Gonadotrophins and ovarian steroids in cattle

I. Pulsatile changes of concentrations in the jugular vein throughout the oestrous cycle

E. Schallengerberger, A. M. Scöndorfer and D. L. Walters

Lehrstuhl für Physiologie der Fortpflanzung und Laktation,
Technische Universität München, 8050 Freising-Weihenstephan, FRG

Abstract. Short-term secretion patterns of LH, FSH, progesterone and oestradiol-17β were evaluated throughout complete oestrous cycles of 6 heifers. Frequent blood samples (in 20-min intervals for 12 continuous h) were taken every 3–5 days from indwelling jugular catheters. There was a high incidence of coincident LH and FSH pulses ranging from 72% at luteolysis to 83–100% during the luteal phase. Almost the same total number of LH and FSH pulses occurred during the early luteal phase (7.0 vs 7.4/12 h, respectively), however, there was an average of one additional FSH pulse in between the synchronous LH/FSH ones during the mid- and late luteal phase (6.9 FSH vs 3.4 LH pulses/12 h). Basal LH and FSH concentrations remained unchanged from the early until the late luteal period. During and after luteolysis frequency of LH and FSH release (14.5 vs 10.5 pulses/12 h) increased considerably as well as basal concentrations and magnitude of LH pulses. Secretion of both gonadotrophins persisted very frequently (13.3 LH and 10.7 FSH pulses/12 h) during pro-oestrous and oestrous when basal FSH concentrations and FSH pulse maxima approached a nadir. During the mid-luteal phase 45% of pulsatile progesterone occurred coincidently with each coinciding LH/FSH pulse and 44% of pulsatile progesterone happened after additional single FSH pulses. Distinct short-term changes of oestradiol concentrations were not observed in the jugular vein but concentrations fluctuated randomly ranging from 2–6 pg/ml throughout the luteal period. Prior to and during heat mean concentrations of oestradiol were approximately 2-fold higher (P < 0.05) than during the other periods of the cycle. It is concluded that the frequency of pulsatile LH release is modulated to a much greater extent than FSH by negative feedback of ovarian steroids. Some pulsatile progesterone secretion resulting from the stimulation of FSH (and LH) is still detectable in the jugular vein whereas of oestradiol-17β is not. The additional frequent monitoring of FSH might be more appropriate reflecting pituitary and hypothalamic function than only measuring LH.

Gonadotrophins are secreted in cows in more or less pronounced fluctuations which are interrupted about once every 3 weeks by a large surge release of LH (and FSH) before ovulation (Schams et al. 1977). The basal (tonic) secretion pattern of LH is characterized by discrete short lasting pulses. In both male (Karg et al. 1976; Schams et al. 1978) and female cattle (Forrest et al. 1980; Schallengerberger & Peterson 1982) the frequency of release is dependent on the gonads and especially on the stage of the oestrous cycle (Rahe et al. 1980; Walters et al. 1984; Walters & Schallengerberger 1984). Regularly fluctuating secretion of LH and FSH is known to occur in intact and gonadectomized males and females of various species such as sheep (Butler et al. 1972; Nett et al. 1974; Galvo et al. 1975; Baird et al. 1976; Baird 1978; Lincoln 1978), pigs (Ellendorff et al. 1975; Edwards & Foxcroft 1983), rats (Gay & Sheth 1972; Gallo 1981), monkeys (Dierschke et al. 1970) and humans.

1 M. R. C. Group in Reproductive Biology, University of Western Ontario, University Hospital, 339 Windermere Road, London, Ontario, N6A 5A5, Canada. This project was supported by the Deutsche Forschungsgemeinschaft. D. L. Walters was a post-doctoral fellow of the Alexander von Humboldt-Stiftung, Bonn.
(Naftolin et al. 1972; Boyar et al. 1972; Santen & Bardin 1973; Smith et al. 1974; Bäckström et al. 1982).

The present study was designed to characterize the short-term secretion pattern of both LH and FSH during the various periods of the oestrous cycle of heifers. An additional objective was to determine if the distribution pattern of ovarian oestradiol-17β and progesterone in the jugular vein reflects the discontinuous gonadotrophic stimulation present in the ovarian drainage (Hixon et al. 1983; Walters et al. 1984).

Materials and Methods

Experimental animals
Six regularly cycling 18–23 months old heifers of the local Braunvieh breed were used. They were tethered indoors, fed twice daily with hay, a mixture of grass and corn silage and some concentrate and had access to water ad libitum.

