Effect of progesterone on basal LH and episodic LH and FSH secretion in heifers

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Summary. Heifers between Days 6 and 10 of the cycle were allocated at random to groups of 8 and treated with (i) a 4% progesterone-releasing intravaginal device (PRID) + oestrogen capsule for 12 days; (ii) 4% PRID for 12 days; (iii) 20% PRID for 12 days; (iv) 4% PRID for 14 days; or (v) 20% PRID for 14 days. Blood was obtained daily during treatment and at 2- or 4-h intervals for 72 h after removal of PRIDs. Some animals were sampled every 20 min for 4·67 h on the 3rd day after PRID insertion, and 1 day before and 36 h after removal of the PRID. During progesterone treatment there was: (i) no correlation between concentrations of progesterone and LH within days; (ii) a significant negative correlation between progesterone and days ($P < 0.01$) and also between progesterone and LH over days ($P < 0.01$); (iii) the overall correlation co-efficient between LH and days was positive ($P < 0.05$). The amplitude of LH or FSH episodes was not affected as progesterone concentrations declined during PRID treatment, but the number of LH (but not FSH) episodes was increased ($P < 0.01$). After PRID removal, the amplitude of both LH and FSH episodes increased ($P < 0.01$). We suggest that progesterone is part of a negative feedback complex on LH secretion in cattle and that this effect is apparently mediated through frequency of episodic LH release.

Introduction

The interactions between ovarian steroid and pituitary gonadotrophic hormones in regulating the oestrous cycle of the cow are not clearly understood. This information would help the development of new and better techniques for artificial control of reproduction in animals. In the ewe, it has been proposed that progesterone, rather than oestradiol, is the major hormone that regulates LH secretion by negative feedback (Hauger, Karsch & Foster, 1977). If this is so, it would have important implications when using progesterone to control oestrus and ovulation in cattle. Doses of exogenous progesterone could be effective in blocking oestrus and the gonadotrophic preovulatory surge, but be insufficient to maintain the low basal LH concentrations found during the luteal phase of the cycle (Hansel, Concannon & Lukaszewska, 1973). Tentative evidence in cattle to substantiate the negative feedback hypothesis for progesterone on LH has been reported by Convey, Beck, Neitzel, Bostwick & Hafs (1977) for ovariectomized heifers and by Roche & Ireland (1981) for heifers given progesterone during the follicular phase of the cycle. Rahe, Owens, Fleeger, Newton & Harms (1980) have reported that marked changes in episodic LH secretion occur during the oestrous cycle of the cow. The aim of this paper was (1) to substantiate further the negative feedback effect of progesterone on LH concentrations in synchronized heifers, and (2) to determine whether the progesterone effect was mediated through effects on pulsatile secretion of LH.

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Materials and Methods

Animals. Forty (40) Holstein heifers between Days 6 and 10 of an oestrous cycle were used. The animals were allocated at random to 5 groups of 8 animals. Animals in Treatment 1 received an effective synchronizing treatment consisting of a progesterone-releasing intravaginal device (PRID: Abbott Laboratories, North Chicago, U.S.A.) containing 4% progesterone with a gelatin capsule containing 10 mg oestradiol benzoate adhered to the coil (Roche, 1978). Animals in Treatments 2 to 5 were given an injection of 30 mg prostaglandin (PG) F-2α at the time of PRID insertion to eliminate endogenous production of progesterone from the corpus luteum and received a PRID containing 4 or 20% progesterone for 12 or 14 days. The five treatments were therefore: 1, 4% PRID + 10 mg oestradiol capsule for 12 days; 2, 4% PRID for 12 days; 3, 20% PRID for 12 days; 4, 4% PRID for 14 days; 5, 20% PRID for 14 days.

Bleeding schedule. Blood was obtained 3 days before and on the day of insertion of the coils. Blood was taken daily while the PRIDs were in the vagina and every 4 h for 24 h and every 2 h from 24 to 72 h after removal of the PRID. Serum was obtained, stored and assayed for LH and progesterone.

