Faculty Papers and Publications in Animal Science   Animal Science Department

1995

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Hormone concentrations in the caudal vena cava during the first ovarian follicular wave of the oestrous cycle in heifers

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Changes in pulsatile secretion of LH, FSH, oestradiol and progesterone were related to the growth phase, early plateau phase and regression phase of the first ovarian dominant follicle of the oestrous cycle in *Bos indicus* heifers. Relationships between these hormones during the three phases were also investigated. Accurate measurements of episodic ovarian steroid secretion were obtained by catheterizing the caudal vena cava via the lateral saphenous vein; the tip of the catheter was positioned just cranial to the ovarian vein using transrectal ultrasonography. Pulsatile secretion of oestradiol was increased only during the growth phase of the dominant follicle and was associated with high frequency release of LH pulses. However, mean concentrations of oestradiol were reduced when the dominant follicle attained its maximum diameter. Between the growth and plateau phases, as the amount of progesterone released increased and oestradiol released decreased, LH pulse frequency and mean concentration of LH decreased. Pulses of LH released were followed within 15 min by increases in mean concentrations of oestradiol ($P < 0.001$); however, there was no apparent relationship between LH and progesterone release ($P = 0.19$). Although there was little evidence of pulsatile release of FSH, mean concentrations of FSH were increased by 0.2 ng ml^{-1} ($P = 0.04$) during the plateau phase, which was on average 2.1 days before the day of emergence of the second dominant follicle of the oestrous cycle. This increase in FSH, in conjunction with the decrease in secretion of oestradiol, may be an indication of the loss of functional dominance by the first dominant follicle of the oestrous cycle.

Introduction

Development of the largest follicle in the bovine ovary has been divided into phases of selection, dominance and atresia (Ireland and Roche, 1987). During the selection phase, several follicles begin to grow, but only one follicle continues to increase in size, becoming larger than all other follicles. This follicle is termed the dominant follicle and is responsible for the increased concentrations of oestradiol in the blood observed during pro-oestrus and early dioestrus (Ireland *et al.*, 1984; Guilbault *et al.*, 1993a). During the luteal phase of the oestrous cycle, the dominant follicle ceases growth and undergoes atresia, being replaced by a second or third dominant follicle (Savio *et al.*, 1988). This sequence of events is mainly under gonadotrophin control (Driancourt, 1991), although a number of intraovarian peptides may also be involved (Findlay, 1993).

Changes in secretion of gonadotrophins during different phases of the bovine oestrous cycle have been examined in a number of studies in which serial blood samples were obtained for periods of 12–24 h. Rahe *et al.* (1980) found that the

frequency of LH pulses decreased and the amplitude of pulses increased during the mid-luteal phase (day 10 or 11) compared with the early luteal phase (day 3) of the oestrous cycle, although there were no differences in mean concentrations of LH. Similarly, Walters *et al.* (1984) found that the frequency of LH pulses was lower during the mid-luteal compared with the early luteal phase of the oestrous cycle, although differences in LH pulse amplitude among different parts of the luteal phase were not detected. The characteristics of the release of gonadotrophin pulses and their relationship to ovarian steroid release have not been examined in relation to the morphological changes of the first dominant follicle that develops during the bovine oestrous cycle.

Venous blood samples have to be collected at a site close to the ovary to obtain accurate measurements of episodic ovarian steroid release. Several methods have been described for the direct sampling of ovarian effluent, including autotransplantation of the ovary and uterus to the neck in ewes (Baird *et al.*, 1976), surgical catheterization of the utero–ovarian vein (Ireland *et al.*, 1984) and cannulation of the medial coccygeal vein (Walters *et al.*, 1984) in cattle. Benoit and Dailey (1991) described a simple technique for catheterization of the caudal vena cava via the lateral saphenous vein. A modification of this technique was used in the current experiment, incorporating

insertion of the catheter under local anaesthesia and positioning of the catheter tip within the caudal vena cava using transrectal ultrasonography.