Oestrous detection and cycle normalization

The animals were observed for visible signs of oestrus 4 times daily and rectal palpations were carried out periodically to assess ovarian changes. The length of the individual oestrous cycles (ranging from 20 to 27 days with a mean of 22.6 ± 2.7 days) was normalized according to the day of ovulation, which was considered day 1 of the cycle. Progesterone concentrations were used as an additional tool for dividing the oestrous cycle into 6 periods (see Table 2) and especially to account for the influences of luteolysis during days 17–19 of the oestrous cycle.

Blood sampling

Samples were withdrawn from indwelling vinyl jugular catheters (outside diameter 2.0 mm, inside diameter 1.0 mm, Dural Plastics, Dural N.S.W., Australia) into heparinized tubes, immediately chilled in an ice-water bath to minimize possible hormone losses (Owens et al. 1980; Reimers et al. 1983), centrifuged at 4°C and the plasma stored at −20°C in aliquots until analysis. Series of frequent bleedings consisted of a total of 36 samples (10 ml) collected every 20 min over 12 continuous h (6 a.m.−6 p.m.) in 3–5 day intervals throughout an entire oestrous cycle.

Hormone determination

All samples were analysed in duplicate by radioimmunoassay twice for LH (Schams & Karg 1969), FSH (Schams & Schallenberg 1976), progesterone (Hoffmann et al. 1973) and the first 4 h period of all bleedings once in

### Table 1

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Antiserum</th>
<th>Standard</th>
<th>Limit of sensitivity (ng/ml)</th>
<th>Recovery* (%)</th>
<th>Binding* (%)</th>
<th>Inter-assay CV* (%)</th>
<th>Intra-assay CV* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>LH-DSA**</td>
<td>1:400 000</td>
<td>43.4 ± 2.7</td>
<td>95.2</td>
<td>17.5</td>
<td>2.25</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>FSH</td>
<td>NIH-FSH-B-***</td>
<td>1:180 000</td>
<td>33.9 ± 3.2</td>
<td></td>
<td></td>
<td>17.5</td>
<td>12.2 ± 0.5</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Crystalline progesterone</td>
<td>1:40 000</td>
<td>51.0 ± 2.1</td>
<td>95.2</td>
<td>9.6 ± 1.2</td>
<td>0.02</td>
<td>14.6 ± 0.7</td>
</tr>
<tr>
<td>Oestradiol-17β</td>
<td>Crystalline oestradiol-17β</td>
<td>1:80 000</td>
<td>31.7 ± 1.8</td>
<td>85.5 ± 0.6</td>
<td>8.7 ± 1.1</td>
<td>0.0016</td>
<td>14.3 ± 2.6</td>
</tr>
</tbody>
</table>

*Mean ± sem. **Biological activity 0.49 times NIH-LH-S1. ***Biological activity 1.0 times NIH-LH-S1.
duplicate for oestradiol-17β. The antisera were characterized in the above mentioned papers. The intra-assay coefficients of variation (CV) were derived from estimates for pooled plasma of 3–8 samples used at least twice in every assay covering the range from very high to very low concentrations. Pooled samples with high hormone concentrations were measured in different aliquots to assure parallelism of unknowns and standard hormone in every run. Details of the assay characteristics are given in Table 1.

Oestradiol-17β was quantified using a modification of the method reported by Moseley et al. (1979). Plasma samples (1 ml) were extracted once with 5 ml dichloromethane (Thibier & Saumande 1975). No purification was necessary as the antisera (E₂/II-11-77) was highly specific for oestradiol-17β. It had a cross-reactivity with oestrone of only 2%, a cross-reactivity of 0.5% with oestradiol-17α and a cross-reactivity of 53% with oestradiol-17β-benzoate. There was no significant cross-reactivity with testosterone, progesterone, cortisol, estradiol, ethylstilboestrol, zeronol or trenboloneacetate. Sensitivity of the assay was 1.6 pg/tube as determined by the lower limit of the 95% confidence interval in the total bindings tubes (zero tubes). The average blank was 1.4 ± 1.2 pg/tube. Oestradiol-17β concentrations of various aliquots (0.25–2.0 ml) of pooled plasma samples were parallel to the standard curve. After adjusting for procedural losses during extraction, 0.25, 0.5, 1.0 or 2.0 ml of plasma were determined to contain 20.8, 23.0, 22.0 and 22.1 pg/ml oestradiol-17β, respectively. The recovery of 3.13–200 pg of exogenous oestradiol-17β added to 1 ml of bovine plasma averaged 95.5 ± 5.3%.