Serum samples were quantified for LH by a previously validated (Oxender, Hafs & Edgerton, 1972; Convey, Beal, Seguin, Tannen & Lin, 1976) homologous double-antibody radio-immunoassay. Highly purified preparations of TSH, prolactin, FSH and GH cross-react $< 0.5\%$ with our antisera. The sensitivity, defined as the lower limit of the 95% confidence interval, was 0·1 ng NIH-LH-B5. Assay recoveries of 0·1, 0·5, 2·0 and 8·2 ng NIH-LH-B5 added to 100 µl serum were 0·1, 0·4, 1·9 and 8·4 ng respectively. Inter- and intra-assay coefficients of variation averaged $< 13 \pm 2\%$ for 6 volumes of serum ranging from 20 to 300 µl across 8 different assays.

Duplicate 100 µl serum samples were used for determination of serum progesterone by a radioimmunoassay described previously (Convey *et al.*, 1977). The progesterone antiserum was produced in rabbits against progesterone-20-oxime–human serum albumin. This antiserum cross-reacts 10% with 20α-hydroxypreg-4-en-3-one, 4·2% with 17α-hydroxyprogesterone, 3·5% with testosterone, 2·5% with androstenedione and $< 0.5\%$ with various oestrogens, corticoids, cholesterol and other androgens. The sensitivity of the assay, calculated as the lower limit of the 95% confidence interval of the total binding in the tubes (binding in absence of hormone), is 10 pg. The intra- and inter-assay coefficient of variation were < 23 and 15% respectively for pools of bovine serum that contained mean $\pm$ s.e.m. values of 0.5 ± 0.04 and 16 ± 0.8 ng progesterone/ml ($n = 10$ assays).

To determine treatment effects on pulsatile LH and FSH secretion, 3 of 8 heifers in each treatment group were bled at 20-min intervals for 280 min 3 days after the PRIDs were inserted, and 1 day before and 36 h after removal of coils. These times were chosen to correspond with high, intermediate and low concentrations of progesterone, respectively. The last time would also be close to or during the LH and FSH surges. The concentrations of LH and FSH in each sample were measured within a single assay. An episodic release (episode) of hormone was defined as any increase at least 3 times greater than the within-assay coefficient of variation followed by an equivalent decrease within 2 h. The height of each episode was defined as the change in concentration from its apex to nadir. Serum concentrations of progesterone were determined in the first, middle and last blood sample collected during each 280-min bleeding schedule.

FSH was measured in duplicate 300 µl serum samples by an homologous double-antibody radioimmunoassay previously validated and adapted for use in our laboratory (Carruthers, Convey, Kesner, Hafs & Cheng, 1980). Highly purified preparations of various hormones including bovine LH and TSH cross-reacted $< 1\%$ with Cheng's antiserum for bovine FSH. The sensitivity, defined as the lower limit of the 95% confidence interval, was the equivalent of 2 ng FSH-NIH-B1/tube. Assay recovery of FSH-NIH-B1 averaged 96% over the range of 10–160 µl serum. Inter- and intra-assay coefficients of variation were $< 18\%$ for 4 different volumes of serum from ovariectomized cows ranging from 20 to 320 µl.

Statistical analyses. One heifer lost the PRID and her data were not included in the statistical analysis. To determine effect of progesterone on concentrations of LH, multiple correlations between LH, progesterone and time (days when PRID was in vagina) were determined. Multiple linear regression was used to determine the correlation within days for changes in concentrations of progesterone and LH.

The effects of progesterone concentration on episodic LH and FSH secretion were also examined. Episodic data were not normally distributed and the following transformations were made: (i) the numbers of peaks of LH and FSH approached normality when transformed to cosign; (ii) peak heights of LH were transformed to natural log LH + 1; (iii) concentrations (ng/ml) of LH were transformed to $1/\sqrt{LH + 0.5}$; (iv) peak heights of FSH were transformed to natural logarithms; (v) concentrations (ng/ml) of FSH were transformed to $\sqrt{FSH + 0.5}$.

