Relationship of secretion of GnRH in vitro to changes in pituitary concentrations of LH and FSH and serum concentrations of LH during lactation in sows

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The objectives of this study were (1) to determine whether release of GnRH in vitro was related to concentrations of LH and FSH in serum and pituitaries and to oestradiol in serum of sows at mid- or late lactation, and (2) to determine whether weaning at mid-lactation changes concentrations of these hormones from values expected at mid-lactation to values expected at late lactation. Multiparous crossbred sows were killed on day 14 (n = 5) or day 28 (n = 5) of lactation or on day 14 post partum after litters had been weaned on day 10 (n = 5). Blood samples were taken every 6 h for 4 days before sows were killed, and the preoptic suprachiasmatic area, medial basal hypothalamus, stalk median eminence, anterior pituitary and ovaries were collected at slaughter. Sows killed on day 14 after having their litters weaned on day 10 had more (P < 0.01) preovulatory follicles (> 6 mm in diameter) than lactating sows killed on day 14 or 28 (7.0 ± 1.2 versus 0.2 ± 0.1 and 1.5 ± 0.8, respectively). Concentrations of LH, FSH and oestradiol in serum during 90 h before slaughter were greater (P < 0.05) in weaned sows and lactating sows killed on day 28 than in lactating sows killed on day 14 (LH: 0.72 ± 0.3 and 0.68 ± 0.3 versus 0.45 ± 0.2 ng ml⁻¹; FSH: 39.3 ± 2.7 and 57.3 ± 4.0 versus 28.8 ± 1.6 ng ml⁻¹; oestradiol: 10.9 ± 1.6 and 5.6 ± 0.7 versus 2.7 ± 0.2 pg ml⁻¹, respectively). Weights of anterior pituitaries did not differ among groups, but concentrations of LH in anterior pituitaries increased (P < 0.05) and concentrations of FSH in pituitaries tended to increase although the increase was not significant (P < 0.10) from day 14 to day 28 of lactation, whereas pituitary concentrations of gonadotrophins in weaned sows were intermediate (LH: 0.45 ± 0.2, 0.94 ± 0.2 and 0.65 ± 0.1 mg g⁻¹; FSH: 8.7 ± 1.2, 13.8 ± 2.6 and 10.9 ± 1.2 mg g⁻¹ for day 14 lactating, day 28 lactating and weaned groups, respectively). Content of GnRH in the preoptic area, and medial basal hypothalamus did not differ among groups. Content of GnRH in stalk median eminence was lower (P < 0.05) in weaned sows, but was similar among sows killed on day 14 or day 28; however, the proportion of residual GnRH released by the stalk median eminence in response to a challenge with K⁺ in vitro was greater (P < 0.05) for sows at day 14 than for weaned sows and sows at day 28 (21.5 ± 4.5 versus 13.7 ± 2.8 and 7.0 ± 1.6%, respectively), indicating an increase in rate of release of GnRH in vivo and a consequent stimulation of synthesis and release of LH and FSH. These results are consistent with the hypothesis that suckling/lactation alters endogenous rate of GnRH secretion, thereby influencing secretion of gonadotrophins and follicular development.

Introduction

Lactation in pigs is characterized by anoestrus that is maintained by the presence of several suckling piglets (Britt et al., 1985; Varley and Foxcroft, 1990; Britt and Sesti, 1991). The principal cause of lactational anoestrus is the suckling-induced inhibition on secretion of gonadotrophins which leads to a diminished follicular development (Britt et al., 1985). Moreover, sows in early lactation manifest a dysfunction of the oestrogen-induced positive feedback mechanism on LH secretion which tends to alleviate lactation proceeds (Elsaesser and Parvizi, 1980; Cox et al., 1988; Sesti and Britt, 1993).

Cox and Britt (1982a) demonstrated that there was a significant increase in GnRH concentration in the stalk median eminence by 60 h after weaning, and that the amount of GnRH reaching the hypophyseal portal area had increased by 96 h after weaning. Follicular development, fertile oestrus and ovulation can be induced in lactating sows by hourly or bihourly pulses of GnRH, indicating an apparent lack of hypothalamic GnRH...
release during lactation in pigs (Cox and Britt, 1982b; Britt et al., 1985). Sesti and Britt (in press) treated primiparous sows with the neuroexcitatory amino acid analogue N-methyl-D,L-aspartic acid (NMA) and reported that the size of the readily-releasable pools of GnRH, as estimated by changes in LH secretion after NMA, increased linearly throughout lactation, indicating a gradual decrease in the suckling-induced inhibition of GnRH secretion. These studies suggest that changes in hypothalamic content of GnRH and pituitary concentrations of gonadotrophins may be occurring throughout lactation rather than just after weaning.

