Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the periovulatory phase of the oestrous cycle in the cow*

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Summary. An injection of 500 µg prostaglandin (PG) analogue was given on Day 12 (mid-luteal phase) of the oestrous cycle to 8 cows. An LH surge occurred 59 ± 2 h later. LH, FSH, prolactin, oestradiol-17β, progesterone and oxytocin concentrations were determined in blood samples collected from the caudal vena cava and/or the jugular vein at 20-min (5 cows) or 5-min (3 cows; only LH and FSH concentrations were determined) intervals for 24 h, beginning 48 h after the PG injection. Oxytocin concentrations were low and similar in the vena cava and the jugular vein. In blood samples collected every 5 min the interpulse interval for LH and FSH during the period before the LH surge was 38–40 min. In the 20-min samples the interpulse interval for oestradiol was similar to that for LH and FSH, but pulse amplitude and basal concentrations steadily increased to reach maximum concentrations 6–8 h before and again during the LH surge. A decrease in oestradiol concentrations, lasting at least 60 min, occurred just before the start of the LH surge. Progesterone concentrations also increased at the same time as the LH surge. The magnitude of the LH surges varied from 7 to 32 ng/ml, but all cows ovulated and had oestrous cycles of normal length. Distinct pulses of LH and FSH were observed throughout the LH and FSH surges. Pulsatile secretion of LH was not detected for a period of up to 6–12 h following the LH surge, but then low-amplitude pulses occurred. In contrast, the pulsatile secretion of FSH remained at a frequency similar to that observed during the descending phase of the FSH surge. Furthermore, a second increase in FSH concentrations occurred, beginning 4–12 h after the LH-surge.

It is concluded that: (1) the frequent, high-amplitude pulses of oestradiol that occur before and during the LH surge are probably due to stimulation by pulses of LH; (2) the LH surge is the result of an increase in frequency and amplitude of the LH pulses; (3) the second increase in FSH that follows the LH and FSH surges appears to be the result of an increase in the amplitude (not frequency) of the FSH pulses; and (4) very little, if any, oxytocin is secreted from the ovary during the periovulatory phase of the oestrous cycle.

Introduction

Changes in pituitary and ovarian hormones during the periovulatory phase of the oestrous cycle of the cow have been described in a number of studies (Chenault, Thatcher, Kalra, Abrams & Wilcox, 1975; Schams, Schallenger, Hoffmann & Karg, 1977; Dobson, 1978), but the exact relationship

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between the pulsatile secretion of gonadotrophins and ovarian steroid secretion has not been reported. After regression of the corpus luteum pulsatile secretion of LH increases concomitantly with oestradiol concentrations (Schallenger, Schams, Bullermann & Walters, 1984). Baird & McNeilly (1981) reported that this increase in oestradiol concentrations is due to stimulation by pulses of LH that cause the preovulatory follicle to secrete high-amplitude pulses of oestradiol.

Numerous studies indicate that high oestradiol concentrations induce the preovulatory LH (Short, Randel, Staigmiller & Bellows, 1979; Kesner, Convey & Anderson, 1981) and FSH (Kesner & Convey, 1982) surges in the cow. The LH surge is apparently composed of very frequent, high-amplitude pulses of LH (Rahe, Owens, Fleeger, Newton & Harms, 1980), but it is not known whether the FSH surge is composed of pulses or a constant release of FSH.

The objective of the present study was to characterize the pulsatile secretion pattern of LH, FSH, prolactin, progesterone, oestradiol-17 β and ovarian oxytocin during the periovulatory phase of the oestrous cycle to determine temporal relationships amongst these hormones.

Materials and Methods

Experimental animals. Eight regularly cyclic cows of the local Braunvieh and Fleckvieh breeds (aged 3–5 years) were used. All cows were non-lactating and were tethered indoors during the experiment. They were fed hay, corn silage and energy supplement twice daily and had access to water *ad libitum*.

Experimental procedure. Cannulae were inserted into the caudal vena cava via the tail vein and into the jugular vein of 5 cows and only into the jugular vein of 3 cows on Day 11 of the oestrous cycle as described previously by Walters, Schams & Schallenger (1984). An i.m. injection of 500 μ g cloprostenol (a prostaglandin (PG) analogue; ICI, Macclesfield, England) was given on Day 12 (mid-luteal phase) of the oestrous cycle. In cows that had vena cava and jugular vein cannulae, blood samples (20 ml) were collected simultaneously from each vein every 20 min for 24 h, beginning 48 h after PG injection. In cows that had only jugular vein cannulae, blood samples were collected every 5 min for 24 h, beginning 48 h after the PG injection. The sampling intervals were chosen as a preovulatory LH surge normally occurs about 60 h after injection of PG in these breeds (Karg *et al.*, 1976). All blood samples were placed into heparinized tubes, cooled, centrifuged and the plasma was stored in 3 aliquots at -20°C until hormone analysis.

