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OESTRADIOL-17β, PROGESTERONE AND 17α-HYDROXYPROGESTERONE CONCENTRATIONS IN JUGULAR VENOUS PLASMA IN COWS PRIOR TO AND DURING OESTRUS

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SUMMARY

Blood samples were collected from the jugular vein of four heifers and a lactating cow at 2-h intervals during the pre-ovulatory phase in order to compare the plasma concentrations of 17-hydroxyprogesterone, progesterone and oestradiol-17β. During this phase, the concentrations of the two C-21 steroids were similar and at a low level: there was no evidence of a surge in the plasma concentrations of either steroid and the concentrations do not seem to be related to that of oestradiol-17β prior to ovulation. The basal levels of progestogens in the follicular phase are very similar to those of castrated bulls. In a heifer in which superovulation was induced, Pregnant Mare Serum Gonadotropin enhanced the secretion of progestogens from the regressing corpus luteum but there was no pre-ovulatory peak.

INTRODUCTION

The pre-ovulatory secretion of progesterone has been well documented in several species including the human [1–5], monkey [6], rat [7–10], hamster [11] and rabbit [12, 13]. This secretion occurs without evidence of corpus luteum formation in the ovary. In the ewe, the pre-ovulatory follicle does not secrete either progesterone or 17-hydroxyprogesterone [14]. In the cow, it has been shown from daily [15, 16] or even more frequent sampling [17, 18] that peripheral plasma concentrations of progesterone remain low during pro-oestrus and oestrus. However, other workers have reported either an elevated concentration [19] or even an increase in this steroid during oestrus [20]. In the latter communication, the specificity of the protein-binding procedure used was not clearly established. These conflicting results suggest either a transitory pre-ovulatory rise in progesterone which could escape detection, or interference by another C-21 steroid such as 17-hydroxyprogesterone. The latter possibility cannot be excluded, as it has been found that homogenates of bovine ovarian tissue [21] or large follicles [22] convert progesterone to 17-hydroxyprogesterone *in vitro*. If 17-hydroxyprogesterone secretion does occur during this short period, it is of interest to try to relate its concentration to the peripheral plasma levels of oestradiol-17β and progesterone, as Strott *et al.*[2] have suggested that plasma 17-hydroxyprogesterone could serve as an index of follicular maturation and corpus luteum function.

MATERIALS AND METHODS

Animals

Five cyclic bovine females (4 heifers, 22, 23, 24 and 26, and 1 lactating cow 20), of French Black and White Breed were used. All showed regular normal oestrus cycles. The time of onset of oestrus, designated as Day 0, was determined by checking oestrus behaviour twice daily (6 a.m. and 6 p.m.) with a vasectomized bull. To increase follicular secretion, one of the heifers (26) was treated to induce superovulation with a single intra-muscular injection of 1600 I.U. of Pregnant Mare's Serum Gonadotrophin (PMSG) on Day 16 (late luteal phase) followed by an intra-muscular injection of 1500 I.U. of Human Chorionic Gonadotrophin (HCG) on Day 0 [23]. This treatment induced 8 ovulations which were controlled by endoscopy [24].

Plasma samples

Every 2 h, jugular blood samples (20 ml) were collected into heparinized tubes. Within 15 min the plasma was separated by centrifugation (4°C) and stored at −15°C until thawed for assay. Sampling began on the 16th day post-oestrus and continued until 24 h (22 and 26), 28 h (24) or 50 h (20 and 23) after the onset of the new oestrus. As ovulation usually occurs at the end or soon after oestrus in the bovine, it is likely that sampling continued until after ovulation in all animals [25].

Assays

Oestradiol-17β: this steroid was measured by radioimmunoassay using an antiserum prepared by immunization of rabbits with oestradiol-17β-6 (O-Carboxy) methyloxime conjugated to bovine serum albumin; the antiserum used was that referred to as L 4386 [26].