A split-plot repeat measurement analysis (Gill & Hafs, 1971) was carried out using progesterone concentration as a covariate (Barr, Goodnight, Sall & Helwig, 1976). The covariate progesterone differed among treatments. After adjusting for progesterone, this analysis indicated that there were no significant treatment effects on serum concentrations of LH and FSH. Differences in LH or FSH were therefore due to associations with changes in serum progesterone concentrations and not to other variables such as length of treatment (12 versus 14 days) or presence of an oestradiol capsule. The data on height and frequency of LH and FSH episodes were therefore pooled according to high (> 2·0 ng/ml), intermediate (1–2·0 ng/ml) or low (< 1·0 ng/ml) concentrations of progesterone and analysed by Scheffe's simultaneous test.

Results

Correlations between progesterone and LH. Although progesterone concentrations differed ($P <$ 0·05) among treatment groups, no effect of treatment on serum concentration of LH was observed. There was no significant correlation between progesterone and LH concentration within any given day while PRIDs were in the vagina (data not shown). There were significant ($P < 0.01$) negative correlations between concentration of serum progesterone and duration of days that PRIDs were in the vagina for animals in all 5 treatment groups (Table 1; Text-fig. 1). There were significant ($P < 0.05$) positive correlations (Table 1; Text-fig. 1) between LH and days that PRIDs were in the vagina for Treatments 1, 3 and 4; the overall correlation coefficient (0·34) was also significant ($P < 0.01$). While PRIDs were in the vagina, a significant ($P < 0.05$) negative correlation (Table 1; Text-fig. 1) existed between serum progesterone and LH concentrations for animals in Treatments 1, 3 and 4; the overall correlation coefficient (−0·31) was also significant ($P < 0.05$).

Table 1. Multiple correlations among concentrations of progesterone and LH in serum and duration of treatment of heifers with progesterone coils containing different amounts of progesterone

Treatment	Progesterone and time	LH and time	LH and progesterone
1, 4% PRID + oestradiol capsule, 12 days	−0·93***	0·82**	−0·78**
2, 4% PRID, 12 days	−0·82**	−0·20	−0·03
3, 20% PRID, 12 days	−0·81**	0·67**	−0·58*
4, 4% PRID, 14 days	−0·88***	0·66*	−0·63*
5, 20% PRID, 14 days	−0·86***	−0·20	−0·11
Overall	−0·70***	0·34**	−0·31*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
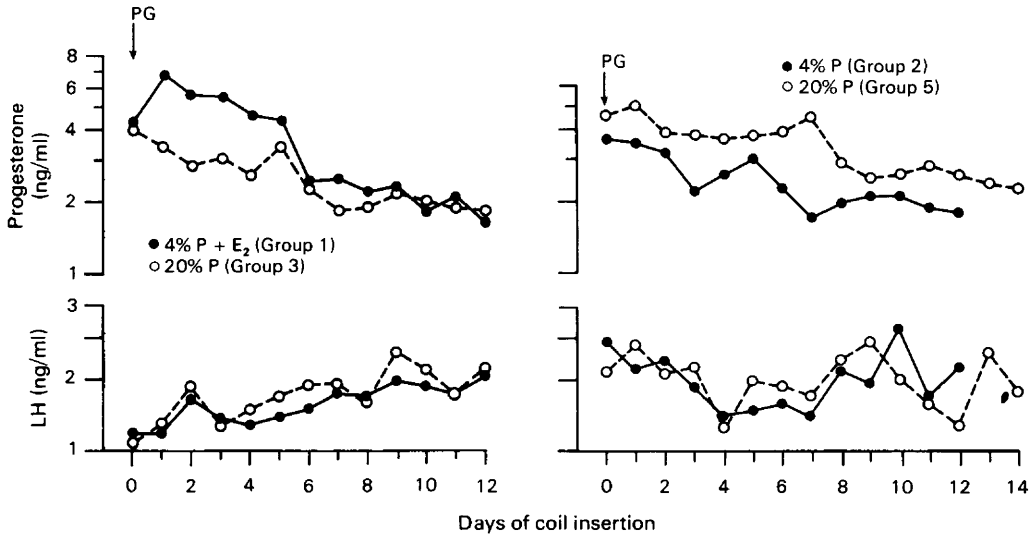
Text-fig. 1. Daily changes in serum concentrations of LH and progesterone while progesterone (P) coils were in the vagina. Each point represents the mean serum hormone value for 8 heifers. The 14-day 4% treatment group (No. 4) was omitted because progesterone and LH serum values were not different from those of the 12-day 4% treated animals (Treatment 2). Day 0 represents the pooled mean serum values for samples taken 3 days before and on the day of coil insertion. The arrow indicates time of PGF-2α injection, which was given only to animals in Treatments 2, 3, 4 and 5.