The aim of this experiment was to evaluate patterns of release of LH, FSH, oestradiol and progesterone in ovarian venous samples during the growth, early plateau and regression phases of the first dominant follicle of the oestrous cycle in Brahman (*Bos indicus*) heifers. Relationships between the patterns of release of these hormones during the different phases of ovarian follicular development were also investigated.

Materials and Methods

Experimental animals

Six Brahman heifers, 2 years of age, weighing on average ($\pm$ SEM) 317.9 $\pm$ 3.1 kg and having oestrous cycles of mean duration 21.1 $\pm$ 0.68 days were used during the months of October and November. All animals were accustomed to daily ultrasound examinations and had previously been tethered indoors. During the course of serial blood sampling periods, heifers were fed 1–2 kg cottonseed meal with access to hay and water *ad libitum*. Between sampling periods, heifers were kept in a small native pasture paddock with access to hay *ad libitum* and an 8% urea–molasses supplement.

Experimental protocol

The ovaries of all heifers were examined daily, between 07:00 h and 08:00 h, for two complete interovulatory intervals, using transrectal ultrasonography (7.5 MHz transducer, Aloka 210DX) commencing on the day of ovulation. Ovulation was determined by the disappearance of a dominant follicle from the ovary and the subsequent formation of a corpus luteum in the same position on the ovary. The size and position of corpora lutea and all follicles ≥ 5 mm in diameter were recorded as described by Savio *et al.* (1988). Day of emergence was defined as the day on which a follicle ≥ 5 mm was first detected.

Three periods of serial blood collection were defined with reference to the growth of the first dominant follicle of the second interovulatory interval: (i) growth phase, when a follicle ≥ 7 mm was first detected (2.5 $\pm$ 0.2 days after ovulation), (ii) plateau phase, the second consecutive day when there had been no increase in the size of the dominant follicle or when the diameter of the first dominant follicle was ≥ 10 mm (5.6 $\pm$ 0.3 days after ovulation), since the maximum diameter of dominant follicles in *Bos indicus* heifers is, on average, 10 mm (Rhodes *et al.*, 1994); and (iii) regression phase, when the dominant follicle started to decrease in size or the second wave of follicles had emerged (8.2 $\pm$ 0.4 days after ovulation).

Catheterization

Before the first day of blood collection from the caudal vena cava, heifers were individually restrained and sedated with an i.m. injection of 0.1–0.2 ml acepromazine maleate (10 mg ml^{-1}). The caudal vena cava was catheterized via the lateral
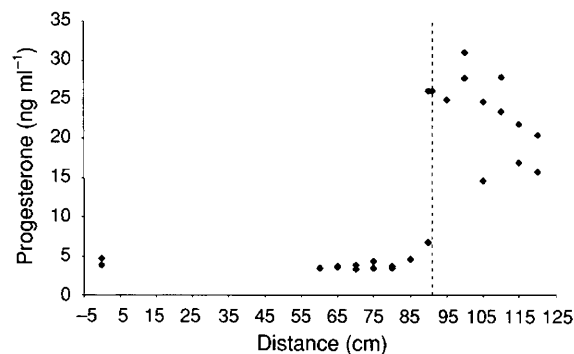

Fig. 1. Concentrations of progesterone in plasma samples obtained from the caudal vena cava at different sites of catheter insertion in one heifer on day 8 after ovulation. Concentrations at 0 cm are jugular samples. Vertical line indicates site of ultrasound placement of catheter tip when positioned medial to the body of the left kidney.

