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LH and FSH secretion, follicle development and oestradiol in sows ovulating or failing to ovulate in an intermittent suckling regimen

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Abstract. The present paper describes LH and FSH secretion, follicle development and ovulation in sows that were subjected to a limited nursing regimen. From Day 14 of lactation, 32 sows were separated from their piglets for 12 h every day (intermittent suckling; IS). Half the sows had boar contact during separation. Nine of 32 sows ovulated spontaneously within 14 days from initiation of IS. The frequency of LH pulses on the first day of IS tended to be higher in anovulatory sows (6.3 *v.* 4.2 pulses per 12 h; $P < 0.10$); other characteristics of LH secretion were similar to sows that ovulated. The characteristics of FSH secretion did not differ over the 8-h sampling period. Boar contact did not influence either LH and FSH secretion or the number of sows that ovulated. Up to 58% of anovulatory sows showed an increase in follicle size after initiation of IS and, 4 days after the initiation of IS, one-third still had follicles similar in size to those in ovulatory sows. However, the oestradiol concentration in anovulatory sows did not increase. We conclude that FSH and LH stimulation in anovulatory sows is not limiting for normal follicle development, but that ovarian follicles are not responsive to increased LH secretion.

Additional keywords: lactation, ovulation.

Introduction

During lactation, a sow typically experiences anoestrus because suckling by the piglets inhibits or strongly suppresses gonadotrophin-releasing hormone (GnRH) and LH secretion (Armstrong *et al.* 1988). Secretion of LH by the pituitary is important for final follicle growth before ovulation (Britt *et al.* 1985). Daily removal of the suckling-induced inhibition of LH secretion (intermittent suckling; IS) can result in ovulation if the hypothalamic–pituitary–axis is sufficiently active (Langendijk *et al.* 2007). During the course of lactation, the GnRH pool in the hypothalamus and the LH and FSH pools in the pituitary are progressively replenished (Sesti and Britt 1993). In addition, during lactation, the positive feedback system that enables a threshold level of oestradiol to induce the preovulatory LH surge is recovered (Bevers *et al.* 1981; De Rensis *et al*. 1991). These factors probably explain why studies in the past have shown considerable variation in both the percentage of sows that showed oestrus and the synchrony of oestrus in sows subjected to an IS regimen depending on the stage of lactation at which IS was initiated, as well as on the parity of the sows, the duration of separation and on boar contact (Smith 1961; Crighton 1970; Rowlinson and Bryant 1982; Stevenson and Davis 1984; Newton *et al.* 1987; Van der Wiel and Booman 1993; Langendijk *et al.* 2000). However, earlier studies on IS during lactation have provided little insight as to why anovulatory sows do not ovulate.

Previous studies from our group have established oestrus and ovulation during lactation in 90–100% of sows from a synthetic dam-line (TOPIGS-40, Topigs, Vught, The Netherlands), by separating sows and piglets for 12 h per day from Day 14 of lactation onwards (Langendijk *et al.* 2007; Gerritsen *et al.* 2008*a*). The sows that failed to ovulate in these studies either did not show follicle growth to preovulatory size, presumably because of insufficient LH secretion (Langendijk *et al.* 2007) or did develop preovulatory follicles but failed to ovulate, probably due to lack of an adequate LH surge (Gerritsen *et al.* 2008*b*). In the sows that ovulated, follicle dynamics following the start of IS and the time of ovulation were similar to sows that were fully weaned. In the present study, a line of sows was used that was less likely to ovulate in a limited nursing regimen and that would give more variable responses to IS in terms of the rate of follicle development and time of ovulation from the start of IS. Therefore, the secretion of LH, FSH and oestradiol and follicle

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development in response to the IS regimen and boar contact were investigated in this population of sows.

Materials and methods

The experiments were approved by the Ethics Committee for Animal Experiments of Wageningen University. Multiparous sows ($n = 32$) of a commercial breed (Topigs20: Yorkshire × Dutch Landrace) were used in the experiments. Parity ranged from 3 to 9 (average 6.8) and bodyweight after farrowing ranged from 245 to 333 kg (average 284 kg). During lactation, sows were housed in farrowing pens (360 × 226 cm) bedded with sawdust. Before parturition, sows were fed 3.5 kg commercial sow feed (8.97 MJ net energy (NE) kg^{-1}). During lactation, sows were fed a lactation diet (9.15 MJ NE kg^{-1}), with feed allowance increasing from 1% of bodyweight at farrowing to the required level 10 days later. The required feeding level was calculated as (bodyweight/100 + 0.45) kg per piglet per day. The daily feed allowance during lactation was distributed over three meals.

Sows farrowed between 114 and 116 days of gestation, but were not all due on the same day. Therefore, the day that most sows farrowed was designated as the start of the experiment, Day 0. Most sows farrowed on Days −1, 0 or 1; one sow farrowed on each of Days −5, −3 and −2. Litter size at birth varied from seven to 15 live piglets and was standardised within 3 days after farrowing. After standardisation, litter size averaged 12.2 ± 0.3 at 3 days after farrowing and, due to mortality, 10.1 ± 0.3 on Day 14. Piglets were offered creep feed (19% milk products; 11.44 MJ NE kg^{-1}; 180 g kg^{-1} crude protein (CP)) from Day 7 onwards.

Treatments

From Day 14 onwards, sows and piglets were submitted to an IS regimen. From 0800 to 2000 hours, sows were separated from their piglets and housed individually in a different unit, where they had no visual, auditory or olfactory contact with their litters. Based on parity and bodyweight at farrowing, sows were allocated to one of two treatment groups: BOAR or NOBOAR. At the start of the treatments (Day 14), bodyweight was still similar across treatment groups. Half the sows ($n = 16$) had fence-line contact with a boar during the time they were separated from their piglets (BOAR). On the first day of IS (Day 14), the boar was introduced at 1200, 1600 and 1930 hours; on later days, the boar was introduced at 0800, 1400 and 1930 hours. Sows were allowed to have nose-to-nose contact with the boar for 15 min on each occasion. The sows that had no boar contact (NOBOAR) were housed in a different unit than the BOAR sows during separation from their piglets.