Plasma samples (5 ml) were extracted once with 25 ml cold dichloromethane (Merck z. Analyse), after addition of a tracer amount of [^{3}H]-oestradiol ([2,4,6,7-^{3}H]-oestradiol 85-105 Ci/mmol; Amersham). No purification was necessary as the antiserum was shown previously to be specific for oestradiol-17β [26] and the dried extracts were dissolved in 0·3 ml of phosphate buffer (0·1 M, pH 7·4). For the assay, 0·1 ml was added to each of 2 plastic tubes and 0·05 ml was counted to estimate recovery. Then 0·1 ml of [^{3}H]-oestradiol in buffer (approximately 8000 d.p.m.) and 0·1 ml antiserum (diluted 1/35 000) were added and the tubes incubated at 4°C for 3 h. The separation of bound and free oestradiol was performed by double antibody immunoprecipitation. The precipitate was washed twice with 1·1 ml buffer, the supernatant decanted into scintillation vials and counted after addition 8·5 ml of toluene triton mixture [27]. The plasma oestradiol values were determined using a Hewlett–Packard programmed calculating machine.

The recovery of the method was 80·4 $\pm$ 1·2% (n = 100). The sensitivity was such that 2·5 pg was significantly different ($P < 0{\cdot}05$) from zero on the standard curve. When taking account of procedural losses and volume of plasma extracted, the sensitivity of the assay was 2 pg/ml plasma. The water blank was usually below the limit of detection of the assay. Some batches of dichloromethane produced blank values of up to 4 pg which were subtracted accordingly. When known amounts of oestradiol-17β were added to 5 ml plasma from a castrated bull, the following results were obtained (pg added, pg recovered $\pm$S.D., n = 5) 200, 207·7 $\pm$ 10·6; 100, 96·1 $\pm$ 6·6; 50, 47·8 $\pm$ 5·8; 25, 24·0 $\pm$ 3·2; 12·5, 13·5 $\pm$ 2·3; 6·25, 4·4 $\pm$ 2·3.

The inter-assay precision, obtained by repeated assay of a pool of bovine plasma was $\pm$5%.

Progesterone and 17-hydroxyprogesterone

Antisera against these 2 steroids were raised in rabbits, immunized against either progesterone-11α-hemisuccinate BSA (Institut Pasteur) or 17-hydroxyprogesterone-3 (O-Carboxy) mechyloxime-BSA (I.F. Sommerville—London). After adding a tracer amount of [^{3}H]-progesterone and [^{3}H]-17-hydroxyprogesterone to the samples ([1α,2α-^{3}H]-progesterone, 53 Ci/mmol; Amersham and [1,2-^{3}H]-17-hydroxyprogesterone, 49·2 Ci/mmol; New England Nuclear) the plasma was extracted with 4 ml of diethyl ether (Purex—S.D.S. Peypin—13). The samples were shaken thoroughly for 30 s on a vortex mixer; frozen in a bath of ethanol and dry ice (−50 C) and then decanted and evaporated to dryness in a stream of nitrogen at 40°C. The two steroids were separated by Sephadex LH 20 micro-column chromatography, the eluates evaporated and 200 μl of antiserum added (the immunological reaction being performed in duplicate). Tubes were then incubated for 30 min at 37°C. Separation of bound and free steroids was achieved by adding 3 ml of toluene, shaking 15 s and pouring the toluene phase including unbound molecules into a counting vial. The results were calculated by plotting the cpm determined against ng progesterone or 17-hydroxyprogesterone.

The specificity of this method is confirmed by no cross-reactions (<1%) with other steroids which might be involved [28]. Mean blank value is 0·06 $\pm$ 0·07 ng/ml for progesterone and is not significantly different from zero for 17-hydroxyprogesterone. Thus from these results and taking into account the volume of aliquot of the sample, the limit of sensitivity may be estimated as 0·11 and 0·05 ng/ml respectively. Accuracy, precision and efficiency of the separation of the two steroids were satisfactory as already reported [16]. Intra-assay precision was estimated by the coefficient of variation of results obtained by repeated determinations. For low levels of 17-hydroxyprogesterone and progesterone, the coefficients of variation are 6% and 9% respectively. Accuracy was determined by addition of known amounts of 17-hydroxyprogesterone or progesterone to plasma. The regression lines were plotted and the slopes were not significantly different from 1.