Relationships between changes in concentration and secretion of GnRH by the hypothalamus and pituitary synthesis and secretion of gonadotrophins during lactation in sows have not been reported. Thus, we examined whether hypothalamic content of GnRH and secretion of GnRH in vitro were related to pituitary and serum concentrations of gonadotrophins and oestradiol in sows at mid- and late lactation and in sows weaned at mid-lactation. We hypothesized that the normal increase in basal secretion of gonadotrophins that occurs as lactation proceeds (Stevenson et al., 1981) is accompanied by changes in GnRH content in the hypothalamus, GnRH turnover and pituitary concentrations of LH and FSH, and that weaning at mid-lactation would accelerate the rate of these changes.

Materials and Methods

Animals and experimental procedures

Fifteen crossbred (Landrace × Large White) multiparous sows with an average weight of 219 ± 21 kg were used in this study. They had produced at least two litters and were nursing at least nine piglets during this study. Sows and litters were kept in individual farrowing crates throughout the experimental period and were fed twice a day (07:00 and 16:00 h) a corn-soybean meal diet supplemented with vitamins and minerals according to NRC (1988) guidelines. Animals were exposed to natural daylight through windows and room lights were on from 07:00 until 08:00 h, except during collection of blood samples when lights were on continuously.

Sows were assigned to one of three experimental groups (n = 5 per group) as follows: (1) lactating sows were killed at day 14 post partum, (2) sows were weaned at day 10 and killed at day 14 post partum, and (3) lactating sows were killed at day 28 post partum. Sows were nonsurgically fitted with indwelling catheters inserted through a jugular vein into the cranial vena cava 120 h before slaughter (Britt et al., 1991). Blood samples were collected every 6 h for 90 h before slaughter; the first sample was collected 6 h after weaning on day 10 for sows killed on day 14. Animals were given a lethal dose of T-61 Euthanasia Solution (Hoescht-Roussel Agri-Vet Co., Sommerville, NJ) which induced an immediate and deep general anaesthesia without excitation, followed, within seconds, by respiratory paralysis, cerebral death and circulatory collapse.

Within 10 min of death, the pituitary gland, a block of brain tissue encompassing the hypothalamus, and the ovaries were excised. The anterior pituitary was trimmed from the whole hypophysis and immediately put into liquid nitrogen. Each hypothalamic block was divided into the preoptic suprachiasmatic area, medial basal hypothalamus and stalk median eminence. The medial basal hypothalamus was defined as a block of tissue bounded rostrally by the optic chiasma, caudally by the mammillary body, laterally by the hypothalamic sulci and dorsally by a cut 5 mm deep. The preoptic suprachiasmatic area was limited rostrally approximately 5 mm anterior to the optic chiasma and caudally by the rostral border of the medial basal hypothalamic area. The stalk median eminence was easily detached from the medial basal hypothalamus without cutting and was then cut away at its junction with the pituitary gland. The stalk median eminence was halved for subsequent incubation as duplicate samples. Ovaries were transported to the laboratory and the follicular population was classified as small (< 4 mm in diameter), medium (4–6 mm in diameter) or large (> 6 mm in diameter; preovulatory).

In vitro incubation of hypothalamus

Immediately after collection of preoptic suprachiasmatic area, medial basal hypothalamus and stalk median eminence, tissues were transferred to glass incubation vials (20 mm × 40 mm) containing 2 ml Krebs–Ringer bicarbonate buffer (Sigma Chemical Co., St Louis, MO), pH 7.4, supplemented with 1 mg glucose ml⁻¹. Vials were transported to the laboratory and upon arrival, approximately 30 min after collection, all tissues were weighed, medium was changed and the incubation period started. Incubation vials were maintained in a water-bath shaker at 37°C with constant shaking (45 cycles min⁻¹) in an atmosphere of 95% O₂ and 5% CO₂. Tissues were incubated for 30 min, and after a change of medium, for another 60 min. Tissues were then exposed to 56 μmol K⁺ l⁻¹ and release of GnRH into medium was estimated during two periods of 30 min. Immediately after each period of incubation, the liquid contents were carefully transferred into plastic tubes and centrifuged at 3000 g for 30 min at 4°C. The supernatant was decanted and frozen at −20°C until concentrations of GnRH in the medium were determined. After the last 30 min incubation, the hypothalamic tissues remaining were homogenized in 2 ml 0.1 mol HC1 l⁻¹, centrifuged at 3000 g for 30 min at 4°C and the supernatant collected and stored at −20°C until determination of cellular content. For all hypothalamic areas, total content and(or) concentrations of GnRH were calculated by using the amount of GnRH released in vitro during all incubation periods including the period for transport of the tissues from the farm to the laboratory plus the cellular content determined after incubation. Content of residual GnRH was estimated as total content minus the amount released during transport and during first two incubation periods before the challenge with K⁺.