### Table 2.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Early luteal</th>
<th>Mid-luteal</th>
<th>Late luteal</th>
<th>Luteolysis</th>
<th>Pro-oestrus oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–4 d</td>
<td>5–8 d</td>
<td>9–12 d</td>
<td>13–16 d</td>
<td>17–19 d*</td>
</tr>
<tr>
<td>Basal concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>FSH</td>
<td>45.5 ± 8.4</td>
<td>48.4 ± 6.0</td>
<td>42.5 ± 7.3</td>
<td>43.2 ± 6.2</td>
<td>45.5 ± 3.2</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0.3 ± 0.1</td>
<td>4.6 ± 0.9</td>
<td>6.3 ± 0.9</td>
<td>6.7 ± 0.7</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>Oestradiol**</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.4</td>
<td>3.9 ± 0.5</td>
<td>3.9 ± 0.5</td>
<td>3.8 ± 1.1</td>
</tr>
<tr>
<td>Pulse maxima</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>1.1 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>FSH</td>
<td>76.2 ± 17.5</td>
<td>68.8 ± 10.5</td>
<td>68.8 ± 14.7</td>
<td>74.3 ± 10.7</td>
<td>56.8 ± 7.7</td>
</tr>
<tr>
<td>Progesterone</td>
<td>5.9 ± 1.1</td>
<td>8.0 ± 1.2</td>
<td>8.4 ± 1.0</td>
<td>6.5 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>Pulses/12 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>7.0 ± 2.3</td>
<td>3.2 ± 0.6</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>FSH</td>
<td>7.4 ± 1.3</td>
<td>7.2 ± 0.8</td>
<td>7.0 ± 0.6</td>
<td>6.5 ± 0.5</td>
<td>6.3 ± 0.9</td>
</tr>
<tr>
<td>Progesterone</td>
<td>6.2 ± 0.5</td>
<td>6.6 ± 0.5</td>
<td>6.8 ± 0.3</td>
<td>6.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Amplitude</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>FSH</td>
<td>31.7 ± 9.3</td>
<td>30.3 ± 5.1</td>
<td>28.5 ± 8.9</td>
<td>33.5 ± 6.1</td>
<td>27.1 ± 8.1</td>
</tr>
<tr>
<td>Progesterone</td>
<td>1.2 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

a Values are means ± SEM of 6 animals bled every 20 min for 12 h.
b Calculations are based only on 4 h periods from each animal.
* Three heifers prior to luteolysis. ** Three heifers during or after luteolysis.
*** Values within preovulatory surge periods are excluded.
proved to provide about the same results as if pulses would have been determined visually from the graphs (Carruthers & Hafs 1980). Only for comparison purposes (see Table 1) average basal concentrations were calculated by excluding all pulsatile increases fitting the above definition from the data pool. The highest value of any individual pulsatile hormone increase was included to compose the average pulse maxima. Statistical significance of means was evaluated by ANOVA and Duncan’s multiple range test was used to differentiate between the time periods.

Results

Tonic secretion of gonadotrophins

Basal LH concentrations began to increase \((P < 0.05)\) during and after luteolysis (Table 2, Fig. 5). They were also high at pro-oestrus and oestrus and decreased \((P < 0.05)\) after the pre-ovulatory LH surge to an about consistent nadir during the entire luteal phase of the cycle (Table 2). The pulse maxima were lowest during the early luteal phase (Table 2) and highest following luteolysis. An average of 7 LH pulses were secreted during the early luteal phase and 3.2–3.5 LH pulses were exhibited during the mid- and 3.5–4.3 during the late luteal phase (Table 2). Fewer, but high amplitude pulses were exhibited during the mid- and late luteal phases (Figs. 2, 3, 4 and Table 2). The frequency increased \((P < 0.05)\) and the amplitude decreased \((P < 0.05)\) when luteolysis occurred (Fig. 5, Table 2) whereas the amplitude remained low after the preovulatory LH surge (Fig. 1, Table 2). Basal and maximal FSH concentrations and amplitudes of pulses remained unchanged \((P > 0.05)\) throughout the entire
oestrous cycle with the exception of pro-oestrus and oestrus when all 3 parameters were lower \((P < 0.05)\) (Table 2, Fig. 6). During the mid-luteal phase of the oestrous cycle there was on average one FSH pulse in between the 96% coinciding LH and FSH pulses (Figs. 2–4, Table 3), thus considerably increasing \((P < 0.05)\) the number of pulses for FSH. During the early luteal phase 83% of LH pulses were parallel to or occurred within 20 min before or after an FSH pulse. During luteolysis the frequency of FSH increased less \((P < 0.05)\) than of LH (Fig. 5, Tables 2 and 3).