Hormone analysis. LH (Schams & Karg, 1969a), FSH (Schams & Schallenger, 1976) and progesterone (Hoffmann, Kyrein & Ender, 1973) were analysed twice in duplicate and prolactin (Schams & Karg, 1969b), oestradiol-17 β (Walters & Schallenger, 1983) and oxytocin (Schams, 1983) once in duplicate. A summary of the assay characteristics for all of the above hormones is provided by Walters *et al.* (1984). Vena cava blood samples were assayed for oestradiol-17 β , progesterone, prolactin and oxytocin while jugular vein blood samples (collected every 20 min) were assayed for all hormones listed above. Blood samples collected at 5-min intervals were assayed only for LH and FSH.

Statistical analysis. A pulse was determined as described by Walters *et al.* (1984). Differences between mean hormone concentrations in the vena cava and jugular vein were tested by using Student's *t* test.

Results

Mean concentrations of oestradiol and progesterone were lower ($P < 0.01$) in the jugular vein than in the vena cava (Table 1). It was not possible to detect easily pulses of oestradiol in the jugular vein (Text-fig. 1a) and no pulses of progesterone were detected in the jugular vein; therefore only data from vena cava plasma were utilized to examine the secretion pattern of these hormones.

Table 1. Mean $\pm$ s.e.m. concentrations of oestradiol, progesterone and oxytocin in vena cava and jugular vein plasma during the periovulatory phase in 5 cows†

Hormone	Vena cava	Jugular vein
Oestradiol (pg/ml)	35.5 $\pm$ 6.7*	9.1 $\pm$ 0.7
Progesterone (ng/ml)	0.7 $\pm$ 0.1*	0.3 $\pm$ 0.1
Oxytocin (pg/ml)	1.4 $\pm$ 0.2	1.3 $\pm$ 0.4

* $P < 0.01$ compared with jugular vein value.

† Samples were collected every 20 min from 48 to 72 h after PG.

Changes before the preovulatory LH surge

The period from the beginning of blood sampling (48 h after PG injection) until the start of the LH surge varied from 6 to 16 h in 7 of the cows. The LH surge had already started in one cow (Text-fig. 2b) and data for this animal were therefore excluded.

LH. An interpulse interval of 61 ± 2 min was calculated from samples collected every 20 min (Table 2; Text-fig. 1). However, an interpulse interval of 40 ± 0.5 min was calculated in samples collected every 5 min (Table 2; Text-fig. 2a). Basal concentrations and pulse amplitude were similar in samples collected every 20 min and every 5 min and did not change during this period.

Oestradiol. The interpulse interval was similar to that of LH (Table 2) with oestradiol pulses generally occurring concomitantly with or 20 min after an LH pulse (Text-fig. 1). However, unlike LH, basal concentrations and pulse amplitude steadily increased and reached maximum concentrations during this period (Text-fig. 1). Basal concentrations and pulse amplitude were higher in cows that had high-amplitude LH surges (Text-fig. 1a) than in cows with low-amplitude LH surges (Text-fig. 1b). In all cows there was a decrease in oestradiol concentrations towards the end of this period that lasted at least 60 min.

FSH. The interpulse interval was similar to that of LH from samples collected either every 20 min (Table 2; Text-fig. 1) or every 5 min (Table 2; Text-fig. 2). Pulse amplitude and basal concentrations were fairly constant during this period in all cows.

Progesterone. Mean concentrations in the vena cava were low (Table 1). The observed changes in concentration in this vessel could only occasionally be classified as pulsatile (Text-fig. 1b).

Changes during the preovulatory LH surge

LH. A surge-type release of LH was observed in all but one cow at 59 ± 2 h after PG injection. The duration of the surge lasted 6–10 h. The magnitude of the surges varied from 7 to 32 ng/ml, but all cows ovulated and had oestrous cycles of normal length (range 18–23 days). Pulses were generally not detected during the surge in samples collected every 20 min, especially in the high-amplitude surges (Text-fig. 1a). However, in samples collected every 5 min, distinct pulses were detected throughout the surge (Text-fig. 2a). The interpulse interval during the surge was similar to that before the surge (Table 2). Pulse amplitude was higher during the surge than before or thereafter.