Blood samples, pituitary tissue and radioimmunoassays

Blood samples (8 ml) were drawn from the catheters every 6 h for 90 h before slaughter and were allowed to clot for 12 h at 4°C and then centrifuged at 1300 g for 30 min. Serum was decanted into plastic tubes and frozen at −20°C until assayed for LH, FSH and oestradiol.

Each anterior pituitary was weighed and homogenized in gel–phosphate-buffered saline (PBS: 0.14 mol sodium chloride...
1−1, 0.01 mol phosphate 1−1 and 0.1% gelatin, pH 7.0) at a concentration of 100 mg tissue ml−1 PBS. Homogenates were centrifuged at 3000 g for 30 min and the supernatants were frozen and stored at −20°C before cellular contents of LH and FSH were measured. All pituitary concentrations were expressed per gram of wet tissue.

Serum and tissue concentrations of FSH were measured by radioimmunoassay as described by Guthrie and Bolt (1983) and modified by Esbenshade and Britt (1985). Purified pFSH (USDA-FSH-B-1) and 125I-labelled pFSH (USDA-FSH-PP1) were used as standards and radiolabelled antigen, respectively. The first antibody was anti-pFSH (USDA-398-048) and the average assay sensitivity was 4.9 ng ml−1 at 95% binding. The intra- and interassay coefficients of variation (CVs) were 10.1 and 14.8%, respectively.

Serum and tissue concentrations of LH were measured by a previously validated radioimmunoassay (Stevenson et al., 1981; Armstrong and Britt, 1987). Purified pig LH (LER-786-3) was used as standard and 125I-labelled LH (USDA-FSH-B-3) was used as radiolabelled antigen. Assay sensitivity at 95% binding was 0.16 ng ml−1 and intra- and interassay CVs were 7 and 12.2%, respectively.

Serum concentrations of oestriadiol were determined on extracts of 200 µl duplicate samples by the method of Cox et al. (1987). The radioiodinated antigen was oestriadiol (Sigma E88-75; Sigma Chemical Co., St Louis, MO) and the antibody was provided by N. R. Mason (Mason and Marsh, 1975). Intra- and interassay CVs were 9.8 and 12.9%, respectively, and the assay sensitivity was 1.7 pg ml−1 at 90% binding.

GnRH secreted into the incubation medium was measured by a single antibody radioimmunoassay slightly modified from that described by Zieck et al. (1989). Synthetic GnRH (acetate salt; Sigma Chemical Co.) was radioiodinated by the iodogen method (Fraker and Speck, 1978) and the labelled hormone was separated from free iodine by ion exchange chromatography (Voet and Voet, 1990). Antiserum (50 µl) diluted at 1:28 000 (dilution at which 30% of added 125I-labelled GnRH was bound) in PBS-EDTA was added to 100 µl unknown and standard samples diluted in PBS, followed 30 min later, by 50 µl of iodinated GnRH (about 25 000 c.p.m.). After incubation for 36–48 h at 4°C, free labelled GnRH was separated from bound by adding 2 ml ice-cold 100% ethanol, incubating for 15 min, centrifuging at 3000 g for 20 min and decanting the supernatant. Bound 125I-labelled GnRH was estimated by counting the pellet. The range of standards used was 0.3125–80 pg per tube and the average assay sensitivity was 5.6 pg ml−1 at 95% binding. Intra- and interassay CVs were 9 and 11.8%, respectively. The anti-GnRH antiserum ESB-297 used in this assay was raised in our laboratory by immunizing pigs against the native GnRH conjugated to BSA (Esbenshade and Britt, 1985). ESB-297 is a polyclonal antibody that does not crossreact (<0.1%) with the GnRH agonists des-Gly10[D-Ala6]–LHRH ethylamide or des-Gly10[D-Phe6]–LHRH (Esbenshade and Britt, 1985; Traywick and Esbenshade, 1988) or with FSH, TRH or CRF (<0.1%; Jayes et al., 1991). ESB-297 is directed against the carboxy terminus of the native GnRH molecule because GnRH fragments lacking the first two or three amino acids showed 100% crossreactivity (Jayes et al., 1993; M. Heit and F. L. Jayes, unpublished results).