Sows that ovulated between Day 14 and Day 28 were designated as 'spontaneous ovulators'. Sows that did not ovulate before Day 28 were treated with exogenous gonadotrophins (400 IU equine chorionic gonadotrophin (eCG) + 200 IU human chorionic gonadotrophin (hCG); PG600; Intervet, Boxmeer, The Netherlands) on Day 28 to induce follicle development and oestrus. From Day 29 onwards, all sows that had been treated with PG600 had boar contact three times a day and were monitored for follicle growth and ovulation until Day 42. Sows that ovulated spontaneously or after PG600 were inseminated and then became part of a different study and were killed on Day 30 of pregnancy (Gerritsen *et al.* 2008*b*).

Oestrus and follicle development

From Day 14 onwards, sows were examined while in their individual stalls at 12-h intervals (at 0800 and 1930 hours) to detect oestrus. First, a back pressure test (BPT) was performed on sows in both groups in the absence of a boar. Then, the boar was introduced to the sows in the BOAR treatment group and the BPT was performed again on the sows in the BOAR treatment. The onset of oestrus, as detected by the BPT in the absence of a boar, was defined as the first time a standing response was shown in response to the BPT minus 6 h. The end of oestrus, as detected by the BPT in the absence of a boar, was defined as the last time a standing response was shown in response to the BPT plus 6 h. The onset and end of oestrus detected by the BPT in the presence of the boar were defined similarly.

Follicle development was monitored using transrectal ultrasound (Soede *et al.* 1992). The diameter of the three largest follicles was recorded daily. The stage of follicle development was quantified as the average of these three diameters. When the diameters of the three largest follicles all surpassed 6 mm, ultrasound was performed every 12 h to estimate the time of ovulation. For sows that ovulated between Day 14 and Day 28, as well as for sows that ovulated after treatment with PG600, the time of ovulation was estimated as the last time preovulatory follicles could be seen plus 6 h.

Sows that did not ovulate between Day 14 and Day 28 were designated as 'anovulatory'. Although anovulatory sows failed to ovulate, several of these sows developed follicles similar in size to those seen in ovulatory sows. Anovulatory sows in which the diameter of the three largest follicles was in the range of that seen for the three largest follicles in ovulatory sows were termed 'anovulatory-large'. The remaining anovulatory sows were termed 'anovulatory-small'. This differentiation was made each day between Day 14 and Day 28.

Blood sampling

Sows were fitted with permanent jugular catheters approximately 14 days before parturition, under general anaesthesia, as described by Langendijk *et al*. (2007). Daily blood samples were taken in the period before IS when catheters were flushed. On the first day of IS, blood samples were taken every 12 min from 0800 to 2000 hours for the determination of LH concentrations. In the BOAR treatment group, the boar was first introduced at 1200 hours on the first day of IS to see whether there were immediate effects on LH secretion. From 1 day after the start of IS until ovulation (or until Day 28), blood samples were taken each day to determine oestradiol concentrations. In addition, FSH was analysed in the 12-min blood samples taken over the first 8 h of separation on the first day of IS. To determine insulin-like growth factor (IGF)-1 concentrations, two blood samples were taken on separate days in the week preceding IS and two blood samples were taken on the first day of IS (at 0800 and 2000 hours). Plasma concentrations of IGF-1 before the onset of IS were calculated as the average concentration of the first two blood samples, whereas

the IGF-1 concentration at the onset of IS was calculated as the average of the latter two blood samples.

Concentrations of FSH, LH, oestradiol and IGF-1

Concentrations of LH were determined in duplicate 100-μL aliquots of plasma by homologous radioimmunoassay (RIA), as validated for pig plasma (Van den Brand *et al.* 2000). Porcine LH-LER 778.4 (kindly supplied by Dr L. E. Reichert, Tucker Endocrine Research Institute LLC, Atlanta, GA, USA) was used for iodination and standards and rabbit anti-porcine (p) LH (UCB A528; Campro Scientific, Veenendaal, The Netherlands; Biogenesis; originally produced by Dr Jean Closset, Sart-Tilman University of Liege, Liege, Belgium; Vandalem *et al.* 1979) was used as the antiserum. The specificity of the RIA was high, as indicated by low cross-reactivity for other pituitary hormones (Van den Brand *et al.* 2000) and by the observed parallelism. The limit of quantitation was 0.2 ng mL^{-1} LH.

Concentrations of 17β-oestradiol were determined using a solid-phase ^{125}I RIA method (Coat-A-Count TKE; Diagnostic Products, Los Angeles, CA, USA), as validated for cow plasma (Dieleman and Bevers 1987). Briefly, 1 mL plasma was extracted with 2 mL diethylether. After evaporation of the diethylether, the residue was dissolved in 250 μL borate buffer, and duplicate 100-μL aliquots were used in the RIA. Extraction efficiency was determined in parallel samples with tritiated steroid. Specificity of the RIA was high, as indicated by low cross-reactivity for other steroid hormones (Dieleman and Bevers 1987) and by the observed parallelism. The limit of quantitation was 2 pg mL^{-1} 17β-oestradiol.

Calculation of all results for LH and oestradiol was done applying the spline approximation for the standard series from RIASmart (Packard Instruments, Meriden, CT, USA). There was a <4% difference between calculated doses and defined doses over the entire range. The intra-assay coefficients of variation (CV) for both LH and oestradiol was <10% and the interassay CV for both LH and oestradiol was <15%.