Episodic releases of LH and FSH. Before removal of PRIDs, the correlations of changes in concentrations of progesterone with LH or FSH (Table 2) indicated that the concentrations of LH during the 20-min sampling time and progesterone were negatively correlated in Treatments 1 and 4; LH and FSH concentrations were positively correlated in all treatments and FSH and progesterone were positively correlated in Treatments 2, 4 and 5. As shown in Table 3, the amplitude of LH or FSH episodes was not increased as progesterone concentrations declined from high to low. There was an increase in the number of LH episodes ($P < 0.05$), but not FSH episodes, as progesterone concentrations declined from high to intermediate or low values. Basal LH but not FSH concentrations were significantly higher ($P < 0.05$) for heifers with low concentrations of progesterone in comparison to heifers with higher concentrations. Following

Table 2. Correlations† of progesterone with LH or FSH before PRIDs were removed from heifers (3/treatment)

Treatment	$1/\sqrt{LH + 0.5}$ and progesterone	$1/\sqrt{LH + 0.5}$ and $\sqrt{FSH + 0.5}$	$\sqrt{FSH + 0.5}$ and progesterone
1, 4% PRID + oestradiol, 12 days	0.45***	−0.67***	0.11
2, 4% PRID, 12 days	0.17	−0.60***	0.24*
3, 20% PRID, 12 days‡	0.09	−0.56***	0.04
4, 4% PRID, 14 days	0.44***	−0.72***	0.62***
5, 20% PRID, 14 days	−0.18	−0.32**	0.60***
Overall	0.35***	−0.51***	−0.04

† Difference of 0.22 among correlations within columns is significant at $P < 0.01$. Because LH data were transformed to a reciprocal, correlations would have opposite sign when arithmetic means are used.

‡ Only 2 heifers.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. Mean (± s.e.m.) LH and FSH concentrations, and number and amplitude of LH and FSH episodes during PRID treatment for different progesterone concentrations, and after PRID removal in heifers which were bled every 20 min for 4·67 h

Progesterone conc. (ng/ml)	No. of heifers	LH (ng/ml)	No. of LH episodes	Amplitude of LH episodes	FSH (ng/ml)	No. of FSH episodes	Amplitude of FSH episodes
During PRID							
>2	9	2·7 ± 0·3[a]	2·2 ± 0·4[a]	2·3 ± 0·4[a]	53 ± 5[a]	1·9 ± 0·4[a]	30 ± 6[a]
1–2	15	2·8 ± 2[a]	3·3 ± 0·3[b]	2·1 ± 0·2[a]	54 ± 3[a]	2·0 ± 0·3[a]	19 ± 1[b]
<1	4	4·7 ± 0·8[b]	3·8 ± 0·3[b]	2·5 ± 0·7[a]	58 ± 4[a]	2·0 ± 0·7[a]	23 ± 4[a]
After PRID							
0·1–0·4	14	21·2 ± 0·7[c]	2·4 ± 0·6[a]	13·5 ± 0·3[b]	99·1 ± 14[b]	1·4 ± 0·4[a]	67 ± 13[c]