RESULTS

For the non-treated animals, C-21 steroids and oestradiol-17β levels prior to oestrus and during oestrus are depicted in Fig. 1 (22), Fig 2 (23), Fig. 3 (24) and in Fig. 4 (20). In all of them results are similar. In heifer 23 the progesterone level is already below 2 ng/ml at the first sampling whereas in the other three this level is reached 24 or 48 h after sampling has started. The beginning of the follicular phase can be defined as when the progesterone level is below 2 ng/ml, since corpus luteum regression is then almost complete. In the four animals, progesterone decreases regularly to basal levels of about 0·3 ng/ml over a period of 2 days (more than 50 h) until the LH peak occurs*. It is of interest to note that progesterone levels remain low after the LH peak for a further period of 48 h. Estradiol-17β levels fluctuate rapidly after progesterone concentrations have decreased. One can see a series of peaks which increase in magnitude as oestrus and ovulation approach. This kind of increase seems to proceed by impulses and emphasizes the necessity of frequent samplings to obtain a good oestradiol-17β secretion picture.

As it has been shown previously with daily sampling [16], the pattern of the two C-21 steroids are very similar. At the same time as progesterone, the concentration of 17-hydroxyprogesterone declines

* It is always difficult to determine the exact onset of oestrus. Therefore we chose the LH peak as a point of reference. This was measured by radioimmunoassay [29].

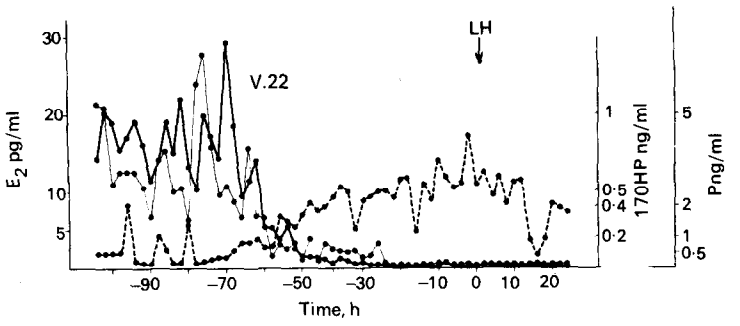

Fig. 1. Plasma 17-hydroxyprogesterone, progesterone and estradiol-17β prior to and during oestrus in heifer 22. (●——●): 17-hydroxyprogesterone (17 OHP); O——O: progesterone (P); O- - -O: oestradiol-17β (E_2); hours according to LH peak.

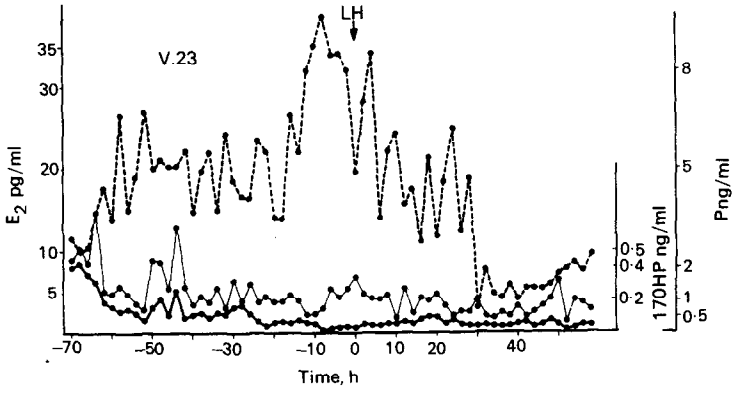

Fig. 2. Plasma 17-hydroxyprogesterone, progesterone and oestradiol-17β prior to and during oestrus in heifer 23. For further explanation see legend to Fig. 1.