Plasma FSH concentrations were quantified by a homologous double-antibody RIA. Purified porcine (p) FSH hormones for iodination and reference standards (pFSH I-1, AFP-10640B; 81.6 × oFSH S1) and the primary antiserum (AFP-2062096) raised in rabbits against purified intact pFSH were kindly supplied by A. F. Parlow (Harbor-UCLA Medical Center, Torrance, AC, USA) and National Institute of Digestive and Diabetes and Kidney Diseases' National Hormone and Peptide program. For iodination, chloramine T and FSH were used at a ratio of 7 μg : 1 μg and a resin column (#140–1431; Bio-Rad Laboratories, Hercules, CA, USA) was used for purification. At the final assay tube dilution of 1 : 400 000 the antiserum bound 40% radiolabelled pFSH in the absence of unlabelled antigen. The assay buffers used were 1% (w/v) bovine serum albumin (BSA; #A7906; Sigma-Aldrich Canada, Oakville, ON, Canada) in phosphate-buffered saline (PBS) containing 2.77 mM monobasic phosphate (#S369–500; Fisher Scientific, Nepean, ON, Canada), 7.22 mM dibasic phosphate (#374B-500; Fisher Scientific), 15 mM sodium azide (#S227; Fisher Scientific) and 139 mM sodium chloride (#ACS 783; BDH, Toronto, Canada), pH 7.0, for buffer and tracer additions and 0.05 M EDTA (#S311; Fisher Scientific), pH 7.0, for primary and secondary antiserum dilutions. Owing to the presence of complement in samples, the assay buffer for curve standards was spiked with blank plasma at a ratio of 0.018 mL blank plasma to 0.3 mL assay buffer. To avoid problems of excessive binding of proteins to plastics, a glass container was used for the dilution of primary and secondary antisera. To avoid assay drift, additions were made into precooled (4°C) assay tubes. Initial additions included 0.3 mL plasma or standard (0.0146 to 30 ng per tube), 0.2 mL assay buffer, 0.2 mL primary antiserum (at a working dilution of 1 : 100 050 in 0.05 M EDTA buffer containing 1 : 600 normal rabbit serum) and 0.1 mL radioiodinated pFSH (diluted in assay buffer to give 10 000 to 15 000 c.p.m.). All tubes were then incubated at room temperature for 24 h. To enhance precipitation of antibody bound hormone, the second antibody (goat anti-rabbit gamma globulin (GARGG); #539845; Calbiochem, San Diego, CA, USA) was diluted 1 : 300 in 6 g% polyethylene glycol (PEG 8000; #P-156; Fisher Scientific) and preincubated for 24 h at 4°C, followed by addition of 0.2 mL GARGG/PEG to the assay tubes and incubation for a further 24–44 h at 4°C. To separate bound and free ^{125}I-labelled FSH, tubes were centrifuged at 1900*g* for 45 min, the supernatant aspirated and the radioactivity in the pellet counted. To achieve consistency with previous publications, potencies are expressed as ng equivalents of pFSH B-1. Sensitivity, defined as approximately 97% of total binding, was 2 ng equivalents of pFSH B-1 per tube (equal to 6.7 ng mL^{-1} in the current assays) based on estimated relative immunopotency of pFSH B-1 and pFSH I-1, AFP 10640B in our assay system. Serial dilutions of standard plasma showed parallelism to the standard curve. The recovery of a known amount of pFSH when added to pig plasma of known potency was 95 ± 2%. Samples from the present experiment were assayed in triplicate in three assays and assays were balanced for treatment groups. The intra-assay CVs in the three assays were 10.75, 9.18 and 11.05%. The inter-assay CV was 17.39%.

Concentrations of IGF-I in porcine plasma were determined using the method of Novak *et al*. (2002) with slight modification. The IGF-I used for iodination and reference standards was GroPep Receptor Grade Human IGF-I (#CU100; GroPep, Thebarton, SA, Australia): the standard has a potency of 0.42× Bachem (Bubendorf, Switzerland) #H555 (the standard used in previous assays). A 100-μL aliquot of plasma was initially extracted with 3 mL acid ethanol and 100 μL neutralised extract was taken to assay. Samples were extracted singly and assayed in duplicate in a single assay. A diluted neutralised plasma control pool showed parallelism with the standard curve. Assay sensitivity, defined as 96.23%B, was 0.00146 ng per tube (equivalent to 2.26 ng mL^{-1} in the current assay). All samples had concentrations higher than the limit of sensitivity. Apparent radioinert recovery was 42.7 ± 0.5%. Results were not corrected for recovery. The intra-assay CV for the single assay run was 7.6%.

A rise in LH concentration was defined as a pulse when: (*1*) the maximum concentration was reached within two samples from the previous nadir; (*2*) there were at least two samples between the maximum concentration and return to basal levels or the next nadir; and (*3*) the maximum concentration was >0.1 ng mL^{-1} above the previous nadir. The definition of LH pulses was based on McLeod and Craigon (1985) and was

Table 1. Oestrus, ovulation and follicle characteristics for sows with (BOAR) or without (NOBOAR) boar contact

[x,y]Values with different superscript letters within a row differ significantly ($P < 0.10$). BPT, back pressure test; IS, intermittent suckling

	BOAR	NOBOAR
Total no. sows in the experiment	16	16
No. sows with spontaneous ovulation	5	4
No. sows with cystic ovaries	1	1
No. anovulatory sows	10	11
Sows with spontaneous ovulation		
Oestrus BPT (*n*)	3	4
Oestrus BPT + boar (*n*)	5	
Onset of oestrus after start of IS[A] (h)	126 ± 14	138 ± 21
Duration of oestrus[A] (h)	36 ± 12	24 ± 7
Duration of oestrus + boar[A] (h)	63 ± 10	
Maximum follicle diameter (mm)	8.2 ± 0.3[x]	7.3 ± 0.3[y]
Day of maximum follicle diameter	6.8 ± 0.2	7.3 ± 0.2
Time of ovulation after IS (h)	153 ± 2	164 ± 8
Follicle diameter at ovulation (mm)	8.0 ± 0.3[x]	7.3 ± 0.2[y]
Anovulatory sows		
No. treated with PG600[B]	9	10
Ovulation after treatment (*n*)	5	8
Cystic (*n*)	1	
Anovulatory (*n*)	3	2

[A]As determined using the BPT (see text for details).