saphenous vein, using a modification of the method of Benoit and Dailey (1991). A tourniquet was placed above the tarsal joint and the area distal to the joint was shaved and disinfected. Anaesthesia of the region was produced after injection of 10 ml of 2% (v/v) lignocaine hydrochloride into the lateral saphenous vein. A 12 gauge 10 cm needle was then introduced into the lateral saphenous vein following incision of the skin. A polyethylene catheter (1 mm i.d., 2 mm o.d. medical grade clear vinyl tubing; Dural Plastics, Auburn, NSW) containing a teflon coated wire guide (length 260 cm; Cook Inc., Bloomington, IN) was introduced through the lumen of the needle into the lateral saphenous vein to a mark at 100 cm. After removing the needle, the tourniquet was released and placement of the catheter tip within the caudal vena cava was confirmed using transrectal ultrasonography (7.5 MHz transducer), as described by Norman and Fields (1993). The aorta was palpated rectally and visualized as a pulsating noncompressible large vessel, and the caudal vena cava was located laterally as an easily compressible thin walled vessel, within which the wire guide could be identified as a thin echogenic line. The tip of the guide was positioned medial to the body of the left kidney, as identified by palpation and ultrasonic visualization. This position had previously been validated as corresponding to the site of maximum concentration of progesterone, when blood samples were obtained sequentially at 5 cm intervals of catheter insertion into the lateral saphenous vein (see Fig. 1). After successful placement of the catheter, the wire guide was removed; the catheter was filled with heparinized saline solution (50 iu ml^{-1} sodium heparin in 0.9% (w/v) saline), and the end was sealed and bandaged to the leg.

Sampling procedures

Heifers were tethered in pairs in adjoining pens, allowed free access to food and water and were able to lie down. Blood samples were withdrawn through remote catheter lines (2 mm i.d., 3 mm o.d.) connected to i.v. catheters, which extended outside the pens to minimize disturbance of the animals. Twice the volume of the sampling catheter was discarded immediately before withdrawal of a 10 ml blood sample and the catheters were flushed with heparin saline solution (12.5 iu sodium

heparin ml^{-1} in 0.9% (w/v) saline) immediately after each sample was collected.

Blood samples were collected at 15 min intervals, commencing between 07:00 h and 08:00 h, for 24 h from each animal on each day of blood collection. In addition, jugular blood samples were collected daily from each animal throughout the interovulatory interval. Samples were immediately placed into heparinized tubes and cooled on ice. Plasma was separated by centrifugation at 3000 g for 15 min within 2 h of collection and stored at − 20°C for subsequent hormone analysis.

Radioimmunoassays

Plasma concentrations of LH were measured by doubleantibody radioimmunoassay, using a modification of the method described by Niswender *et al.* (1969). Purified bovine LH (USDA-bLH-B-6, AFP-11743-B) was provided by D. Bolt (USDA Animal Hormone Program, Beltsville, MD). The antiserum used was NIDDK-anti-oLH-1 (AFP-192279), supplied by NIDDK, Bethesda, MD. Iodination of bLH was performed using a modification of the chloramine-T method described by Bolt and Rollins (1983). All assay reagents were prepared in 0.04 mol phosphate buffered saline l^{-1} (pH 7.2) containing 50 mg egg albumin ml^{-1} (RIAD; McNeilly *et al.*, 1976). Duplicate 100 µl of standards or samples were incubated with 50 µl of normal rabbit serum (diluted 1:250 in RIAD), 50 µl of antiserum (1:400 000 dilution in RIAD) and 50 µl of tracer (12 000 c.p.m.) for 24 h. Donkey anti-rabbit serum (50 µl IDS; Boldon, Tyne and Wear), diluted 1:25 in RIAD, was then added and the tubes incubated for 18 h at 4°C. After addition of 300 µl of RIAD, the tubes were centrifuged at 3000 g for 30 min at 4°C. Supernatants were decanted and the radioactivity of the pellet was determined. Increasing volumes (3.125–100 µl) of plasma in three pools produced displacement of the radioligand that paralleled the standard curve. Quantitative recovery of added LH was evaluated by adding 0, 0.31, 0.63, 1.25, 2.50 and 5.0 ng USDA-bLH-B-6 to three plasma pools. There was a linear relationship between concentrations of LH recovered and LH added. The interassay coefficients of variation (CVs) for two bovine plasma pools of 0.373 and 1.886 ng ml^{-1} were 10.2 and 6.7%, respectively. The intraassay CVs for the same plasma pools were 9.8 and 5.2%, respectively. Assay sensitivity, defined as concentration at 90% of maximum binding, was 0.2 ng ml^{-1}.