Frequency of LH pulses was also greater \((P < 0.05)\) than of FSH during the pro-oestrous and oestrus period (Fig. 6, Tables 2 and 3), whereas during the initiation of luteal function only few additional FSH pulses were present (Fig. 1, Tables 2 and 3). During the luteal phase and during luteolysis there was occasional massive FSH release due to higher amplitude pulses. Frequency of release remained unchanged (Fig. 5, h 5–12). No change of LH occurred during these several h lasting periods of high FSH concentrations.

**Secretion of ovarian steroids**

There was considerable variation in progesterone secretion during the luteal phase of the oestrous cycle. Basal as well as maximum concentrations increased \((P < 0.05)\) steadily until initiation of luteolysis (Table 2). Almost every parallel LH and FSH pulse was either followed after 20–40 min by or was concomitant to a progesterone pulse during the luteal phase (Fig. 2). Also 44% of progesterone pulses occurred in close correlation with individual FSH pulses, thus 89% of progesterone pulses were parallel to gonadotrophin pulses (Table 3). The

---

**Fig. 3.** Secretion pattern of LH, progesterone, FSH and oestradiol-17β on day 12 of the oestrous cycle of the same heifer as depicted in Figs. 1 and 2.

**Fig. 4.** Secretion pattern of LH, progesterone, FSH and oestradiol-17β on day 15 of the oestrous cycle of the same heifer as depicted in Figs. 1–3.
Fig. 5.
Secretion pattern of LH, progesterone, FSH and oestra-
diol-17β on day 18 of the oestrous cycle of the same
heifer as depicted in Figs. 1–4. Note the FSH increase
between h 5–12.

Fig. 6.
Secretion pattern of LH, progesterone, FSH and oestra-
diol-17β in between oestrus and ovulation on day 21 of
the oestrous cycle of the same heifer as depicted in Figs.
1–5.

Table 3.
Association of gonadotrophin and progesterone pulses during the oestrous cycle of 6 cows.

<table>
<thead>
<tr>
<th>Comparison of pulses</th>
<th>Days of oestrous cycle</th>
<th>1–4</th>
<th>5–16</th>
<th>17–19</th>
<th>20–21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n*</td>
<td>%*</td>
</tr>
<tr>
<td>Total: concomitant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH: LH/FSH</td>
<td></td>
<td>35:29</td>
<td>82.9</td>
<td>55:53</td>
<td>96.4</td>
</tr>
<tr>
<td>FSH: LH/FSH</td>
<td></td>
<td>37:29</td>
<td>78.4</td>
<td>114:53</td>
<td>46.5</td>
</tr>
<tr>
<td>Progesterone: Progesterone/</td>
<td></td>
<td>–</td>
<td>–</td>
<td>113:51</td>
<td>45.1</td>
</tr>
<tr>
<td>LH/FSH</td>
<td></td>
<td>–</td>
<td>–</td>
<td>113:50</td>
<td>44.2</td>
</tr>
<tr>
<td>Progesterone: Progesterone/FSH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Three heifers prior to luteolysis. ** Three heifers during or after luteolysis.
amplitude of the progesterone pulses was highest during the mid- and late luteal phases (Figs. 3 and 4, Table 2) whereas pulsatile release vanished during luteolysis (Fig. 5). No fluctuations were measurable during oestrus and the early luteal period (Figs. 6 and 1). Oestradiol-17β did not appear to circulate in a pulsatile fashion in the peripheral plasma. Variations occurred irregularly and did not seem to be related to the pulsatile release of LH, FSH or progesterone. Basal concentrations were rather constant throughout the cycle (Table 2, Figs. 1-5) except during pro-oestrus and oestrus when a significant (P < 0.05) increase was obvious.

