CHANGES IN THE HYPOTHALAMIC-HYPOPHYSAL-OVARIAN AXIS OF PRIMIPAROUS SOWS FOLLOWING WEANING OR PULSATILE GONADOTROPIN RELEASING HORMONE ADMINISTRATION AND WEANING

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ABSTRACT

Lactating primiparous sows were used to examine relationships among hypothalamic gonadotropin releasing hormone (GnRH), serum, and anterior pituitary gonadotropins and follicular development after weaning or after administering GnRH pulses (1.5 ug) once hourly for 72 h before weaning. Control sows were either slaughtered at 0 or 72 h after weaning or were cannulated for collection of blood samples until 24 h after estrus. Sows pulsed with GnRH were either slaughtered 72 h after beginning of GnRH treatment or were cannulated for collection of blood samples until 24 h after estrus. Exogenous GnRH pulsed hourly during 72 h prior to weaning stimulated follicular growth as demonstrated by an increase in number of surface follicles >5 mm in diameter and a decrease in number of follicles <5 mm in diameter. Interval (h) from weaning to an increase in estradiol (>16 pg/ml) was less in GnRH-pulsed than in control sows (P < 0.05), but hours from weaning to estrus were similar between groups. Amounts of GnRH in the medial basal hypothalamus (MBH), stalk median eminence (SME), and hypophyseal portal area (HPA) were similar among control sows killed at 0 or 72 h and sows pulsed with GnRH. Serum concentrations of luteinizing hormone (LH) and frequency of release of LH were similar between GnRH-pulsed and control sows, but concentrations of LH and follicle stimulating hormone (FSH) in anterior pituitary were lower in GnRH-pulsed sows than control sows. Administration of GnRH for 72 h prior to weaning in primiparous sows stimulated follicular growth as manifested by increased secretion of estrogen; however, the amount of follicular growth was apparently inadequate to hasten the onset of estrus after weaning.
INTRODUCTION

Suckling by the litter suppresses secretion of GnRH and thereby limits the amount of LH and FSH secreted during lactation in pigs (1). As a consequence, ovarian follicular development is lower during lactation than after weaning. The largest follicles normally observed during lactation seldom exceed 5 mm in diameter, but preovulatory follicles larger than 10 mm usually appear within 4 to 7 d after weaning (1). Follicular development during lactation can be stimulated by exogenous gonadotropins or by endogenous gonadotropins released by hourly pulse of exogenous GnRH (1, 2).

In previous studies, we observed that hourly pulses of GnRH would stimulate follicular development and estrus in lactating (2) or chronically anestrous weaned sows (3). In all cases, GnRH was given until 24 h after onset of estrus or for 7 d, whichever came first. It is unclear whether shorter periods of GnRH treatment during lactation would initiate follicular development that would continue after weaning.

Therefore, the objective of this experiment was to determine if hourly pulses of GnRH for 72 h prior to weaning would stimulate follicular growth and hasten onset of estrus after weaning in primiparous sows. We also compared changes in the hypothalamic-hypophyseal-ovarian axis occurring spontaneously after weaning to changes occurring after pulsatile GnRH prior to weaning.

MATERIALS AND METHODS

Landrace × Yorkshire primiparous sows (n = 26) that farrowed in March 1983 and lactated 24.8 ± 0.3 d were used. Litter sizes were equalized within 3 d of parturition and average litter size at weaning was 10.0 ± 0.2 pigs. Temperature within the farrowing house was maintained at about 20°C by providing supplemental heat. Lights in the farrowing house were on during feeding and sample collection, but length of photoperiod depended primarily on sunlight from windows on both sides of the house. During lactation, sows were fed ad libitum a corn-soybean meal diet supplemented with vitamins and minerals according to National Research Council (4) guidelines.

After farrowing, sows were randomly assigned to control or GnRH treatment groups. Control sows were slaughtered either at weaning (n = 5) or at 72 h after weaning (n = 6), or they were checked for estrus twice daily beginning at weaning (n = 5). Sows assigned to receive GnRH pulses were administered 1.5 µg GnRH/h intravenously (i.v.) for 72 h before weaning and were slaughtered at weaning (n = 5) or checked for estrus twice daily beginning with initiation of GnRH (n = 5). All control sows and GnRH-treated sows that were not slaughtered were cannulated. Cannulas were inserted through a 12-ga needle under local anesthesia and were taped to the skin surface with elastic tape. Ovaries, anterior pituitaries, and areas of the brain encompassing the hypothalamus were obtained at slaughter. Number of surface ovarian follicles with diameters <5 mm or >5 mm were recorded.