The concentrations of FSH were determined by doubleantibody radioimmunoassay (Wolfe *et al.*, 1989) using rabbit anti-serum against ovine FSH (JAD-RaOFSH 17-6,7,9) and highly purified ovine FSH (LER-1976-A2) as the labelled hormone and standard. The intra- and interassay CVs were 3.6% and 17%, respectively. The assay sensitivity was 0.23 ng ml^{-1}. The concentrations of oestradiol in plasma were determined by radioimmunoassay following ether extraction (Kojima *et al.*, 1992). Intra- and interassay CVs were 2.7% and 15.7%, respectively. The assay sensitivity was 0.08 pg ml^{-1}.

The concentrations of progesterone in plasma were determined in unextracted plasma samples using a modification of the Danazol method (McGinley and Casey, 1979) described by Jolly (1992). Samples were diluted as necessary using charcoalextracted steer plasma. The interassay CVs for two bovine

serum pools of 0.98 and 3.93 ng ml^{-1} were 14.9 and 13.2%, respectively. The intra-assay CVs for the same serum pools were 13.5 and 11.9%, respectively. The assay sensitivity, as defined by concentration at 90% of maximum binding, was 0.04 ng ml^{-1}.

Statistical analyses

The mean concentration, basal concentration, frequency and amplitude of pulses for each of the four hormones during the separate periods of serial blood collection were determined using a modified version of the algorithm developed by Merriam and Wachter (1982), adapted for IBM-compatible personal computers (PULSAR: R. Lazarus, Department of Community Medicine, Westmead Hospital, NSW). The 'G' parameters used were those derived empirically for LH profiles by Merriam and Wachter (1982), namely 3.8, 2.6, 1.9, 1.5 and 1.2 for G(1) to G(5), respectively. Precision profiles were derived separately for each hormone.

Variation in the characteristics of hormone release between growth and plateau phases, and between plateau and regression phases were evaluated using paired *t* tests. The percentage of pulses of oestradiol and progesterone that followed pulses of LH within 60 min were compared for the three sampling periods using logistic regression analysis. A chi-squared analysis was used to determine whether pulses of oestradiol or progesterone were more likely to occur in the 60 min following a pulse of LH compared with sampling times not following a pulse of LH. Relationships between peaks of LH and concentrations of oestradiol and progesterone were determined using analysis of variance, with the main effects of animal, period, sampling time relative to the LH peak and all first order interactions included in the model; terms were dropped if non-significant ($P > 0.05$).

Results

The three sampling periods were, on average (± SEM), 2.5 ± 0.2, 5.6 ± 0.3 and 8.2 ± 0.4 days after ovulation, for the growth, plateau and regression phases, respectively. The second dominant ovarian follicle emerged 7.7 ± 0.5 days after ovulation. In one heifer, the catheter became blocked following the first period of serial sampling; the results for this animal were therefore excluded from the analyses. The mean diameter of the largest follicle increased from 6.8 ± 0.5 to 9.8 ± 0.2 mm ($P < 0.01$) and the corpus luteum from 11.2 ± 1.0 to 16.0 ± 1.3 mm ($P < 0.001$) between growth and plateau phases, respectively; but there was no change in diameter between the plateau and regression phases for the largest follicle ($P = 0.26$) or corpus luteum ($P = 0.12$). Representative profiles of daily changes in the diameter of the corpus luteum and largest follicles from one heifer are shown (Fig. 2).