[B]Of the 21 anovulatory sows, three sows were removed from the experiment because of illness before treatment with PG600 (Intervet, Boxmeer, The Netherlands).

described earlier by Van den Brand *et al*. (2000). To describe the pulsatile release pattern of LH, three parameters were defined, namely the amplitude and frequency of pulses and the average LH concentration. The amplitude of an LH pulse was the difference between the maximum of a pulse and the previous nadir. The average LH concentration was calculated as the average of all samples in a given period, including the LH pulses.

Similar criteria were used to analyse FSH secretion patterns, except that any rise ($>0\,ng\,mL^{-1}$) in FSH was accepted as a pulse as long as the other pulse criteria were met. Alternatively, $5\,ng\,mL^{-1}$ was used as a threshold in preliminary analyses, but did not result in any different interpretation of the results.

Statistics

Differences between treatments (BOAR *v.* NOBOAR) and differences between sows that ovulated and those that did not within 14 days from the start of IS were tested for each day using the GLM procedure of SAS (SAS/STAT 1990), with the following model:

$$Y = \mu + \text{treatment (or ovulation status)} + e$$

where Y is one of the LH or FSH characteristics, IGF-1, oestradiol, follicle diameter, onset and duration of oestrus, or time of ovulation. In addition, for LH and FSH, a 'sliding-window' approach was used to analyse within-day changes in basal and mean concentration of these hormones for ovulating *v.* non-ovulating sows. The sampling period on the first day of IS was divided into 4-h blocks and the mean or basal concentration during these periods was analysed using a nested model, with sows nested within ovulation status. A Chi-squared test was used to test for differences between treatment groups for the number of sows that ovulated. Pearson correlation coefficients were calculated for the relationship between continuous variables.

Results

Ovulation and oestrus

Nine of 32 (28%) sows ovulated spontaneously within 14 days after initiation of IS (Table 1). Two sows developed cystic ovaries during this period. The remaining 21 sows showed varying degrees of follicle growth (Fig. 1), but did not ovulate within the first 14 days (anovulatory sows). All sows that ovulated or developed cystic ovaries showed oestrous behaviour, except for one sow in the NOBOAR treatment group. Of the 21 anovulatory sows, only one showed oestrus between Day 14 and Day 28. Boar contact did not increase the percentage of sows that ovulated within 14 days. The sows that ovulated did not differ from anovulatory sows in terms of feed intake from Day 0 to Day 21 (81% *v.* 82% of allowance, respectively), bodyweight loss from Day 0 to Day 21 (23.2 ± 7.2 *v.* 16.8 ± 2.6 kg, respectively), litter size after cross-fostering (11.9 ± 0.7 *v.* 12.1 ± 0.3, respectively), litter size at the start of IS (9.7 ± 0.5 *v.* 10.2 ± 0.5, respectively) or in piglet weight gain in the first (154 ± 11 *v.* $162 \pm 9\,g\,day^{-1}$, respectively), second (232 ± 12 *v.* $220 \pm 11\,g\,day^{-1}$, respectively) and third (143 ± 16 *v.* $180 \pm 15\,g\,day^{-1}$, respectively) weeks of lactation. There was also no difference between sows that ovulated and anovulatory sows in terms of plasma

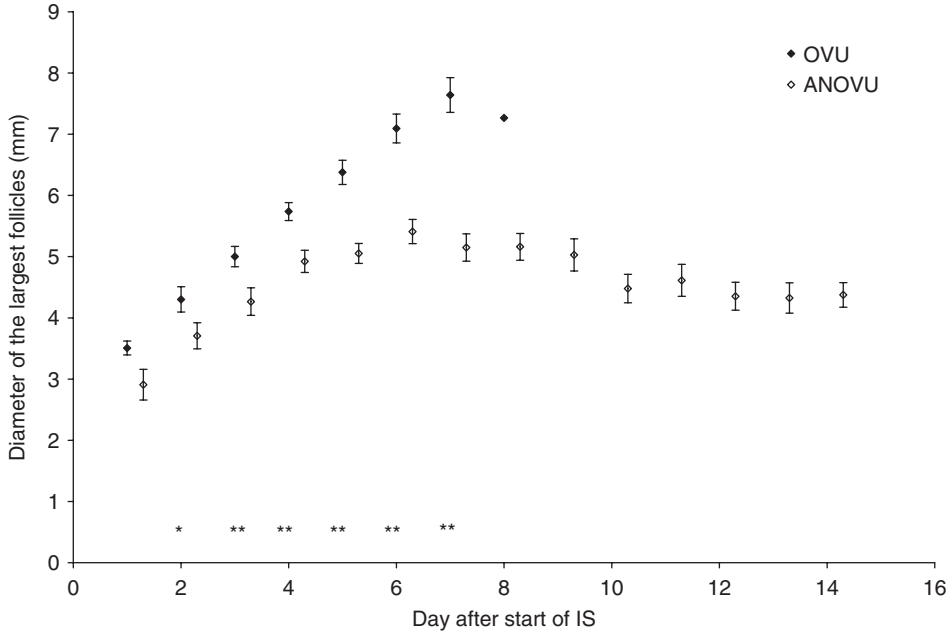

Fig. 1. Change in follicle diameter of the largest follicles (means and standard errors) after the start of intermittent suckling (Day 14 of lactation), for ovulatory (OVU) and anovulatory (ANOVU) sows. *$P < 0.10$. **$P < 0.05$.

IGF-1 concentrations during the week before IS (50.2 ± 4.9 *v.* 50.1 ± 4.6 ng mL^{-1}, respectively) or on the first day of IS (43.4 ± 4.0 *v.* 45.9 ± 3.2 ng mL^{-1}, respectively). Bodyweight and back fat loss during the first 2 weeks of lactation were not related to plasma concentrations of IGF-1.