Recoveries of GnRH in the incubation system were estimated by adding 500 pg native GnRH to vials containing 2 ml incubation media and a known amount of hypothalamic tissue. After incubation for 30 min under the normal conditions described above, medium was assayed and 470 ± 18 pg (mean ± SEM; n = 6) or 94% of the added GnRH was recovered.

Statistical analyses

Data were analysed by least squares analyses of variance using General Linear Models of the Statistical Analysis System (SAS, 1988). Hormonal profiles for LH, FSH and oestriadiol during the 4 day sampling, number of small, medium and large follicles, and amount of GnRH secreted in incubation medium were compared among the different experimental groups using appropriate one-way or split-plot models. Means were separated by Student–Newman–Keul's test (SAS, 1988). GnRH concentrations in media before and after a challenge with K+ and different sizes of follicle on the ovaries were analysed by one-way analysis of variance. Repeatability of percentage of the total content of GnRH that was released in medium from the stalk median eminence halves after the K+ challenge was calculated by the PROC NESTED procedure using stage and sow (sow nested within stage) as class variables (SAS, 1988).

Results

Profiles of oestriadiol and gonadotrophins

Concentrations of oestriadiol in serum rose linearly (P < 0.01; R2 = 0.92) from the time of weaning on day 10 (3.3 ± 0.4 pg ml−1) until slaughter (17.6 ± 4.0 pg ml−1) on day 14 in weaned sows (Fig. 1). Lactating sows at day 28 had
and higher serum versus 4.5 sampling 0.2 did greater lactating five of day Fig. (54.3 14 lactation 1 ng ml⁻¹; Fig. 1). In weaned sows, average concentrations of LH decreased (P < 0.05) from 0.94 ± 0.07 ng ml⁻¹ at −84 h to 0.62 ± 0.2 ng ml⁻¹ at −72 h and stayed low for the remainder of the sampling period (Fig. 2). Concentrations of FSH decreased slowly, but linearly (P < 0.05; R² = 0.87), from weaning (54.3 ± 8.4 ng ml⁻¹) until the time of slaughter (13.2 ± 4.5 ng ml⁻¹; Fig. 2).

Serum LH concentrations during 90 h before slaughter were higher (P < 0.05) in weaned sows and lactating sows on day 28 than in lactating sows on day 14 (0.72 ± 0.3 and 0.68 ± 0.3 versus 0.45 ± 0.2 ng ml⁻¹, respectively; Fig. 2). Likewise, serum concentrations of FSH were greater (P < 0.05) in weaned sows and in those killed on day 28 of lactation than in those sows killed at day 14 of lactation (overall means: 39.3 ± 2.7 and 57.3 ± 4.9 versus 28.8 ± 1.6 ng ml⁻¹, respectively; Fig. 2).

Weights of anterior pituitaries did not differ among groups (570 ± 105, 640 ± 53 and 618 ± 97 mg for day 14 lactating, day 14 weaned and day 28 lactating groups, respectively); however, LH concentrations were lower (P < 0.05) in lactating sows on day 14 than on day 28, whereas concentration of LH in the weaned group was intermediate (Fig. 3). Concentrations of FSH in anterior pituitaries showed a tendency (P < 0.10) to increase from mid- to late lactation (Fig. 3).

**Ovarian follicular population**

There was a shift in size of follicles on the ovaries during lactation. Lactating sows on day 14 had more (P < 0.01) small and fewer (P < 0.01) medium-sized follicles than did sows at late lactation. Weaning the litter at day 10 of lactation induced a rapid and significant (P < 0.01) increase in the number of large, preovulatory follicles (Fig. 4).