The frequency of pulses of LH, oestradiol and progesterone tended to change in a coordinated fashion; they were lowest during the plateau phase of the dominant follicle (Table 1). The amplitude of pulses of oestradiol decreased between the growth and plateau phases ($P = 0.03$), but did not change between the plateau and regression phases ($P = 0.41$). The frequency of pulses of LH decreased between the growth and
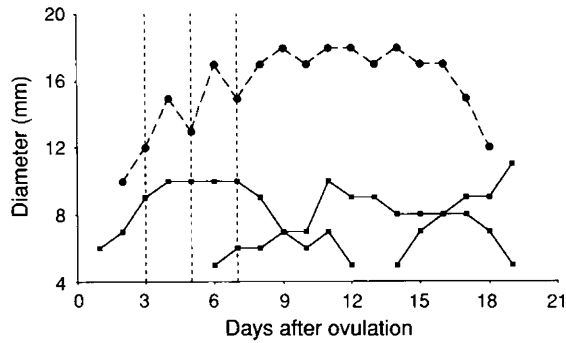
Fig. 2. Diameter of dominant follicles (■) and corpus luteum (●) during a complete interovulatory interval for one heifer. Days of collection of serial samples are indicated by vertical lines.

plateau phases ($P = 0.06$, Table 1). However, there was little indication of pulsatile release of FSH, with eight of the 15 periods of serial blood collection having no pulses of FSH (data not shown).

Changes in mean concentrations of LH reflected changes in frequency of pulses of LH; the mean concentration of LH decreased between the growth and plateau phases ($P = 0.04$, Table 2). Similarly, mean concentrations of oestradiol in ovarian venous samples decreased between the growth and plateau phases ($P = 0.03$, Table 2). Mean concentrations of progesterone in the caudal vena cava increased between the growth and plateau phases, whereas mean concentrations of FSH were 0.2 ng ml^{-1} greater between the plateau and regression phases ($P < 0.05$, Table 2).

Changes in concentrations of oestradiol and progesterone in the jugular vein were lower but mirrored changes in concentrations of samples collected from the caudal vena cava (Table 2). Concentrations of progesterone in the caudal vena cava were > 2.5 times higher than jugular concentrations in all experimental heifers and were, on average, 6.8 times greater than those in the jugular vein. However, it was only during the growth phase that concentrations of oestradiol were greater, by a factor of 2.8, in the caudal vena cava than in the jugular vein.

There was no effect of period of serial blood collection on the percentage of LH pulses that were followed by either a pulse of oestradiol ($P = 0.25$) or progesterone ($P = 0.32$) within 60 min of the peak release of LH. Overall, 56% of LH pulses were followed by pulses of oestradiol, which was greater than the percentage expected by chance, given the mean observed frequency of oestradiol pulses of 0.37 h^{-1} ($\chi^2_{(3)} = 7.63$, $P = 0.05$). Examination of concentrations of oestradiol in all plasma samples obtained following pulses of LH revealed an increase in mean concentration of oestradiol of 2.81 ± 0.82 pg ml^{-1} within 15 min of the peak release of LH ($P < 0.001$, Fig. 3).

The percentage of LH pulses followed by pulses of progesterone was 35%, which was no different to the percentage expected by chance, given the observed frequency of pulses of progesterone of 0.32 h^{-1} ($\chi^2_{(3)} = 0.02$, $P = 0.99$). In addition, mean concentrations of progesterone did not change following a pulse of LH ($P = 0.19$, Fig. 4). The relationships between LH pulses and concentrations of oestradiol or progesterone did not differ among days of serial blood collection ($P = 0.48$ and 0.61, respectively).

Discussion

The frequency of pulses and mean concentrations of LH were greatest during the growth phase of the first dominant follicle of the oestrous cycle, when secretion of oestradiol was greatest and peripheral concentrations of progesterone were < 1.0 ng ml^{-1}. Concentrations of LH decreased during the plateau phase in association with a decrease in the frequency of pulses of LH, probably owing to increased peripheral concentrations of progesterone. Price and Webb (1988) found that treatment of ovariectomized heifers with progesterone alone decreased the frequency but did not change the amplitude of pulses of LH. In contrast, treatment with oestradiol alone suppressed release of LH by decreasing the amplitude but not the frequency of LH pulses. Similarly, Stumpf et al. (1993) reported that the frequency of pulses of LH was significantly lower in ovariectomized cows treated with progesterone alone, compared with those treated with oestradiol alone.