Discussion

LH, FSH and progesterone were all detectable in a pulsatile manner during the various periods of the oestrous cycle studied in the plasma of the jugular vein. Changes in the secretory hormone patterns could not be classified as diurnal rhythms as they did not appear to be related to the time of day, variation of light, temperature or to incidences of feeding. When blood samples have been collected continuously (Terqui et al. 1982) or even 10 min (Rahe et al. 1980) the pattern of LH secretion in cows was similar to the present data. The release pattern of both gonadotrophins may reflect the discontinuous stimulation of the pituitary gland by the hypothalamic gonadotrophin releasing hormone (GnRH). McNeilly et al. (1984) could recently demonstrate that the administration of anti-serum to GnRH leads to an immediate cessation of pulsatile LH in ewes. It was also proven that GnRH in pituitary stalk blood and LH in the jugular vein of conscious sheep are secreted concomitantly (Levine et al. 1982; Clarke & Cummins 1982). Both groups measured more frequent GnRH pulses than LH pulses although the extra GnRH pulses were generally lower in amplitude. There was more frequent FSH than LH release detected in the present study except during pro-oestrus and oestrus (see Tables 2 and 3), when the frequency of gonadotrophin secretion reaches a maximum which cannot be properly quantified when the presently used sampling interval is applied (Walters & Schallenberger 1984). There is no reason not to assume that the extra FSH pulses might be a result of GnRH pulses since every high amplitude LH pulse during the luteal phase was always parallel to an FSH one and, using more frequent blood samplings (Walters et al. 1984), low amplitude short lasting LH pulses might occur on the occasion of extra FSH pulses. Thus, discontinuous GnRH release might trigger either parallel high amplitude LH/FSH pulses, high amplitude FSH/low amplitude LH pulses or separate FSH pulses depending either upon the amplitude of the GnRH release or ovarian steroid feedback. Detecting more frequent FSH secretion is not only depending on the chosen blood sampling interval but rather upon the reproductive status as it cannot be observed in ovariectomized cattle (Schallenberger & Peterson 1982). Monitoring FSH might therefore be a better indicator of GnRH release than only monitoring pulsatile LH release.

The present data confirm that progesterone exerts a potent negative feedback effect on pulsatile LH release (Ireland & Roche 1982) reducing mainly the frequency of release (Rahe et al. 1980) whereas amplitude is increased. After luteolysis frequency of LH secretion increased reaching a threshold necessary for the expression of the surge (Kesner et al. 1981; Walters & Schallenberger 1984). Although basal and maximal FSH concentrations were fairly constant from ovulation through the luteal phase of cycle, FSH concentrations declined after luteolysis similar to sheep (Baird et al. 1981) with the lowest basal levels occurring during oestrus. An interesting observation concerning FSH was made during various bleeding periods (see example in Fig. 5). FSH concentrations sometimes increased considerably for several h during the luteal and early follicular phase of the oestrous cycle while continuing to exhibit unaltered pulsatile release. This resembles the augmentation in amplitude but not frequency of FSH release 4-12 h after the preovulatory gonadotrophin surge (Walters & Schallenberger 1984). These several h lasting periods of increased concentrations of FSH are assumed to contribute to the wave-like FSH release pattern observed when blood samples were collected at less frequent (4-6 h) intervals during the oestrous cycle (Schams et al. 1977; Lahlou-Kassi et al. 1984). These increased FSH concentrations appeared to coincide with changes in follicular growth and atresia (Schams et al. 1976; Matton et al. 1981).

Mean oestradiol concentrations did increase before and during oestrus (Glencross et al. 1973; Schams et al. 1977; Butler et al. 1983) but re-
mained relatively unchanged during the other periods of the cycle studied. The pronounced changes reflecting follicular activity in the ovarian venous drainage (Walters et al. 1984; Walters & Schallengerber 1984) have disappeared in the jugular circulation. Unlike oestradiol, pulses of progesterone were detected in the periphery but amplitude was much less than in the ovarian vein (Hixon et al. 1983) or vena cava caudalis (Walters et al. 1984). Pulsatile secretion seemed to have a close temporal relationship with LH as well as FSH release (Tables 2 and 3, Figs. 3 and 4).

FSH has been shown to stimulate progesterone synthesis from granulosa cells in vitro (Thanki & Channing 1978; Dorrington & Armstrong 1979; Ntimrod & Lindner 1979). It had been demonstrated earlier that FSH interfered considerably in the ovarian ascorbic acid depletion assay (OAAD) (Brüggenmenn et al. 1965; Rosemberg et al. 1965) and in a cytohistochemical assay (Schallengerber & Schams 1980) when one tried to measure LH in unextracted plasma. It appears that FSH has an important role in progesterone synthesis and release, but its mechanism of action is not understood. The physiological significance of pulsatile progesterone secretion and its potential effect(s) on feedback regulation is yet unclear. A synergistic action of parallel short-term releases of oestradiol and progesterone on LH negative feedback might be important (Walters et al. 1984).

Acknowledgments
We thank the NlAMDD for generous supply with NIH-FSH-B2, and I. Redl, U. Oerterer and S. Prokopp for expert technical and experimental assistance.

References


Received on September 10th, 1984.