Follicle development

In the nine sows that ovulated within 14 days of the start of IS, there was an increase in the average diameter of the largest follicles from 3.5 mm (range 3.2–4.4 mm) on the first day of IS to 7.6 mm (range 6.3–9.4 mm) on the seventh day of IS (Fig. 1). Follicle size in these sows at the last ultrasound examination before ovulation (at most 12 h before ovulation) was 7.7 mm on average and ranged from 6.8 to 9.1 mm. Ovulating sows with boar contact (five of nine ovulating sows) had larger follicles than ovulating sows without boar contact on the sixth (7.5 ± 0.3 *v.* 6.6 ± 0.3 mm; $P < 0.04$) and seventh (8.1 ± 0.3 *v.* 7.1 ± 0.4 mm; $P < 0.08$) days of IS, as well as at ovulation (8.0 ± 0.3 *v.* 7.3 ± 0.3 mm; $P < 0.09$). The nine sows that ovulated did so between 150 and 186 h after the start of IS. The ovulation rate did not differ between sows with and sows without boar contact (26 ± 1 *v.* 27 ± 2 corpora lutea, respectively).

Anovulatory sows showed a varying degree of follicle growth from the start of IS (Fig. 1). On the first day of IS, maximum follicle diameter ranged from 1 to 5 mm between sows; on the sixth day of IS, average follicle size had increased to 5.4 mm, but ranged between 3.5 and 7.6 mm. Although the average follicle diameter of anovulatory sows differed significantly from that in sows that ovulated from the second day of IS onwards (Fig. 1), a considerable percentage of anovulatory sows had a follicle size within the same range as the ovulatory sows. On the first 8 days of IS, the percentage of anovulatory sows with 'large' follicles was 47%, 58%, 47%, 35%, 33%, 37%, 10% and 0%, respectively, indicating a progressive increase in the difference between ovulatory and anovulatory sows in terms of follicle size.

Secretion of LH and FSH

The secretion of LH on the first day of IS was characterised by a high frequency of LH pulses (5.7 pulses in 12 h; range 1–11 pulses), with low amplitude (0.18 ng mL^{-1}; range 0.12–0.29 ng mL^{-1}). Figure 2 shows individual LH and FSH secretion patterns for eight representative sows. Boar contact did not affect LH or FSH secretion patterns and the introduction of a boar did not seem to have an immediate effect on LH or FSH secretion. Averaged over the entire sampling period, ovulatory sows tended to have a lower frequency of LH pulses than anovulatory sows ($P < 0.10$); other LH secretion characteristics were similar (Table 2). The mean FSH concentration across all groups was 19.3 ± 2.6 ng mL^{-1} and there was no difference between sows that ovulated and sows that failed to ovulate in the number of FSH pulses, the amplitude of FSH pulses, 8-h mean FSH or the initial (first hour) concentration of FSH (Table 3). There was no correlation between FSH secretion characteristics and LH secretion characteristics, except for a positive ($r = 0.40$; $P < 0.05$) correlation between the number of FSH pulses and LH amplitude. In some sows, LH pulses seemed to coincide with FSH pulses (Fig. 2) and analysis of all LH pulses of all the animals in the study established that approximately 50% of LH pulse maxima were matched by an FSH pulse maximum within one or two samples.

If analysed as two 4-h sampling blocks, ovulatory sows showed a decrease in FSH concentration, whereas anovulatory