Different superscripts within columns indicate statistically significant means ($P < 0.05$).

removal of the PRID and the subsequent decline of progesterone, the amplitudes of LH and FSH episodes were significantly increased ($P < 0.05$), as were concentrations of LH and FSH ($P < 0.05$) before and during the surge. Text-figure 2 shows an example of the changes in episodic LH and FSH secretions.
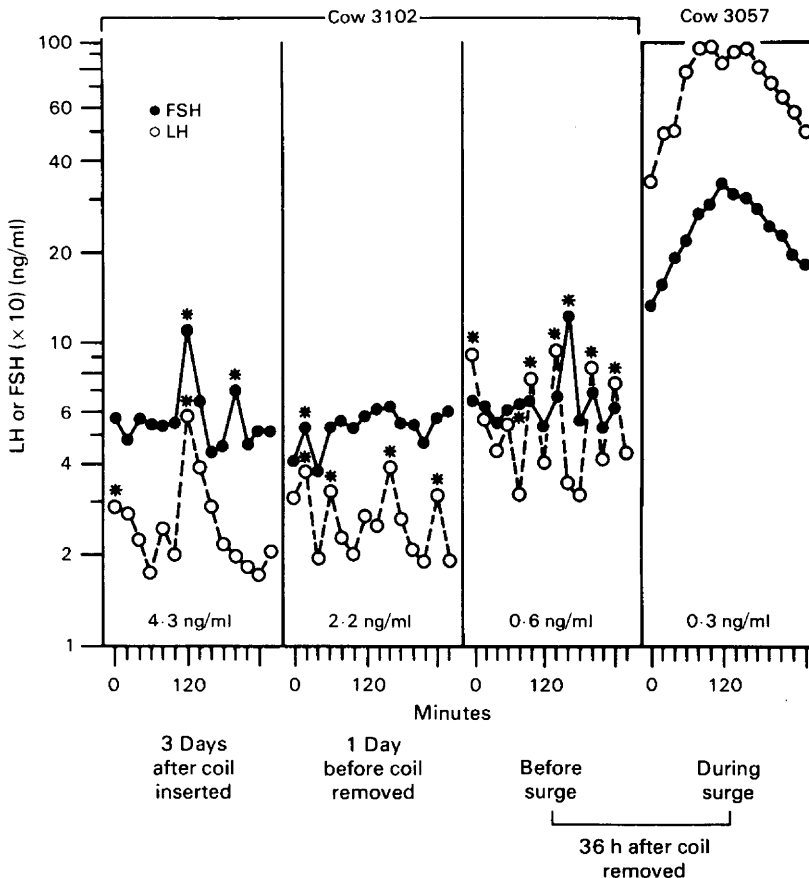
Text-fig. 2. Acute changes in serum concentrations of LH and FSH release in 2 heifers while progesterone coils were in the vagina and 36 h after removal (see Table 2). Asterisks indicate episodic release as defined in 'Materials and Methods'. The mean progesterone concentration during the time of frequent sampling is indicated at the foot of each panel. Blood samples were taken at 20-min intervals for 280 min.

Discussion

These results indicate that progesterone is not the only hormone involved in controlling LH secretion in cattle. LH concentrations on any given day were not correlated with changes in progesterone concentrations and a low, although significant, negative correlation between progesterone and LH concentrations was obtained in 3 of 5 treatments. Moreover, episodic changes in LH and progesterone were negatively correlated in only 2 of 5 treatments and episodic release of FSH and progesterone were positively correlated in only 2 of 5 treatments. The above data strongly suggest that other unidentified factors regulate LH and FSH secretion in cattle. Oestradiol is known to affect LH levels in cattle and sheep (Goding *et al.*, 1969; Legan, Karsch & Foster, 1977), and this is probably an equally important hormone for LH regulation. Likewise, Beck, Smith, Seguin & Convey (1976) have shown that a combination of oestradiol and progesterone is more effective in blocking the post-ovariectomy rise of LH in cattle. That the oestradiol capsule did not affect LH and FSH in Treatment 1 may be because sampling was not frequent enough to pick up an effect. The effect of oestradiol on FSH secretion in cattle is unknown.