**GnRH concentrations in hypothalamus and secretion in vitro**

Tissue concentrations of GnRH in the preoptic supra-hypothalamic area and medial basal hypothalamus were lower (P < 0.01) than in the stalk median eminence (Fig. 5). Sows weaned at day 10 and killed at day 14 had a lower (P < 0.04)
GnRH and gonadotrophins in lactating sows

Fig. 4. Number of small (<3 mm; □), medium (4–6 mm; □) and large (>6 mm; □) follicles per ovary in sows killed at days 14 or 28 of lactation and in sows killed at day 14 post partum after their litters had been weaned on day 10. Sows at day 14 of lactation had more (P < 0.01) small and fewer (P < 0.01) medium-sized follicles than sows at day 28 of lactation. Weaned sows had a greater (P < 0.01) number of large follicles. Values are means ± SEM of ten observations (two ovaries per sow).

Fig. 5. GnRH concentrations in three hypothalamic areas in sows killed at days 14 (□) or 28 (■) of lactation and in sows slaughtered at day 14 post partum after their litters had been weaned on day 10 (□). Weaned sows had lower (P < 0.04) total content of hypothalamic GnRH than lactating sows. Values are means ± SEM of five observations.

GnRH in medium, if any, was below the sensitivity of the assay. Percentage of the residual tissue concentration of the decapptide that was released in medium was greater (P < 0.05) in stalk median eminence from sows at day 14 of lactation than for sows at day 28 (Table 1). Repeatability for the amount of GnRH released in medium after K+ for the stalk median eminence halves was 0.61 indicating that each stalk median eminence half produced reliable secretion data.

Discussion

Results from this study show that changes in hypothalamic concentrations of GnRH and release of GnRH in vitro are related to the dynamics of secretion of gonadotrophins and follicular growth during lactation. Readily releasable pools of GnRH, as assessed by in vitro secretion after K+, decreased as lactation proceeded, indicating an apparent increase in endogenous rate of release of GnRH. Concentrations of LH and FSH in the anterior pituitary increased during the 28 day lactation, and increased acutely during 4 days after weaning litters at day 10 post partum. Similarly, serum concentrations of gonadotrophins increased from mid- to late lactation and apparently acutely after weaning on day 10, leading to an increase in the number of medium and large follicles on the ovaries and to higher secretion of oestradiol.

Concentrations of GnRH in the preoptic suprachiasmatic area and medial basal hypothalamus did not differ among the three experimental groups, but GnRH content in stalk median eminence of sows weaned at day 10 and killed on day 14 were lower than those of sows at mid- and late lactation. Concentration of GnRH in these brain areas apparently does not change during early lactation in ewes (Moss et al., 1980), beef cows (Cermak et al., 1983; Moss et al., 1985) or dairy cows (Carruthers et al., 1980). It would therefore appear that concentrations of GnRH in the hypothalamus at a single point in time are not correlated strongly with changes in gonadotrophin secretion during lactation in ewes, cows and sows.

The release of GnRH from stalk median eminence tissue after a challenge with K+ in vitro decreased significantly from mid- to late lactation. According to Fink and Shewad (1989) the relationship between the amount of GnRH released into the portal blood and that stored in the hypothalamus provides an index of the endogenous rate of release of the neuropeptide. Although direct measurements of secretion of GnRH into the hypophysial portal blood were not performed in this study, the data from the K+-induced release of GnRH in vitro from stalk median eminence of sows at mid- or late lactation provide indirect evidence of changes in the endogenous rate of release of the decapptide during lactation in sows. The fact that stalk median eminence from sows at late lactation released less GnRH than did those from sows at mid-lactation, whereas there was no difference in total content of GnRH, could be explained by an increase in endogenous rate of release of GnRH from day 14 to day 28 of lactation. This increase in basal release of GnRH would have reduced the readily available stores of GnRH in the axon terminals in stalk median eminence, resulting in less GnRH being released after the K+ stimulation. In addition, we have estimated that the size of the readily-releasable pools of GnRH in primiparous sows increases linearly as lactation progresses...
Table 1. Residual content and $K^+$-stimulated GnRH release in vitro from stalk median eminence of sows at day 14 or 28 of lactation and sows at day 14 post partum after their litters were weaned at day 10

<table>
<thead>
<tr>
<th>Stage of lactation</th>
<th>Residual content</th>
<th>$K^+$-stimulated release</th>
<th>Percentage released from residual content*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14, lactating</td>
<td>18.1 ± 2.5b</td>
<td>3.9 ± 1.2b</td>
<td>21.5 ± 4.5b</td>
</tr>
<tr>
<td>Day 14, weaned</td>
<td>10.4 ± 1.9c</td>
<td>1.1 ± 0.2c</td>
<td>13.7 ± 2.8c</td>
</tr>
<tr>
<td>Day 28, lactating</td>
<td>17.4 ± 2.7b</td>
<td>1.1 ± 0.3c</td>
<td>7.0 ± 1.6c</td>
</tr>
</tbody>
</table>

Values are means ± SEM of ten observations (two stalk median eminence halves per sow).
*Calculated from mean percentage released by each half of stalk median eminence.