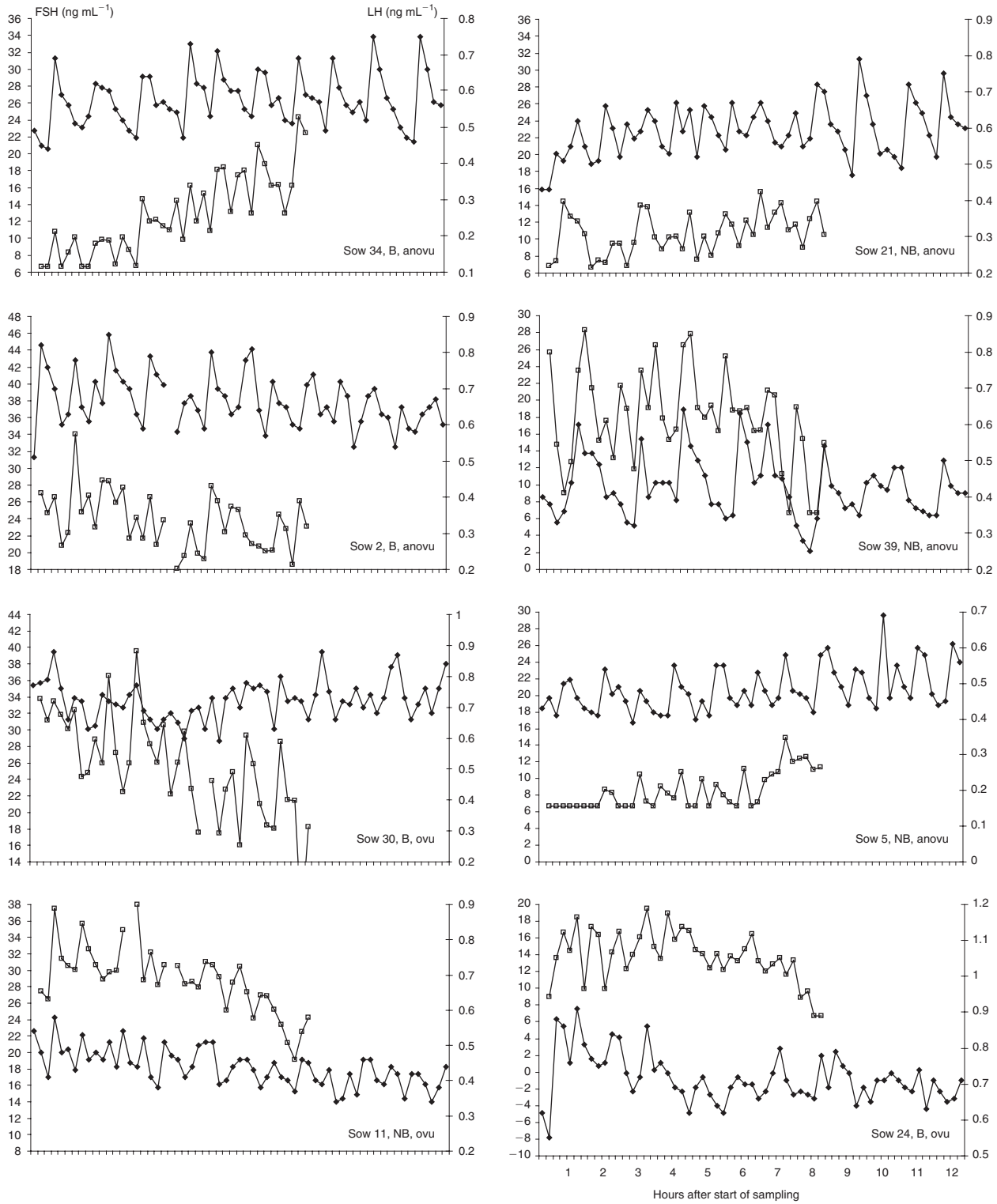

Fig. 2. FSH (open symbols, left axis; ng mL^{-1}) and LH (closed symbols, right axis; ng mL^{-1}) for eight individual sows during the 12-h period immediately after the start of intermittent suckling. Sows 30, 11 and 24 ovulated (ovu), whereas other sows did not (anovu). All axes are scaled such that the magnitude of different profiles is comparable. Note that for sows 34, 2 and 39 in particular, distinct pulses of LH seem to coincide with FSH pulses. For other sows this is less clear. Secretion of LH did not differ between ovulatory and anovulatory sows; however, note that in ovulatory sows FSH becomes inhibited whereas in anovulatory sows FSH is stable or increases.

Table 2. Secretion of FSH and LH on the first day (12 h for LH, 8 h for FSH) of intermittent suckling in ovulatory and anovulatory sows

Data are the mean ± s.e.m. [x,y]Values with different superscript letters within a row differ significantly ($P < 0.10$)

	Ovulatory sows ($n = 9$)	Anovulatory sows ($n = 21$)
FSH secretion		
No. pulses in 8 h	4.50 ± 0.89	5.12 ± 0.39
Amplitude (ng mL^{-1})	5.96 ± 0.71	6.39 ± 0.40
Mean FSH (ng mL^{-1})	18.73 ± 3.35	19.60 ± 2.74
Average first four samples (ng mL^{-1})	17.78 ± 3.41	16.27 ± 2.39
LH secretion		
No. pulses in 12 h	4.22 ± 0.62^{x}	6.33 ± 0.67^{y}
Amplitude (ng mL^{-1})	0.18 ± 0.02	0.17 ± 0.01
Mean LH (ng mL^{-1})	0.75 ± 0.10	0.66 ± 0.05
Basal LH (ng mL^{-1})	0.68 ± 0.08	0.58 ± 0.05

Table 3. Concentrations of FSH and LH during 4-h windows of the sampling period in in ovulatory and anovulatory sows

Data are the least square mean ± s.e.m. based on a nested model (Y = μ + ovulation (yes/no) + sow (ovulation) + window + window × ovulation + e). [a,b]Different superscripts within a row indicate significant differences ($P < 0.05$). [a,b,x,y]Different superscripts within columns for each hormone indicate significant difference ([a,b]$P < 0.05$, [x,y]$P < 0.10$, respectively)

	0–4 h	4–8 h	8–12 h
FSH mean (ng mL^{-1})			
Ovulatory	19.4 ± 0.9	18.2 ± 0.9^{x}	
Anovulatory	18.4 ± 0.6^{a}	20.6 ± 0.6^{b,y}	
Basal LH (ng mL^{-1})			
Ovulatory	0.70 ± 0.01	0.69 ± 0.01	0.66 ± 0.01
Anovulatory	0.58 ± 0.01	0.59 ± 0.01	0.56 ± 0.01
LH mean (ng mL^{-1})			
Ovulatory	0.78 ± 0.01^{a}	0.76 ± 0.01^{a}	0.72 ± 0.01^{a}
Anovulatory	0.65 ± 0.01^{b}	0.66 ± 0.01^{b}	0.64 ± 0.01^{b}

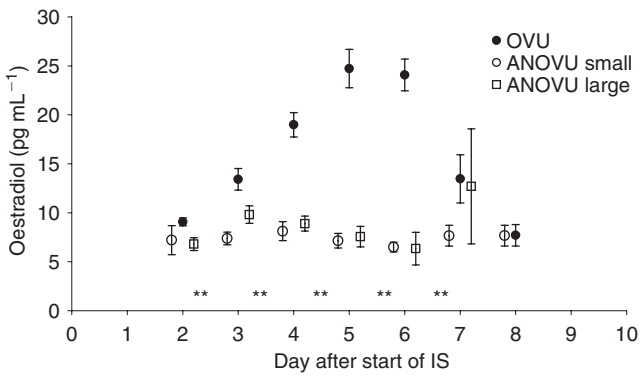

Fig. 3. Change in serum oestradiol concentrations (means and standard errors) after the start of intermittent suckling (Day 14 of lactation), for ovulatory sows (OVU), anovulatory sows with a maximum follicle diameter comparable to that of ovulatory sows (ANOVU large) and anovulatory sows with a maximum follicle diameter smaller than that in ovulatory sows (ANOVU small). $^{**}P < 0.05$.

sows showed an increase (Table 3). There was no change in LH secretion (baseline or mean) throughout the sampling period, but ovulating sows had a consistently higher mean LH over the three 4-h periods. This difference was approximately 0.10 ng mL^{-1}.