Hypothalamic tissue was dissected into the medial basal hypothalamus (MBH) and stalk–median eminence (SME), and the hypophyseal portal area (HPA) was separated from the remainder of the anterior pituitary as previously described (5). All tissues were stored in liquid nitrogen until subsequent analyses. Tissues for determination of GnRH (MBH, SME and HPA) were processed as
previously described (6). Anterior pituitaries were processed as described previously (5) except that pituitary extracts were diluted 1:9000 before LH and FSH assays were done.

Blood samples were obtained via indwelling vena cava cannulas at 12-h intervals from 72 (GnRH-treated) or 0 (control) h before weaning until 24 h after the onset of estrus. Blood samples were also obtained at 15-min intervals for 4 h beginning at 0, 24, 48, 72 and 96 (GnRH-treated only) h after weaning or initiation of GnRH, respectively.

Concentrations of FSH (7, 8), LH (9) and GnRH (5, 10) were quantified by radioimmunoassay. Average coefficients of variation (CVs) were 12.3 and 11.6% for FSH and GnRH, respectively. Average intra- and inter-assay CVs for seven LH assays were 11.9 and 16.2%, respectively. Samples collected at 12-h intervals were subjected to radioimmunoassay for estradiol-17 beta (2). Samples collected from sows not exhibiting estrus were analyzed for progesterone (9). Intra- and inter-assay CVs for six estradiol assays were 13.8 and 14.9%, respectively. Concentrations of estradiol less than two times the assay sensitivity were designated less than basal (<16 pg/ml).

Least-squares analyses of variance were by procedure General Linear Models of the Statistical Analysis System (11). Comparisons of tissue content of GnRH, LH and FSH and follicle numbers between control and GnRH-pulsed sows were made by Student’s t-test (12). Content of GnRH in MBH was transformed to log 10 to remove (P > 0.2) heterogeneity of variance (13). Duncan’s multiple-range test (12) was used to separate means (0 h, 72 h, and GnRH). Differences in serum levels of LH and estradiol and frequency of LH release were analyzed using split-plot analysis of variance (14). Models included treatment group, hours from weaning, and the treatment x hour interaction. Frequency of episodic release of LH was determined by a subjective method as described previously (15).

RESULTS

One sow in the GnRH-treated group failed to exhibit estrus and ovulate following weaning; thus, progesterone and estradiol remained less than 1.0 ng/ml and 9 pg/ml, respectively, and all data for that sow were deleted. Intervals (h) from weaning to estrus were similar between control (93 ± 5, X ± SEM) and GnRH-treated sows (n = 4, 69 ± 14). Estradiol was elevated (>16 pg/ml) prior to weaning in two of four GnRH-treated sows (Figure 1), but intervals from weaning to estrus were similar (range 24 to 96 h) in all four GnRH-treated sows. Average interval (h) from weaning to elevated estradiol was greater in control (43 ± 9) than GnRH-treated (15 ± 9, P = 0.07) sows. Also, concentrations of estradiol before and/or after weaning were elevated above basal (>16 pg/ml) for a longer period (h) in GnRH-treated (84 ± 4) than control sows (53 ± 6, P<0.05).

Concentrations and frequency of release of LH in control and GnRH-treated sows are shown (Table 1). Mean concentration and frequency of episodic release were similar between groups and did not change significantly with interval from weaning or beginning of GnRH treatment.

Data from control sows slaughtered at 0 or 72 h after weaning and for GnRH-treated sows slaughtered immediately after weaning are presented (Table 2). Levels of GnRH in the MBH, SHE and HPA were not affected by interval from
weaning or GnRH treatment. However, anterior pituitary concentration of LH was lower in GnRH-pulsed sows than in control sows killed at 0 h (P = 0.06) or 72 h (P = 0.09). Similarly, pituitary FSH concentration was lower in GnRH-pulsed sows than in 0-h (P = 0.03) or 72-h (P = 0.07) control sows.

![Graph showing estradiol levels over time]

Figure 1. Average concentrations of estradiol in weaned control sows (n = 5, ---) and sows pulsed with GnRH in which estradiol increased (n = 2,----) or remained basal (n = 2,----) before weaning. Standard errors ranged from 0 to 9 and 0 to 23 for control and GnRH-treated sows, respectively.