Oestradiol. Basal concentrations increased and decreased concomitantly with the LH surge (Text-fig. 1). Frequent, high-amplitude pulses were observed throughout this period in the vena cava but not in the jugular vein (Text-fig. 1a). Peak oestradiol concentrations were highest in cows that had high magnitude (~ 30 ng/ml) LH surges (Text-fig. 1a) and were lowest in cows with low-magnitude (~ 7 ng/ml) LH surges (Text-fig. 1b; $r^2 = 0.8$; $P < 0.01$).

FSH. A surge-type release of FSH occurred concomitantly with the high-magnitude (> 10 ng/ml) LH surges (Text-fig. 1a) but not with the low-magnitude LH surges (Text-fig. 1b). Pulses were not generally detected during the FSH surge in samples collected every 20 min, but in samples collected every 5 min distinct pulses were detected throughout the surge (Text-fig. 2) with an inter-

Text-fig. 1. Hormone profiles from a cow with (a) a high-magnitude LH surge and (b) a low-magnitude LH surge. Blood samples were collected from the jugular vein (LH, FSH and oestradiol (□)) and vena cava (oestradiol (●) and progesterone) at 20-min intervals for 24 h (beginning 48 h after an injection of a prostaglandin analogue on Day 12 (mid-luteal phase) of the oestrous cycle).

pulse interval similar to that obtained for LH (Table 2). In contrast to LH, however, an increase in pulse amplitude was not consistently observed during the ascending and plateau phases of the FSH surge.

Progesterone. Mean concentrations in the vena cava increased and decreased concomitantly with the LH surge in 4 out of 5 cows (Text-figs 1b vs 1a), but this increase was not detected in the jugular vein. The increase in progesterone concentrations occurred whether cows had large or small LH surges, although there was no relationship between the magnitude of the progesterone increase and the magnitude of the LH surge.

Changes after the preovulatory LH surge

LH. Pulsatile secretion was not detected for a period of 6–12 h, after which low-amplitude pulses were observed (Text-fig. 2; Table 2). Basal concentrations were lower than those observed before the LH surge.

Oestradiol. Pulsatile secretion was abolished during this period in cows with high-magnitude LH surges (Text-fig. 1a). Mean concentrations were then lower ($P < 0.01$) than before or during the LH surge (Text-fig. 1a).

FSH. In cows that had a surge-type FSH release, basal concentrations were lower immediately after than before the surge (Text-fig. 1a). The interpulse interval was short and relatively constant during the entire period of this study (Table 2). In contrast to LH, a distinct increase in basal FSH concentrations was observed beginning 4–12 h after the LH surge (Text-fig. 2), primarily due to an increase in pulse amplitude.

Progesterone. Basal concentrations were similar to those observed before the LH surge (Text-fig. 1).

Table 2. Mean $\pm$ s.e.m. interpulse intervals for LH, FSH and oestradiol before, during and after the preovulatory LH surge of 8 cows

Hormone	Sampling interval (min)	No. of cows	Interpulse interval (min)				
			Before*	During†			After‡
				Ascending	Plateau	Descending	
LH	5	3	40 $\pm$ 0.5	38 $\pm$ 2	23 $\pm$ 0.4	29 $\pm$ 4	65 $\pm$ 7
	20	5	61 $\pm$ 2	—	—	—	—
FSH	5	3	38 $\pm$ 0.5	31 $\pm$ 3	24 $\pm$ 2	29 $\pm$ 4	30 $\pm$ 0.6
	20	5	69 $\pm$ 2	—	—	—	—
Oestradiol	20	5	62 $\pm$ 5	—	59 $\pm$ 2	—	—

* A period of 6–16 h before the LH surge.

† A period of 6–10 h during the LH surge. Interpulse intervals were determined during the ascending, plateau and descending phases only for LH and FSH.

‡ A period of 6–20 h after the LH surge. No LH pulses were observed during a 6–12-h period after the end of the LH surge.

Changes in oxytocin and prolactin concentrations

Oxytocin. Mean concentrations were low and similar ($P > 0.05$) in the vena cava and the jugular vein (Table 1). Low-amplitude pulses were only detected in 2 cows (Text-fig. 3). Basal concentrations did not increase at the time of the LH surge, but instead remained relatively constant throughout the periovulatory phase studied.

Prolactin. Mean concentrations were not different ($P > 0.05$) between the vena cava and the jugular vein. Basal concentrations and pulse amplitude were higher ($P < 0.05$) in samples collected during the night than in samples collected during the day (Text-fig. 3). However, basal concentra-

Text-fig. 2. LH and FSH profiles from jugular vein blood samples collected at 5-min intervals to characterize the period (a) before and during the gonadotrophin surge (samples collected from 48 h after the PG injection) and (b) after the gonadotrophin surge. The cow in (b) had exhibited the LH surge by 48 h after PG.