None of the LH or FSH characteristics was related to follicle size on any of the first 5 days following the start of IS. The secretion of LH and FSH on the first day of IS in anovulatory sows with large follicles did not differ from LH and FSH secretion in anovulatory sows with smaller follicles, nor in ovulatory sows. None of the LH or FSH characteristics was related to IGF-1 concentrations.

Oestradiol secretion

In ovulatory sows, plasma concentrations of oestradiol increased concomitantly with the increase in the diameter of the large follicles. On the second day of IS, the concentration of oestradiol in these sows was higher than in anovulatory sows (Fig. 3). Ovulatory sows with boar contact tended ($P < 0.10$) to reach preovulatory peak concentrations of oestradiol earlier than sows without boar contact. On the sixth day of IS, the average concentration of oestradiol for sows with boar contact was already decreasing and tended to be lower than for sows without boar contact (21.7 ± 1.9 *v.* 27.1 ± 2.0 pg mL^{-1}; $P < 0.10$), whereas sows without boar contact reached maximum oestradiol concentrations on this day.

In anovulatory sows, plasma concentrations of oestradiol did not increase between Day 14 and Day 28 (Fig. 3), and remained between 3 and 15 pg mL^{-1}. The plasma concentration of oestradiol was already higher in ovulatory sows than in anovulatory sows on the second day of IS; even anovulatory sows with 'large' follicles did not exhibit an increased concentration of oestradiol (Fig. 3). Therefore, in anovulatory sows morphological changes in the ovary (increase in follicle size) were not accompanied by functional changes (increased oestradiol secretion). There was no correlation between oestradiol concentration on Days 15, 16 or 17 with any of the LH secretion parameters on Day 14.

Discussion

This study presents data on follicle growth, follicle function (in terms of oestradiol output) and ovulation in a line of sows that was expected to be less responsive to an IS regimen than a line of sows used in earlier studies (Langendijk *et al.* 2007; Gerritsen *et al.* 2008*a*). Indeed, only nine of 32 sows ovulated spontaneously during the IS regimen and boar contact did not increase the number of sows that ovulated. The expectation was that most sows would eventually ovulate, but with a considerable variation in the time of ovulation. Surprisingly, the sows that ovulated did so rather synchronously, within 6–7.5 days after the start of IS, whereas the anovulatory sows did not ovulate for 14 days after the start of IS. However, the anovulatory sows showed a varying degree of follicle growth, some even up to ovulatory size, but failed to ovulate and failed to show an increase in plasma oestradiol concentrations.

The metabolic condition of the sows may be one of the factors that contributed to the failure to ovulate. The loss of absolute bodyweight during the first 3 weeks of lactation (20 kg on average) confirms the substantial state of catabolism of the sows.

Around the onset of IS, IGF-1 concentrations were low (40–50 ng mL^{-1}), similar to concentrations reported in a previous study with lactating sows subjected to IS (Langendijk *et al.* 2008), and much lower than in weaned, primiparous sows (up to 250 ng mL^{-1}; Langendijk *et al.* 2008). However, there was no difference in feed intake, weight loss or IGF-1 concentrations between ovulatory and anovulatory sows. Although these data suggest that the metabolic status did not determine differences in ovarian responsiveness to IS, two points need to be emphasised. First, the third week of lactation was the first week of the limited nursing regimen and sows still received their full feed allowance as if they were suckled continuously, whereas milk production would have dropped to approximately 50% of that during full lactation based on estimations by Berkeveld *et al.* (2007). Consequently, sows were changing to a less catabolic state during the first week of IS, even though they were still losing weight during this week (−7 kg). In the second week of IS, sows were gaining weight. Second, the same pattern and magnitude of weight change (weight loss in the first 3 weeks of lactation and then becoming anabolic) were observed in a previous experiment (Gerritsen *et al.* 2008*a*), in which 90–100% of sows ovulated within 1 week of IS.

As in other studies in which sows were either transiently separated from their piglets (Armstrong *et al.* 1988; Langendijk *et al.* 2007) or weaned (Van den Brand *et al.* 2000), LH secretion on the first day of IS was characterised by a high frequency of LH pulses with low amplitude, as opposed to a low frequency of LH pulses with high amplitude normally observed in suckled sows. The LH and FSH profiles obtained adequately characterised the gonadotrophic status of the sows used in the present experiment. In a similar study in which blood samples were taken over 4 days and 4 nights continuously, Langendijk *et al.* (2007) showed that the LH secretion pattern before the start of IS was typical for sows in continuous lactation (only one or two pulses with high amplitude over an 8-h sampling period), whereas immediately after the first separation of sows and piglets, the LH secretion changed to a pattern with a high frequency and low amplitude of LH pulses. When suckling resumed, LH secretion was again inhibited. After 2 days of the IS regimen, LH secretion became inhibited due to negative feedback of increasing oestradiol concentrations, even during non-suckling periods. Based on these findings, the use of a sampling period before the start of IS was considered unnecessary to establish a similar and predictable pattern of LH secretion, and the one sampling period during the first 12 h of separation was thought to be indicative of the LH secretion potential for each sow. The LH pulse frequency for the anovulatory sows (6.3 per 12 h) was lower compared with sows subjected to an IS regimen in a different study (7.8 pulses per 12 h; Langendijk *et al.* 2007), but higher than the ovulating sows in the present study (4.3 per 12 h) and within the range shown by primiparous sows on their first day after weaning that eventually ovulated (2–12 pulses per 12 h; Van den Brand *et al.* 2000). The amplitude of the LH pulse was lower than observed previously (Langendijk *et al.* 2007) in sows subjected to an IS regimen (0.17 *v.* 0.4 ng mL^{-1}) and mean LH was 0.1 ng mL^{-1} lower in anovulatory sows compared with ovulatory sows. The lower pulse frequency in sows that ovulated compared with the anovulatory sows was probably due to the acute increase in secretion of oestradiol, exerting modulatory feedback on LH secretion at the hypothalamic–pituitary level. Oestradiol concentrations were already elevated in the first sample taken after the start of IS. Boar contact did not increase either the secretion of LH and FSH or the percentage of sows that ovulated, although boar contact has been reported previously to improve the number of sows that show oestrus and ovulate after weaning (Langendijk *et al.* 2000, 2006) and Van der Wiel and Booman (1993) reported an increase in LH secretion a few hours after boar contact was initiated in three sows that had not ovulated for 21 days after weaning. It is not clear why boar contact did not stimulate LH release and ovulation in the present study.