(Sesti and Britt, in press). These readily releasable pools presumably comprise GnRH-containing secretory granules that are located close to the plasma membrane of axon terminals in the stalk median eminence and are promptly released upon depolarization of neurones (Mellon et al., 1990).

An increase in endogenous rate of release, without changes in total content of GnRH in the hypothalamus, would explain the increase in serum and pituitary gonadotrophin concentrations found in this experiment. Studies with female rats demonstrated that very low rates of synthesis and release of GnRH are required to maintain normal pituitary—gonad function (Fink, 1988). It would appear that the increase in endogenous rate of release of GnRH in the hypothalamus, observed in this study, was probably due to the gradual decrease in suckling intensity that normally occurs as lactation progresses in sows (Britt et al., 1985).

This study is apparently the first to show that pituitary concentrations of LH increase during lactation in pigs. Concentrations of LH in the anterior pituitary were significantly greater at day 28 than at day 14 and concentrations of FSH tended to increase from mid- to late lactation. Concentrations of both gonadotrophins increased acutely, but not significantly, in sows whose litters were weaned at day 10. Increases in pituitary LH content during lactation have been reported in rats (Taya and Greenwald, 1982; Smith, 1978), beef cows (Moss et al., 1985) and ewes (Moss et al., 1980). Early evidence for changes in gonadotrophin secretion in lactating and/or weaned sows was derived from bioassays (Melampy et al., 1966; Crighton and Lamming, 1969). Crighton and Lamming (1969) suggested that LH synthesis and secretion is initiated by weaning, because concentrations of LH in the anterior pituitary increase by 3–4 days afterwards. Subsequently, Cox and Brit (1982a), using radioimmunoassay, demonstrated that anterior pituitary stores of LH were significantly higher at 60 and 96 h after weaning than at 0 h. No changes in concentrations of FSH in the anterior pituitary were found among lactating and weaned sows in the studies by Crighton and Lamming (1969) and Cox and Brit (1982a), but Kirkpatrick et al. (1965) reported a gradual increase in the amount of FSH in the pituitary throughout lactation in pigs. On the basis of these earlier reports, McNeilly (1988) concluded that anterior pituitary content of LH is low during lactation but increases after weaning, and that FSH concentrations are high throughout lactation and do not change after weaning. These conclusions are not surprising when it is considered that in none of the aforementioned studies were pituitary concentrations of gonadotrophins compared contemporarily among sows at different stages of lactation. Thus, the present study shows quite clearly that synthesis of both gonadotrophins increases progressively as lactation proceeds and that there is an additional acute response to weaning.

The increase in serum concentrations of LH and FSH beyond the third week of lactation in this study is in agreement with previous studies showing an increase in gonadotrophins in both intact (Stevenson and Brit, 1980; Stevenson et al., 1981; Kirkwood et al., 1984) and ovariectomized lactating sows (Stevenson et al., 1981). This increase is attributed mainly to a decrease in suckling intensity by the litter as lactation progresses (Varley and Foxcroft, 1990). Secretion of LH in sows increases immediately after weaning at day 21 post partum (Shaw and Foxcroft, 1985), and this rise in LH is associated with an increase in LH pulse frequency. The sampling regimen used in our study did not allow characterization of LH pulsatility, but the basal LH concentrations at 6 h after weaning (90 h before slaughter) in the day 14 weaned sows was higher than in the day 14 lactating herdmates, indicating an apparent acute increase in LH pulse frequency after the litters were weaned. This was followed by a gradual decline, indicative of negative feedback of oestradiol from growing follicles. Moreover, higher concentrations of LH in late lactation compared with the values in mid-lactation are consistent with an increase in LH pulsatility during late lactation before weaning (Varley and Foxcroft, 1990).