Text-fig. 3. Oxytocin and prolactin profiles from one cow. Blood samples were collected from the vena cava at 20-min intervals for 24 h beginning 48 h after PG. The time of the day and the duration of the LH surge are indicated.

tions, pulse amplitude and frequency varied considerably between cows sampled during various seasons of the year.

Discussion

From the results of the present study it is clear that the increase in oestradiol concentrations that occurs before and during the LH surge in cows (Chenault *et al.*, 1975; Schams *et al.*, 1977) is a result of frequent, high-amplitude pulses of oestradiol. The interpulse intervals for oestradiol and LH were virtually identical during the period before the LH surge, indicating a possible association between these two hormones. The oestradiol pulses may be a result of stimulation of the preovulatory follicle by pulses of LH as has been demonstrated for the ewe (McNeilly, O'Connell & Baird, 1982). The interpulse intervals for oestradiol and LH were fairly constant before the LH surge, but during this time the oestradiol pulse amplitude increased while the LH pulse amplitude remained the same. This increase in oestradiol pulse amplitude probably represents an increase in follicular responsiveness to LH as a result of the increased number of LH receptors in the preovulatory follicle during this period (Walters *et al.*, 1982; Staigmiller, England, Webb, Short & Bellows, 1982). The physiological relevance of the corresponding FSH pulses is not clear, although according to Richards *et al.* (1976) FSH is required for induction of LH receptors in rats.

During this period before the LH surge the high oestradiol concentrations appear to exert a positive effect on the pituitary and a negative effect on the hypothalamus. The positive feedback effect on the pituitary results in an increased ability of the pituitary to release LH and FSH in response to GnRH (Kesner *et al.*, 1981; Kesner & Convey, 1982). The highest oestradiol concentrations in the present study were observed 6–8 h before the LH surge at a time when the pituitary reaches maximum responsiveness to GnRH (Kesner *et al.*, 1981; Kesner & Convey, 1982). However, during this period of high oestradiol concentrations and enhanced pituitary responsiveness to GnRH the amplitude of the LH and FSH pulses did not increase and was in fact lower than the LH and FSH pulse amplitude during the luteal phase of the cycle (Baird & McNeilly, 1981; Schallenberger *et al.*, 1984). An increase in the amplitude of the LH and FSH pulses was probably prevented at this time by the negative feedback effect of the high oestradiol concentrations on the hypothalamus. Several studies have demonstrated that injections of oestradiol will initially suppress basal LH and FSH concentrations for a period of 8–10 h and then stimulate a preovulatory-like LH and FSH surge in ovariectomized and intact (follicular phase) cows (Kesner *et al.*, 1981; Kesner & Convey, 1982; Schallenberger, Oerterer & Hutterer, 1982). Schallenberger *et al.* (1982) found that an injection of oestradiol benzoate in ovariectomized cows suppressed LH concentrations by reducing the amplitude, but not the frequency, of the LH pulses. It is therefore possible that the amplitude of the endogenous GnRH pulses was reduced by the high oestradiol concentrations. This assumption is supported by data of Sarkar & Fink (1980) from rats that demonstrated that the amplitude (but not frequency) of endogenous GnRH pulses was reduced after an injection of oestradiol. Also, in ovariectomized ewes it has been shown that the amplitude of endogenous GnRH pulses is highly correlated with the amplitude of the corresponding LH pulses (Clarke & Cummins, 1982; Levine, Pau, Ramirez & Jackson, 1982).

It is not known what actually triggers the onset of the LH and FSH surges. From the studies of Kesner *et al.* (1981) and Kesner & Convey (1982) it is clear that the LH and FSH surges are not induced by a sudden increase in pituitary responsiveness to GnRH. Based on the present observations an alternative proposal is suggested. In all cows examined there was a consistent decline in oestradiol concentrations just before the onset of the LH surge. Its basal concentrations remained low for at least 60 min and then the LH surge began and oestradiol concentrations increased concomitantly with the LH surge. A similar pattern has been reported for monkeys (Marut *et al.*, 1981), sheep (Baird & McNeilly, 1981), rats (Butcher, Collins & Fugo, 1974) and pigs (van de Wiel, Erkens, Koops, Vos & van Landeghem, 1981). Therefore, it is suggested that it is the decrease in

oestradiol concentrations that actually triggers the onset of the LH surge by removing the inhibitory effect on the hypothalamus. This would allow the amplitude of the GnRH pulses to increase and thereby stimulate a maximally responsive pituitary to release frequent, large-amplitude pulses of LH as observed in the present study. Thus, the mechanism for the initiation of the LH surge may be compared to a switch that is turned on by the decrease in oestradiol concentrations and cannot be turned off when oestradiol concentrations increase again due to stimulation by the rising LH concentrations.