Table 1. Characteristics of pulsatile release of hormones in five heifers during three periods of serial blood collection from the caudal vena cava (mean ± SEM), with significance of difference between periods (P)

| Parameter | Period of collection[a] | | | P | |
	1	2	3	Period 1 and 2	Period 2 and 3
Pulse frequency h^{-1}					
LH	0.41 ± 0.06	0.23 ± 0.03	0.28 ± 0.08	0.06	0.52
FSH	0.03 ± 0.03	0.06 ± 0.02	0.03 ± 0.02	0.34	0.43
Oestradiol	0.50 ± 0.11	0.23 ± 0.05	0.38 ± 0.11	0.16	0.30
Progesterone	0.39 ± 0.09	0.19 ± 0.06	0.38 ± 0.07	0.14	0.22
Pulse amplitude					
LH (ng ml^{-1})	0.29 ± 0.02	0.38 ± 0.03	0.48 ± 0.12	0.12	0.36
Oestradiol (pg ml^{-1})	11.97 ± 2.44	3.41 ± 0.78	6.14 ± 2.77	0.03	0.41
Progesterone (ng ml^{-1})	7.26 ± 3.14	11.73 ± 2.18	34.25 ± 11.43	0.32	0.17

[a]Periods 1, 2 and 3 are the growth, plateau and regression phases of the first dominant follicle, respectively.

Table 2. Concentrations of hormones in the caudal vena cava (CVC) and jugular vein of five heifers during three periods of serial blood collection (mean ± SEM), with significance of difference between periods (P)

| Parameter | Period of collection[a] | | | P | |
	1	2	3	Period 1 and 2	Period 2 and 3
Mean concentration (CVC)					
LH (ng ml^{-1})	0.43 ± 0.05	0.36 ± 0.04	0.34 ± 0.03	0.04	0.25
FSH (ng ml^{-1})	1.77 ± 0.07	1.86 ± 0.03	1.66 ± 0.08	0.29	0.04
Oestradiol (pg ml^{-1})	11.49 ± 2.67	3.07 ± 0.21	4.35 ± 1.71	0.03	0.46
Progesterone (ng ml^{-1})	6.74 ± 2.98	13.26 ± 3.81	26.97 ± 5.70	0.05	0.10
Jugular vein concentration					
Oestradiol (pg ml^{-1})	5.28 ± 0.85	2.77 ± 0.27	2.68 ± 0.57	0.02	0.83
Progesterone (ng ml^{-1})	0.89 ± 0.22	2.60 ± 0.23	3.87 ± 0.66	0.01	0.16

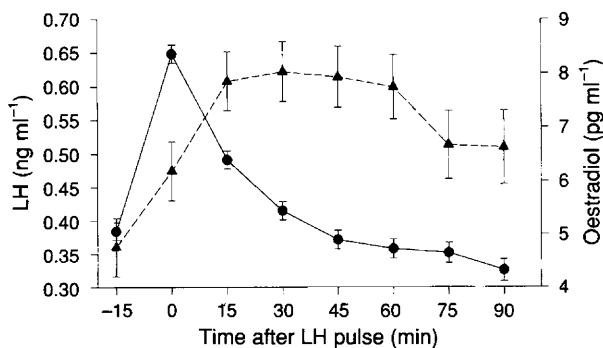[a]Periods 1, 2 and 3 are the growth, plateau and regression phases of the first dominant follicle, respectively.