Table 1. Mean concentration of LH and frequency of release of LH in weaned control sows and sows pulsed with GnRH for 72 h prior to weaning

<table>
<thead>
<tr>
<th>Hours from weaning or GnRH</th>
<th>LH, ng/ml&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Frequency, peaks/4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GnRH</td>
</tr>
<tr>
<td>0</td>
<td>1.08 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ± 0.05</td>
</tr>
<tr>
<td>24</td>
<td>0.99 ± 0.03</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>48</td>
<td>1.79 ± 0.26</td>
<td>1.19 ± 0.07</td>
</tr>
<tr>
<td>72</td>
<td>0.71 ± 0.02</td>
<td>0.89 ± 0.42</td>
</tr>
<tr>
<td>96</td>
<td>1.15 ± 0.91</td>
<td>1.15 ± 0.91</td>
</tr>
</tbody>
</table>

<sup>a</sup>Average of 17 samples/sow/4 h.
<sup>b</sup>Mean ± SEM.
Characteristics of populations of surface ovarian follicles are presented (Table 2). Number of follicles < 5 mm in diameter was lower in 0-h than in 72-h control sows (P < 0.05). Number of small follicles was lower in GnRH-treated sows than in either group of control sows. Number of follicles with diameters > 5 mm was greater in GnRH-pulsed than in 0-h controls (P < 0.05), with 72-h controls having an intermediate number (Table 2).

DISCUSSION

Results from this experiment indicate that hourly pulses of GnRH for 72 h prior to weaning stimulated ovarian follicular growth in lactating primiparous sows. Follicular growth, as estimated from number of follicles of different sizes, was greater in GnRH-pulsed sows than in sows slaughtered 0 or 72 h after weaning. Previous studies have demonstrated that the number of small follicles decreases concomitant with an increase in the number of large follicles during the period from weaning to estrus (1).

This study revealed that the duration of GnRH pulses necessary for stimulation of sufficient follicular growth to induce estrus during lactation in primiparous sows is greater than 72 h. Giving 72 hourly pulses of GnRH prior to weaning induced some follicular growth, but the onset of estrus after weaning was not significantly different from that observed for controls. Follicular changes in sows given GnRH were similar to those that occurred during 72 h after weaning in control sows. Two of four GnRH-pulsed sows had elevated estradiol levels within 48 h after beginning GnRH, and estradiol levels remained above basal after weaning in these sows (Figure 1). In previous studies in which estrus was induced in lactating (2, 16) or chronically anestrous (3) sows, GnRH was administered until onset of estrus or 24 h after onset of estrus, which occurred between 72 and 123 h after onset of GnRH treatment.

We cannot determine why elevated estradiol during lactation in two GnRH-treated sows did not induce estrus before weaning or result in shorter intervals from weaning to estrus (Figure 1). Suckling-induced increases in glucocorticoids or endogenous opioid peptides (EOPs) may have altered the ability of estradiol to induce estrus. For example, elevated estradiol levels during Day 16 to 42 of lactation were not associated with estrus (17), and estradiol treatment of sows at weaning resulted in only weak signs of estrus, although estradiol was elevated (>40 pg/ml) for 7 d (18). A suckling-induced rise in cortisol may have blocked estrus until after weaning. A synthetic glucocorticoid blocked estrus in estradiol benzoate-treated gilts in spite of estradiol levels similar to control gilts exhibiting estrus (19). Concentration of EOPs are elevated during lactation in the sow (20) and blocking EOP receptors facilitated estrus in the estrogen-primed rat (21).