The follicular development observed in this experiment reflects the escape of the hypothalamic—pituitary axis from the inhibitory effects of suckling. Higher amounts of gonadotrophins during lactation and after weaning at mid-lactation induced an increase in the number of medium and large Graafian follicles present on the ovaries. Similarly, Palmer et al. (1965b) reported that the number of follicles >5 mm in diameter increased 3–4 days after weaning. This increase in follicular size during lactation and after weaning has also been associated with
a reduced incidence of atresia (Palmer et al., 1965a; Kunavongkrit et al., 1982; Britt et al., 1985).

Concentrations of oestradiol in serum were higher between days 10 and 14 postpartum in weaned sows than in suckled herdmates, and in the weaned group oestradiol increased linearly throughout the sampling period. A positive relationship between follicular diameter, follicular fluid volume and concentrations of oestradiol in follicular fluid has been demonstrated in pigs (Foxcroft and Hunter, 1985; de Rensis et al., 1991), so differences in oestradiol concentrations among the three experimental groups are probably due to the differences in follicular development.

In summary, the results demonstrate that pituitary and serum concentrations of LH and FSH increase from mid- to late lactation and after weaning the litter at day 10 post partum. This leads to an enhanced follicular development and consequently increased serum concentrations of oestradiol. It is suggested that a gradual increase in synthesis, processing and release of GnRH in the hypothalamus stimulates synthesis and secretion of gonadotrophins by the anterior pituitary from day 14 to day 28 of lactation in sows.

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References


Cox NM and Britt JH (1982a) Relationships between endogenous gonadotropin-releasing hormone, gonadotropins, and follicular development after weaning in sows Biology of Reproduction 27 70–78


Cox NM, Ramirez JL, Matamoros IA, Bennett WA and Britt JH (1987) Influence of season on estrus and luteinizing hormone responses to estradiol benzoate in ovarioseparated sows Theriogenology 27 395–401

Cox NM, Ramirez JL, Matamoros IA and Bennett WA (1988) Estrus induces estrus unaccompanied by a preovulatory surge of LH in suckled sows Biology of Reproduction 38 592–596


de Rensis F, Hunter MG, Grant SA, Lancaster RT and Foxcroft GR (1991) Effect of estrogen administration on endogenous and luteinizing hormone–releasing hormone-induced luteinizing hormone secretion and folliculogenesis in lactating sow Biology of Reproduction 44 975–981


Ebenshade KE and Britt JH (1985) Active immunization of gilts against gonadotropin-releasing hormone: effects on secretion of gonadotropins, reproduction function and responses to agonists of gonadotropin-releasing hormone Biology of Reproduction 33 569–577


Fraker PJ and Speck JC (1976) Protein and cell membrane iodinations with a sparingly soluble chloramine. 1,3,4,6-tetraiodo-3,5,6-dihenylpygocuril Biochemical and Biophysical Research Communications 80 849–857


Jayes FL, Britt JH and Esbenshade KE (1991) Gonadotropin-releasing hormone (GnRH) and luteinizing hormone profiles in peripheral serum of sows from weaning to estrus Journal of Animal Science Supplement 1 69 429 (Abstract)


Mason NR and Marsh R (1975) Cyclic AMP in the rat ovary: effects of exogenous LH secretion Endocrine Research Communications 21 357–364

Melampy RM, Henricks DM, Anderson LL, Chen CL and Shultz GR (1966) Pituitary follicle stimulating hormone and luteinizing hormone concentrations in pregnant and lactating pigs Endocrinology 78 801–804


Palmer WM, Teague HS and Venzke WG (1965a) Histological changes in the reproductive tract of the sow during lactation and early postweaning Journal of Animal Science 24 1117–1125

Palmer WM, Teague HS and Venzke WG (1965b) Microscopic observations on the reproductive tract of the sow during lactation and early postweaning Journal of Animal Science 24 541–545


Sesti LAC and Britt JH (1995) Agonist-induced release of gonadotrophin-releasing hormone, luteinizing hormone and follicle-stimulating hormone and their associations with basal secretion of luteinizing hormone and follicle-stimulating hormone throughout lactation in sows Biology of Reproduction (in press)

Smith MS (1978) The relative contribution of suckling and prolactin to the inhibition of gonadotropin secretion during lactation in the rat Biology of Reproduction 19 77–83
Stevenson JS and Britt JH (1980) Luteinizing hormone, total estrogens and progesterone secretion during lactation and after weaning in sows Theriogenology 14 453–462