituitary stores of releasable LH were lower on Day 20 than on Day 10.

Apparently then, suckling plays the principal role in suppressing LH during lactation, possibly as a means of preventing ovulation too soon after parturition. With LH, but not FSH, suppressed by suckling, excess follicular growth might occur if FSH secretion were not inhibited. Apparently the ovaries (follicles) provide for suppression of FSH until after weaning. After weaning, LH rises and the synergistic action of LH and FSH leads to normal follicular maturation, ovulation, and initiation of estrous cycles.

### *Prolactin Secretion During Lactation*

Serum prolactin concentrations in the present study were similar to previous observations in lactating pigs (Threlfall et al., 1974; van Landeghem and van de Wiel, 1978) but higher than concentrations observed by others (Bevers et al., 1978; Mulloy and Malven, 1979). These variations in magnitude were probably due to differences in purity of standards and/or specificities of antiserum. Prolactin declined when litters were separated from sows and increased dramatically when litters were replaced and allowed to nurse (Fig. 5), similar to previous findings (Bevers et al., 1978). Reasons for consistently higher prolactin secretion in intact compared with ovex sows are not clear (Fig. 4), but may be due to some factor produced by the ovary.

Prolactin tended to decline from Day 10 to Day 20 (Fig. 4) and to Day 30 (day of temporary separation of four litters, Fig. 5A). Minaguchi and Meites (1967) suggested that suckling acts on the rat hypothalamus to suppress release of LHRH and of prolactin inhibiting factor (PIF), subsequently leading to decreased LH and increased prolactin secretion. Divergence between LH and FSH secretion during lactation observed in monkeys (Weiss et al., 1976) and humans (Rolland et al., 1975) occurs when elevated prolactin secretion is accompanied by constant low LH, but rising FSH levels. In the present study, no acute effects on LH secretion were observed when suckling was interrupted for 4 h even though prolactin concentrations declined dramatically (Fig. 5). Parvizi et al. (1976) failed to show any relationship between serum LH and periods of nonsuckling in the miniature pig. However, we recently noted that basal serum FSH concentrations increased before LH when suckling periods were limited to 12 h/day between Days 21 and 28 of lactation (Stevenson and Britt, unpublished observation).

Studies in lactating rats have demonstrated that suckling contributes more than high levels of prolactin to suppression of gonadotropins during early lactation, but during later lactation prolactin may account for decreased gonadotropin secretion (Smith, 1978c). However, suckling appears to be necessary for the inhibitory effect of prolactin to be manifested.

### *Control of Postweaning Gonadotropin Surges*

Interval from weaning to estrus in this study was consistent with results reported for sows following lactations of similar duration (Allrich et al., 1979). Relationships between changes in serum estrogens, progesterone, and LH after weaning (Fig. 7) were similar to those in previous work (Stevenson et al., 1978; Dyck et al., 1979), and concentrations around estrus were not different from those reported for cycling females (Niswender et al., 1970; Rayford et al., 1971; Henricks et al., 1972). Concentrations of FSH in serum increased during Days 1 and 2 after onset of the postweaning estrus, similar to the increase observed in cycling gilts on Days 2 and 3 following estrus (Rayford et al., 1974).

Patterns of estrogen secretion following weaning were remarkably similar in intact sows (note standard errors in Fig. 7A). The decline in total estrogens after weaning suggests that some factor associated with lactation stimulated estrogen secretion. Suckling causes an increase in adrenal corticoid secretion in cattle (Smith et al., 1972), and it is possible that the pig adrenal secretes estrogen or an estrogen precursor in respone to the suckling stimulus (Heap et al., 1966). This concept is supported by our observation that ovariectomy during lactation did not affect level of estrogens in serum. The synchronous rise in estrogens during Day 4

after weaning in intact sows preceded the onset of estrus and the surges of LH and FSH that were observed. This suggests that estrogen secreted from preovulatory Graafian follicles around estrus was a key signal for inducing the preovulatory surge in LH and the subsequent rise in FSH. This casual relationship between increased serum estrogens and subsequent changes in LH and FSH secretion at weaning was solidified by our studies in ovex sows. When ovex sows were given estradiol-17$\beta$ in doses to mimic the postweaning changes in estrogens, subsequent changes in LH and FSH paralleled those in intact sows following weaning. Furthermore, duration of the induced estrus, time to the onset of the LH peak, and duration and magnitude of the LH peak were not different from that observed in intact sows (Table 3).

The fact that total estrogens were not elevated more than twofold during estradiol administration was unexpected. Although blood was not collected until 6 h after each successive estradiol injection, serum estrogens never exceeded 100 pg/ml in individual samples. Slow release of estradiol from injection sites or rapid metabolism or conjugation to inactive steroids may have diluted the overall increase in serum estrogens. When estrogen treatment ended, blood level of estrogens declined slowly over the next 5 days, also suggesting that release from injection sites may have been slow.

Estrogen treatment caused an increase in prolactin. This is consistent with previous reports that serum prolactin increases at estrus in pigs (Brinkley et al., 1973; Bevers et al., 1978), rats (Wuttke et al., 1971), and sheep (Kann and Denamur, 1974). Since estrogen can apparently induce prolactin release in pigs (Fig. 8), in rats (Coppings and McCann, 1979), and cattle (Schams, 1974), it is suggested that surges of LH, FSH, and prolactin at estrus are caused by the same trigger, i.e., estrogen.

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