The question as to why LH and FSH were always positively correlated with each other, but correlations of LH or FSH with progesterone were not always positive, is raised by the present data. The gonadotrophins may be positively correlated if LH-releasing hormone controls the release of both hormones. However, other data show that pituitary release of gonadotrophins in cattle can occur independently of each other (Roche & Ireland, 1981). Thus the frequency and amplitude of LH and FSH pulses may be modulated by progesterone, oestradiol and perhaps by inhibin. Goodman & Karsch (1980) have shown that oestradiol decreases the amplitude of LH pulses, whereas progesterone reduces the frequency of LH pulses in sheep. Since factors other than progesterone modulate episodic gonadotrophin secretion, correlations of episodic release and progesterone will depend on the interactions with other factors.

The rise in basal LH concentrations that occurred as concentrations of progesterone declined over time was probably due to an increase in the number of LH pulses released rather than through an effect on amplitude of each pulse as shown by Rahe *et al.* (1980). This fact suggests that the negative feedback effect of progesterone and/or other hormones on LH may be mediated by an effect on release of LH-RH rather than by a direct effect on the pituitary gland. In contrast, when progesterone levels were very low 36 h after removal of the PRIDs, the preovulatory surge of LH resulted from an increase in amplitude of LH releases. No increase in frequency of episodic LH releases was observed but a sampling interval of 20 min may not be frequent enough at this time to pick up such an effect. Since progesterone was low at this time and oestradiol was high 36 h after PRID removal (Roche & Ireland, 1981), oestradiol may exert its positive feedback effect on LH by altering the amplitude of episodic LH release. The hypothesis that progesterone in the cow mediates a negative feedback effect on LH by reducing the frequency of episodic LH releases, while a positive feedback effect of oestradiol is mediated through an increase in amplitude of episodic LH release, is attractive but seems over-simplified. Other factors, as yet unidentified, appear to be involved, especially in negative feedback regulation of LH secretion. Neither frequency nor amplitude of FSH releases consistently decrease as progesterone declines. However, decline in FSH in the follicular phase of the oestrous cycle of the ewe has been reported (L'Hermite, Niswender, Reichert & Midgley, 1972). Perhaps an increase in concentration of inhibin or oestradiol from the developing follicle blocks an increase in episodic FSH release and thus blocks a pre-surge rise in FSH.

The significant rise in basal LH concentrations as progesterone declined during PRID treatment may be an important factor when poor fertility occurs in synchronized cattle (Roche, 1974). Prolonged elevated LH concentrations during progesterone treatment could alter steroid synthesis and thus upset the physiological interplay between ovary, oviduct and uterus, as has been reported for rabbits (McCarthy, Foote & Maurer, 1977). However, basal LH

concentrations in the presence of increased progesterone values, and a 1–2 day rise in basal LH prior to the LH peak are required to support the development of preovulatory follicles by increasing endogenous androgen production, follicular aromatase activity, and theca and granulosa cell LH receptors (Bogovich & Richards, 1980). Moreover, the ratio of serum LH to FSH values can affect follicle growth and function in rats (Ireland & Richards, 1978), and these ratios can be altered in cattle (Roche & Ireland, 1981). Control of FSH secretion must also be considered when devising new methods for synchronizing ovulation. As proposed by Roche & Ireland (1981), optimal concentrations of hormones to control oestrus in cattle must be sufficient to block behavioural oestrus and prevent a rise in basal LH and FSH concentrations during the entire treatment regimen.

The present data present circumstantial evidence that progesterone is part of a negative feedback complex on LH secretion in cattle. Further work is required to delineate the role of progesterone in this complex and also to determine how progesterone and other factors affect episodic LH secretion in cattle.

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