Fig. 3. Concentrations of LH (ng ml^{-1} ●) and oestradiol (pg ml^{-1} ▲) in the caudal vena cava of five heifers around the time of detection of all LH pulses (least squares means ± SEM).
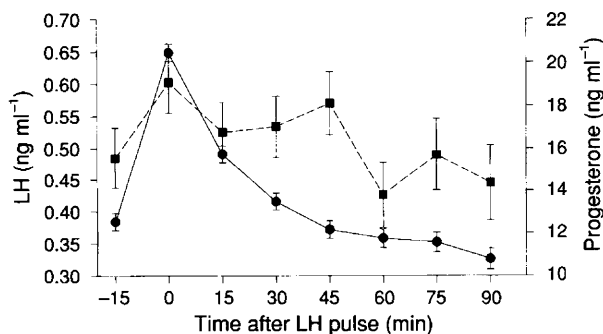

Fig. 4. Concentrations of LH (ng ml^{-1} ●) and progesterone (ng ml^{-1} ■) in the caudal vena cava of five heifers around the time of detection of all LH pulses (least squares means ± SEM).

The amplitude of pulses and mean concentration of oestradiol were greatest during the growth phase of the first dominant follicle of the oestrous cycle and were significantly reduced when the dominant follicle attained its largest diameter. This increased secretion of oestradiol was associated with a high frequency release of LH pulses, supporting the conclusion of Walters *et al.* (1984) that oestradiol production is determined by the frequency of LH pulses. Comparing the plateau and regression phases, there were no differences in the amplitude or frequency of oestradiol pulses, or in the mean concentration of oestradiol in the caudal vena cava. These findings are in agreement with *in vitro* results from bovine ovarian follicles collected 5, 8 or 12 days after behavioural oestrus (Badinga *et al.*, 1992). Concentrations of oestradiol in the follicular fluid of dominant follicles were greater on day 5 compared with day 8 or 12, which corresponded with a small increase in plasma oestradiol on day 4. The decrease in production of oestradiol by dominant follicles when they attained maximum diameter may also be due to a decrease in the numbers of receptors for LH between the growth and plateau phases, as reported by Rollosson *et al.* (1994). As dominant ovarian follicles increase in size, the ability to bind human chorionic gonadotrophin increases (Ireland and Roche, 1983) and as a single ovarian follicle becomes dominant, increased secretion of oestradiol by this follicle occurs and unequal concentrations of oestradiol in the two utero–ovarian veins is the net result (Ireland *et al.*, 1984). It was not possible to determine from the current study the relative contribution of dominant and subordinate follicles to concentrations of oestradiol in the caudal vena cava; however, it is possible that during the regression phase the emerging group of follicles made some contribution. Sunderland *et al.* (1994) reported that follicular fluid concentrations of oestradiol were greater in small, newly emerged follicles than in dominant follicles of beef heifers on day 12 of the oestrous cycle.

In agreement with the current findings of a decreased release of oestradiol by the dominant follicle when it had attained its largest diameter, Kaneko *et al.* (1991) reported that peripheral concentrations of oestradiol in the plasma of cows increased with growth of the dominant ovarian follicle, but decreased 6 days after the preovulatory surge of LH, although the dominant follicle continued to increase in size. Similarly, Guilbault *et al.* (1993b) found that peak concentrations of oestradiol occurred about 4 days before the cessation of growth of the first dominant follicle of the oestrous cycle. During the period of follicular dominance, growth of secondary follicles is halted, indicating that dominance of the largest follicle, as defined by its ability to suppress the growth of other ovarian follicles, is not solely related to the secretion of oestradiol.

The association between pulses of LH and oestradiol in the current study was not as great as that reported by others. On average, 56% of LH pulses were followed by a pulse of oestradiol within 60 min of peak release of LH. The temporal relationship between pulses of LH and oestradiol was first detected in ewes during the breeding season by Baird *et al.* (1976), who found that LH pulses were followed, within 5 min, by an increased release of oestradiol, which reached a peak in 30 min. Walters *et al.* (1984) reported that > 90% of gonadotrophin pulses were associated with pulses of oestradiol in cows, with a mean time-lag of approximately 24 min. It may be that the smaller dominant follicle of *Bos indicus* heifers (Rhodes *et al.*, 1994), compared with *Bos taurus* cows, secretes less oestradiol in response to stimulation by LH. The nearly sevenfold difference in the concentrations of progesterone in the caudal vena cava compared with jugular concentrations indicates that incorrect catheter placement could not have been the cause of the lower detection rate of gonadal hormone pulses in the current compared with previous studies. Nevertheless, there was a significant increase in the mean concentration of oestradiol following each LH pulse, which reached a maximum between 15 and 30 min after the peak release of LH. In addition, this relationship did not vary with the period of serial blood collection, in agreement with the findings of Walters *et al.* (1984), who report that pulses of oestradiol were associated with 90% and 96% of pulses of LH during the early and mid-luteal phases of the oestrous cycle, respectively, in spite of a significant decrease in the amplitude of pulses of oestradiol. The temporal relationship between release of LH and oestradiol, therefore, does not appear to be influenced by the stage of oestrous cycle.