Factors associated with suckling may have influenced the responsiveness of the hypothalamic-hypophyseal axis of primiparous sows to GnRH and estradiol. Although direct comparisons have not been made, intervals from beginning of GnRH pulses to estrus are typically longer in lactating primiparous (16) than multiparous sows (2). Moreover, it is well established that duration of postweaning anestrus is longer in primiparous than in multiparous sows (22, 23). This difference between primiparous and multiparous sows apparently is associated with suckling, because interval from onset of GnRH pulses to estrus in weaned, chronically anestrous, primiparous sows (3) was similar to intervals for GnRH-treated lactating multiparous sows (2). Responsiveness of the anterior
Table 2. Content of GnRH in the hypothalamus, concentrations of LH and FSH in the anterior pituitary, and follicular development for control sows slaughtered at weaning or 72 h after weaning and for sows slaughtered after 72 h of GnRH treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>GnRH, ng total</th>
<th>Anterior pituitary</th>
<th>No. follicles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MBH</td>
<td>SHE</td>
<td>HPA</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>85.4 ± 66.0a</td>
<td>57.3 ± 7.3a</td>
<td>24.9 ± 11.2a</td>
</tr>
<tr>
<td>72 h</td>
<td>11.2 ± 2.0a</td>
<td>44.2 ± 10.5a</td>
<td>18.5 ± 7.3a</td>
</tr>
<tr>
<td>GnRH</td>
<td>9.1 ± 0.9a</td>
<td>56.7 ± 11.2a</td>
<td>15.8 ± 5.1a</td>
</tr>
</tbody>
</table>

All values are mean ± SEM.

a,b,c Means in same column with different superscripts are different (P < 0.09).
pituitary and hypothalamus to GnRH (24) and estradiol benzoate (25), respectively, changes as lactation progresses. Therefore, differences may exist in response of the hypothalamic-hypophyseal-ovarian axis to endocrine stimulation.

Hourly pulses of GnRH may have affected follicular growth independent of the hypothalamic-hypophyseal axis, because the number of small follicles (<5 mm in diameter) was lower in GnRH-pulsed than in either group of control sows. This observation is consistent with a recent report (26) that demonstrated a possible physiological role of GnRH or GnRH-like material as an atretic signal in the ovary.

Although serum levels of LH were similar between control and GnRH-pulsed sows, concentrations in the anterior pituitary were greater in controls (Table 2). Different frequencies of GnRH stimulation of the anterior pituitary may have affected pituitary stores of LH, because Clarke and Cummins (27) recently reported that releasable pools of anterior pituitary LH increased with decreased frequency of GnRH stimulation. Frequency of release of LH in GnRH-pulsed sows was not significantly greater than in control sows (Table 1). Cox and Britt (2) reported that LH peaked from 5 to 10 min after GnRH in lactating sows. In our study, samples were not obtained until 15 min after GnRH; thus, we may not have detected all episodes of release of LH induced by exogenous GnRH.

Serum FSH levels were not measured in this study because serum and pituitary FSH are similar before and after weaning (1). In addition, anterior pituitary content of FSH reflects physiological changes in secretion, because in a companion study (28), we found that sows weaned during summer had greater serum FSH but lower anterior pituitary FSH than sows weaned during winter.

The observation that sows with greater levels of pituitary FSH also had a greater number of small follicles (Table 2) may indicate a greater output of inhibin from small follicles and thus a greater inhibition of FSH release from the anterior pituitary (29). Cauterization of ovarian follicles >3 mm in diameter resulted in elevated serum FSH levels 24 to 36 h later in lactating sows (30). Differences in pituitary FSH were probably not directly related to GnRH pulsing because hypothalamic content of GnRH and serum and pituitary LH increased from 0 to 96 h after weaning while serum and pituitary FSH remained unchanged (1). Stevenson et al. (9) demonstrated that LH and FSH are under divergent control during lactation in the pig. They found that FSH but not LH increased following ovariectomy in lactating sows.

The observation that content of GnRH in the hypothalamus and concentration of LH in the anterior pituitary did not change between 0 and 72 h after weaning in control sows was unexpected. Previously, we reported that hypothalamic GnRH and levels of LH in the anterior pituitary increased from 0 to 60 h after weaning (5). In this study, values of GnRH in MBH were highly variable. In particular, one sow in the 0-h control group had an extremely high amount of GnRH in the MBH (382 ng). In the initial studies, animals were not sacrificed until the fifth or sixth week of lactation and litter sizes were only 60 to 70% as large as in the present study. Also, multiparous sows were used in the initial study. Therefore, the ability of the hypothalamus to respond to weaning by increasing synthesis of GnRH may have been greater in the initial studies.

Exogenous GnRH, pulsed at hourly intervals for 72 h prior to weaning, apparently stimulated follicular growth in primiparous sows; however, interval
from weaning to estrus was not influenced by GnRH treatment. This was apparently because the period of stimulation was inadequate to promote a continuation of follicular growth after cessation of GnRH treatment.

REFERENCES