The mean FSH concentration was low in the present study (approximately 20 ng mL^{-1}) compared with studies with split-weaned sows (approximately 65 ng mL^{-1}; Degenstein *et al.* 2006) and weaned sows (approximately 48 ng mL^{-1}; Willis *et al.* 2003), but comparable to early weaned (Day 14 of lactation) sows (approximately 27 ng mL^{-1}; Willis *et al.* 2003). Nevertheless, at the onset of IS there were no differences in FSH concentrations between ovulatory and anovulatory sows, which suggests that FSH was not limiting for the development of oestrogenic follicles. The rise in FSH in the second 4-h window of sampling in anovulatory sows may have been the consequence of separating sows from their piglets, whereas in the ovulatory sows this rise was inhibited by increasing feedback from the developing follicles.

It is not clear whether FSH and LH secretion is a limiting factor in the sows that failed to ovulate. Comparison with other studies indicates that LH pulse frequency and FSH secretion are not limiting. Moreover, FSH and LH secretion was not related to follicle diameter or oestradiol secretion after the start of IS, indicating that sows with small or non-oestrogenic follicles were not those with a lower level of FSH or LH secretion. This suggests that follicular responsiveness to gonadotrophic stimulation, rather than differences in circulating gonadotrophins, is a key factor determining the development of oestrogenic and ovulatory follicles in the sows studied. Conversely, boar contact failed to increase the level of LH and FSH secretion, whereas several authors have suggested that, after weaning, the stimulatory effect of boar contact on the attainment of oestrus is through increased secretion of, at least, LH (Van der Wiel and Booman 1993; Langendijk *et al.* 2006). In the present study, IS commenced at a stage of lactation at which the secretory capacity of gonadotrophins by the pituitary is still increasing. Sesti and Britt (1993) showed that, in multiparous sows, a challenge with GnRH results in a higher release of FSH and LH at 21 days of lactation than at 7 days. Therefore, in the present study, the FSH and LH secretion capacity was probably not sufficient to be stimulated by boar contact, but the level of secretion on the first day of IS was not necessarily limiting for normal follicle development.

Surprisingly, the anovulatory sows that showed follicle growth comparable to ovulatory sows in terms of maximum follicle size did not show an increase in oestradiol concentration. Such an uncoupling of follicle growth and oestradiol secretion has not been described before. Foxcroft and Hunter (1985) described a positive relationship between follicle size and the concentration of oestradiol in follicular fluid, aromatase activity

and binding of ^{125}I-labelled hCG to granulosa cells. In a study by Foxcroft *et al*. (1987), 10 sows were classified at 48 h after weaning as having oestrogenic follicles or not if the folliclar fluid contained more or less than 100 ng mL^{-1} oestradiol, respectively. In sows with oestrogenic follicles ($n = 5$), the average follicle diameter of the largest 10 follicles (determined macroscopically after sows had been killed) was 6.9 mm, compared with 5.9 mm in sows without oestrogenic follicles. In sows with no oestrogenic follicles, but also in non-oestrogenic follicles of sows that also had oestrogenic follicles, granulosa cell aromatase activity was not different (Foxcroft *et al*. 1987). However, ^{125}I-hCG binding to granulosa cells was lower and, overall, follicular fluid oestradiol concentrations were correlated with ^{125}I-hCG binding by granulosa cells. Therefore, as discussed by Foxcroft and Hunter (1985), a lack of LH receptors may be a limiting factor for the production of oestradiol in non-oestrogenic follicles. As follicles develop during a normal cycle, they acquire more LH receptor mRNA (Liu *et al.* 1998) and the formation of LH receptors in early porcine antral follicles is stimulated by FSH *in vitro* (Loeken and Channing 1985). In the present study, oestradiol concentrations were already higher in ovulatory sows on the second day of IS, indicating an early differentiation in follicle development between ovulatory and anovulatory sows, a differentiation that may already have been present at the start of IS. Therefore, an explanation for the failure of anovulatory sows to secrete increasing concentrations of oestradiol may be that their follicles were not equipped to respond to LH (and FSH) at this stage of lactation. Unfortunately, there are no studies in pigs that describe the development of receptor populations in ovarian follicles during the course of lactation. Britt *et al*. (1985) summarise several studies in which pregnant mare's serum gonadotropin or GnRH, injected at various stages of lactation, had variable effects on the number of sows exhibiting oestrus, suggesting that there is a window in early lactation during which the ovaries are not responsive to gonadotrophins. However, none of these studies incorporated a longitudinal comparison of the effect of gonadotrophins at different stages of lactation. This makes it hard to draw conclusions on the development of ovarian responsiveness to gonadotrophins during the course of lactation. Nevertheless, we hypothesise that, in the present study, on Day 14 of lactation the early antral follicle pool was unresponsive to LH (and FSH) in anovulatory sows. However, this still does not explain the uncoupling between the increase in follicle diameter and oestrogen secretion in anovulatory sows or why after the start of IS the sows in the anovulatory group still did not acquire oestrogenic potential with time.

In conclusion, only a limited number of sows used in the present study showed a normal ovarian response to increased secretion of LH and FSH in an IS regimen, initiated on Day 14 of lactation. Anovulatory sows did show a substantial increase in follicle diameter (some even up to ovulatory size), but failed to show an increase in oestradiol and their follicles regressed. Although FSH concentrations were comparatively low in the present study, neither FSH nor LH seemed to be limiting for the development of oestrogenic follicles. Our findings warrant future studies on the acquisition of responsiveness to increased LH and FSH secretion by early antral follicles during the course of lactation.

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