The frequency of progesterone pulses did not vary with the period of serial blood collection and there was no apparent relationship between the mean concentrations of progesterone and peak release of LH, in agreement with the findings of Baird *et al.* (1976) in ewes. Secretion of progesterone by corpora lutea was reported to be episodic in a number of other studies (Walters *et al.*, 1984; Benoit and Dailey, 1991; McNeilly *et al.*, 1992). Moreover in heifers, Peters *et al.* (1993) reported that stimulation by LH pulses is required for the normal function of corpora lutea from day 2 to day 12, but not from day 12 to day 17 of the oestrous cycle. In contrast, Scottish Blackface ewes treated with an antagonist of GnRH on day 4 or day 11 of the oestrous cycle have continued episodic secretion of progesterone in the absence of pulsatile secretion of LH, and do not differ in the timing of luteolysis compared with control animals (McNeilly *et al.*, 1992). Therefore, the cause of pulsatile release of progesterone remains unknown, but may lie within the corpus luteum itself. More than 80% of the progesterone secreted by the corpus luteum is produced by large luteal cells, which seem to be independent of LH stimulation (Niswender *et al.*, 1994).

There was little evidence of FSH pulses in the current experiment compared with the study of Walters *et al.* (1984), who reported a high degree of association between pulses of FSH and progesterone. However, pulses of FSH and LH were also closely associated and it has been suggested that there might have been significant crossreactivity between FSH and LH in the assay system used in the previous study (Butler *et al.*, 1983).

The concentrations of FSH during the plateau phase (mean = 5.6 days after ovulation) were greater than during the regression phase (mean = 8.2 days after ovulation). This increase in FSH occurred approximately 2 days before the day of detection of the second dominant follicle, in agreement with the findings of previous authors. Adams *et al.* (1992) reported high concentrations of circulating FSH 1–2 days before the emergence of a new wave of follicles and concluded that a surge in release of FSH necessarily precedes the emergence of a new wave of ovarian follicular growth in cattle. Similarly, Sunderland *et al.* (1994) reported transient increases in serum concentrations of FSH before the selection of a dominant follicle. In addition, Turzillo and Fortune (1990) showed that treatment of heifers with bovine follicular fluid from 12 to 60 h after the onset of behavioural oestrus abolishes the postovulatory rise in FSH and delays the appearance of the first dominant ovarian follicle. It is hypothesized that, in cattle, increases in circulating concentrations of FSH initiate the development of new phases of dominant follicle growth (Sunderland *et al.*, 1994).

In conclusion, mean concentrations and the amplitude of pulses of oestradiol were found to be high only during the growth phase of the largest ovarian follicle and were significantly reduced when this follicle attained maximum diameter, during the plateau phase. In addition, increased secretion of oestradiol was associated with increased frequency of pulsatile release of LH by the pituitary. Compared with the regression phase, mean concentrations of FSH were increased during the plateau phase of dominant follicle development, which occurred approximately 2 days before the day of emergence of the second dominant follicle. This increase in FSH, in conjunction with the decrease in the release of oestradiol, may be an indication of the loss of functional dominance by the largest ovarian follicle of the first wave of follicular growth, following the decreased release of LH during the oestrous cycle of bovine females.

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