In the majority of the cows the LH surge was associated with a surge of FSH. However, in the 2 cows that had small (~ 7 ng/ml) LH surges a corresponding FSH surge was not detected. Possibly, the lower oestradiol concentrations in these 2 cows may have inadequately primed the pituitary to respond to GnRH resulting in smaller LH surges and no detectable FSH surges. The smaller LH surges appear to resemble the type of LH surge often observed when post-partum cows resume cyclic function (Schams *et al.*, 1978). When a large gonadotrophin surge did occur the relative FSH increase above basal concentrations was ~ 2 – 3 -fold, much less than the ~ 20 -fold increase observed for LH.

In addition to the increase in oestradiol secretion during the LH surge, there was also in most cows a significant increase in progesterone concentrations in the vena cava that changed concomitantly with the LH surge. A similar increase in progesterone concentrations in ovarian vein plasma has been reported by Wheeler, Baird, Land & Scaramuzzi (1975) during the LH surge in sheep. We have confirmed by HPLC analysis (data not shown) that the increase in radioimmunologically measurable hormone in our studies was in fact progesterone and not a related progestagen.

Oxytocin has been reported to be present in high concentrations in corpora lutea of ewes and cows (Wathes & Swann, 1982; Schams, Walters, Schallenger, Bullermann & Karg, 1983) and appears to be secreted in a pulsatile fashion concomitantly with progesterone into the ovarian vein (Flint & Sheldrick, 1983; Walters *et al.*, 1984). However, in the present study mean concentrations of oxytocin were not higher in the vena cava than in the jugular vein, suggesting that little, if any, oxytocin is secreted from the ovary when a functional corpus luteum is not present despite the fact that Schams *et al.* (1983) measured relatively high concentrations of oxytocin in the follicular fluid of large bovine follicles.

Unlike results for the luteal phase, it was not possible to detect clearly pulses of LH and FSH during the surge using the 20-min sampling procedure due to the extremely short interpulse interval of both hormones. However, in agreement with Rahe *et al.* (1980) it was evident from the 5-min sampling procedure that pulses of both LH and FSH did occur throughout the LH and FSH surges. Pulses of LH were almost always secreted concomitantly with pulses of FSH before and during the LH surge, suggesting that GnRH is the sole hormone responsible for their release during this period.

LH pulses were not detected for 6–12 h after the LH surge, although low-amplitude pulses of LH were observed after 12 h. In contrast, FSH pulses continued to be secreted at approximately the same frequency as that observed during the surge. Furthermore, FSH basal concentrations and pulse amplitude increased 4–12 h after the LH surge, resulting in a distinct second increase in mean FSH concentrations, as reported by Dobson (1978) and Peters, Kingsley & Riley (1981). Baird & McNeilly (1981) suggested for the ewe that the marked reduction in oestradiol concentrations after the LH surge reduces the negative feedback effect of this steroid, thus allowing FSH concentrations to increase. However, in the rat, it was not possible to inhibit the second rise in FSH concentrations when oestradiol concentrations were increased exogenously (Chappel & Barraclough, 1977). Therefore, the second FSH increase may be the result of a decrease in inhibin concentrations that allows basal FSH secretion to increase (DePaolo, Shander, Wise, Barraclough & Channing, 1979; Elias, Kelch, Lipner & Blake, 1982). However, the fact that the second rise in FSH concentrations was composed of frequent, high-amplitude pulses would seem to indicate that it is a result of stimulation of the pituitary by pulses of GnRH, as observed during the preovulatory LH and FSH surges. In addition, distinct pulses of LH continue to be secreted in ovariectomized cows after an oestradiol

benzoate-induced LH surge (Schallenger *et al.*, 1982), indicating that GnRH continues to be secreted in a pulsatile manner. It is therefore possible that the responsiveness of the pituitary is altered after the LH and FSH surges so that a very small quantity of GnRH can cause the selective release of FSH.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft. D.L.W. was a postdoctoral research fellow of the Alexander von Humboldt-Stiftung, Bonn, FRG. We thank the NIAMDD for supplying NIH-FSH-B1 and NIH-P-B3; Professor D. Schams and his technicians for the determination of oxytocin concentrations; Dr H. H. D. Meyer for the HPLC analysis of progesterone; and I. Redl and P. Hempe for expert technical and experimental assistance.

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Received 25 October 1983