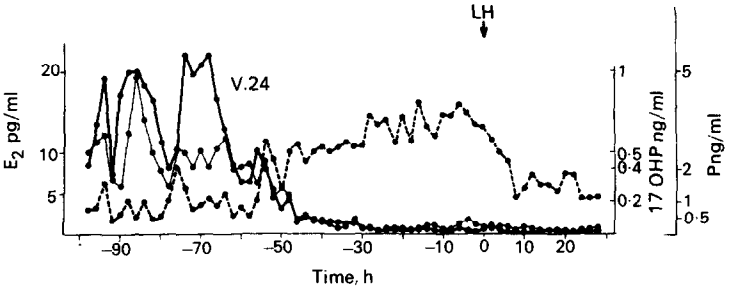

Fig. 3. Plasma 17-hydroxyprogesterone, progesterone and oestradiol-17β prior to and during oestrus in heifer 24. For further explanation see legend to Fig. 1.

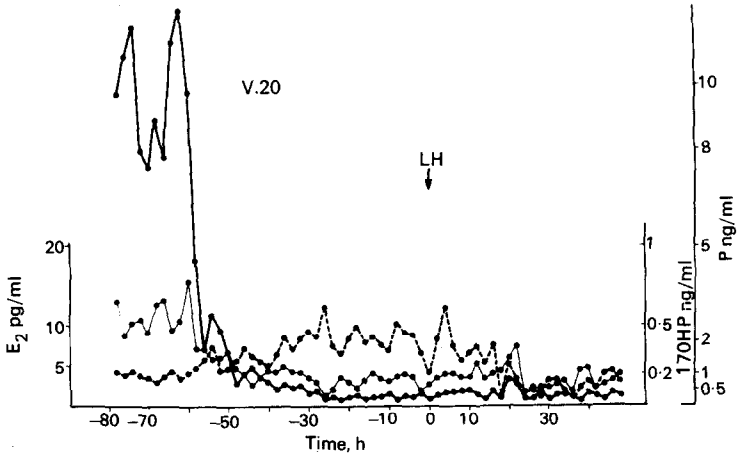

Fig. 4. Plasma 17-hydroxyprogesterone, progesterone and oestradiol-17β prior to and during oestrus in a lactating cow. For further explanation see legend to Fig. 1.

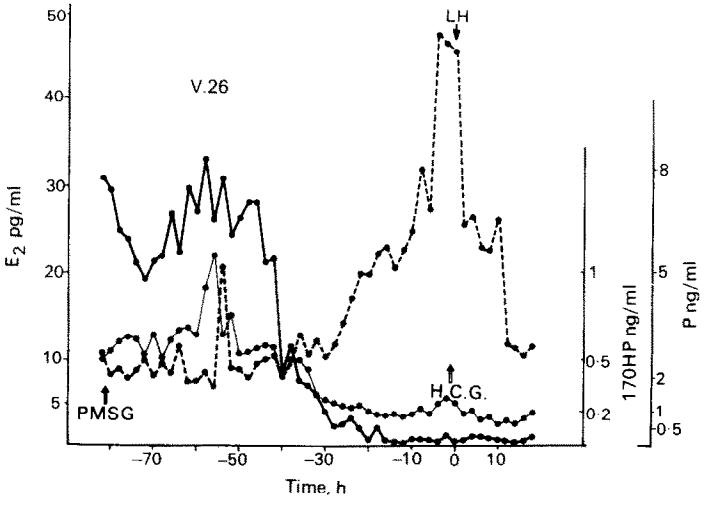

Fig. 5. Plasma 17-hydroxyprogesterone, progesterone and oestradiol-17β prior to and during oestrus in a superovulated heifer. For further explanations see legend to Fig. 1.

regularly to basal levels of 0·1–0·2 ng/ml where it remains for the rest of the follicular phase.

Progesterone concentrations had begun to fall when the first samples were taken from the superovulated heifer (26, Fig. 5), but PMSG seems to enhance the secretion of progestogens from the regressing corpus luteum after a 12 h delay. Within 24 h, an induced progesterone peak is reached (8·29 ng/ml), accompanied by an increase in 17-hydroxyprogesterone to a maximum of 1·11 ng/ml, observed 26 h after PMSG treatment. Apart from a very short peak of oestradiol-17β of 21 pg/ml 2 h after that of 17-hydroxyprogesterone, no variation of the oestrogen concentration can be seen during this prolonged luteal phase. Following the decrease in progestogens, oestradiol-17β concentration increases much more rapidly than in the normal animals, to reach concentrations of 48 pg/ml. These probably reflect the incidence of stimulated follicular growth. But during this estradiol increase, 17-hydroxyprogesterone, like progesterone, remains at a low level apart from a small increase (0·29 ng/ml of 17-hydroxyprogesterone) occurring at −2 h, when oestradiol-17β is the highest.

From more than 250 plasma samples we found no evidence of a 17-hydroxyprogesterone or progesterone discharge (during the pro-oestrus and oestrous phases) in any animal.

Throughout the follicular phase, 17-hydroxyprogesterone and progesterone levels are low but different from zero. In order to estimate the ovarian contribution to these levels we have compared the results with those obtained in the peripheral plasma of castrated bulls (Table 1). Progesterone and 17-hydroxyprogesterone levels during the follicular phase appear similar to those of castrated bulls.

DISCUSSION

Daily sampling during the oestrus cycle has shown that 17-hydroxyprogesterone levels are correlated with progesterone levels ($r = 0·9$, $P < 0·01$) [16]. With more frequent sampling during the preovulatory phase, these steroids are still closely associated. We found no relationship during the follicular phase between the C-21 steroids and oestradiol-17β secreted by the growing follicle(s). In the superovulated heifer, the results obtained during the follicular phase follow the same trend: 17-hydroxyprogesterone seems to be associated only with progesterone i.e. corpus luteum activity. The effect of PMSG on the level of progesterone has already been demonstrated in the cow [30]. Our results show that this stimulation is also reflected in the level of 17-hydroxyprogesterone. The induced peak of progestogens can be explained by the LH-like activity of PMSG. It can be assumed that 1, 600 I.U. of PMSG are equivalent to 320 μg LH, and this was sufficient to obtain a corpus luteum response. The bovine corpus luteum has been shown to be extremely sensitive to LH [31]. Such an effect of PMSG on corpus luteum secretion has also been shown in the rat [32].

Table 1. Progestogens basal levels in castrated bulls and during follicular phase in cows

	Progesterone (ng/ml)		17-hydroxyprogesterone (ng/ml)	
	Castrated bull*	Cows†	Castrated bull*	Cows†
	0·25	0·22	0·10	0·08
	0·30	0·28	0·15	0·17
	0·29	0·25	0·16	0·09
	0·33	0·31	0·15	0·16
	0·39	0·22	0·09	0·17
	0·22		0·11	
Average	0·29	0·26	0·13	0·13

* Six castrated bulls of 30 months old (mean of 3 successive jugular venous plasma samples).

† Respectively 22, 23, 24, 20 and 26.

During pro-oestrus and oestrus, the two progestogens remained at basal levels and showed no pre-ovulatory peak. In the superovulated heifer it was only when oestradiol-17β reached its maximum value that we found a small but significant rise both for progesterone and 17-hydroxyprogesterone. However, this was probably an indication of the secretion of intermediates in the stimulation of estradiol-17β biosynthesis rather than a physiological discharge.

The progesterone measured during the preovulatory phase is probably of adrenal origin since similar levels of progestogens were found in castrated bulls. In addition, the corpus luteum has clearly regressed by this stage in the cycle [33] there is a very little ovarian interstitial tissue in the cow [34] and adrenals are known to produce progesterone and 17-hydroxyprogesterone [35].

Since there is no 17-hydroxyprogesterone surge prior to ovulation, steroid secretion in the cow is different from that in women and in the Rhesus monkey [1–6]. If a pre-ovulatory progestogen release does occur in the bovine then it is either too short to be evidenced by sampling every two hours or the secreted progestogen is a steroid other than progesterone or 17α-hydroxyprogesterone.

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