Comparison of the rate of decline in plasma progesterone concentrations at a natural and progesterone-synchronized oestrus and its effect on tonic LH secretion in the ewe

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Summary. The pattern of change in plasma progesterone and LH concentrations was monitored in Clun Forest ewes at a natural oestrus and compared to that observed after removal of progesterone implants. The rate of decline in plasma progesterone concentrations after implant withdrawal ($1.8 \pm 0.2 \text{ ng/ml h}^{-1}$) was significantly greater ($P < 0.001$) than that observed at natural luteolysis ($0.2 \pm 0.1 \text{ ng/ml h}^{-1}$), and this resulted in an abnormal pattern of change in tonic LH secretion up to the time of the preovulatory LH surge. This more rapid rate of progesterone removal was also associated with a shortening of the intervals from the time that progesterone concentrations attained basal values to the onset of oestrus ($P < 0.05$) and the onset of the preovulatory LH surge ($P < 0.01$). However, there were no significant differences in the duration of the LH peak, preovulatory peak LH concentration, ovulation rate or the pattern of progesterone concentrations in the subsequent cycle. It is suggested that the abnormal patterns of change in progesterone and tonic LH concentrations may be one factor involved in the impairment of sperm transport and abnormal patterns of oestradiol secretion known to occur at a synchronized oestrus.

Introduction

The use of intravaginal pessaries containing either synthetic progestagens or progesterone to synchronize oestrus in the ewe has been reported to result in detrimental effects on both sperm transport and sperm survival within the female reproductive tract (Quinlivan & Robinson, 1969; Hawk, 1971; Hawk & Conley, 1971a, b; Pearce & Robinson, 1985). These effects are observed even when the synthetic progestagen is given via the feed (Hawk & Conley, 1971a), indicating that it is not the result of a local response of applying the progestagen via the intravaginal route. The use of synthetic progestagen pessaries changes the pattern of oestradiol secretion (Smith & Robinson, 1970; Echternkamp, Bolt & Hawk, 1976) and hence cervical mucus production (Smith & Allison, 1971) at the synchronized oestrus as well as resulting in differences in the cytochemistry of the endometrium relative to a natural oestrus (Kruip, 1972). It has been suggested that such factors are directly responsible for the impairment of sperm transport and survival (Allison & Robinson, 1972). However, the precise endocrine basis for the abnormal patterns of oestradiol secretion is poorly understood.

Recent developments in our understanding of the endocrine control of the oestrous cycle in the ewe indicate that the regulation of tonic LH secretion is determined by the combined negative feedback effects of oestradiol-17 β and progesterone (Karsch, Legan, Ryan & Foster, 1978). Furthermore, the tight temporal relationship between episodic LH secretion and oestradiol secretion rate from the ovary (Baird, Swanston & Scaramuzzi, 1976; Scaramuzzi & Baird, 1979) indicates that tonic LH secretion is a major factor responsible for driving oestradiol secretion by developing

follicles. This is further supported by the observation of parallel increases in plasma LH and oestradiol concentrations over the follicular phase of the oestrous cycle (Karsch *et al.*, 1978).

Preliminary observations (W. Haresign, unpublished data) had suggested that insertion of progestagen-impregnated sponges on Day 8 of the oestrous cycle followed by their withdrawal 12 days later resulted in a more rapid rate of increase in tonic LH secretion than that observed over the follicular phase at a natural oestrus. However, it was not clear whether such a difference was a function of using a synthetic progestagen rather than natural progesterone, or was a reflection of differences in rates of removal of progestational activity.

The purpose of the present study was therefore to determine whether the rate of decline in plasma progesterone concentrations results in any change in the pattern of tonic LH secretion which might explain the abnormal patterns of oestradiol secretion that have previously been reported to occur when oestrus is synchronized with progestagen pessaries (Echternkamp *et al.*, 1976).

Materials and Methods

Animals and management. Twelve Clun Forest ewes showing regular recurrent oestrous cycles were selected from the School of Agriculture flock and randomly allocated to one of two equal-sized groups.

During the periods of daily blood sampling they were kept at pasture with a colour-marked vasectomized ram, but during the period of intensive blood sampling over the periovulatory period they were housed in individual pens and had free access to hay and fresh water. A jugular vein catheter was inserted into all ewes on Day 12 of the natural oestrous cycle (control ewes) or 2 days before removal of the progesterone implants (treated ewes). During the period of frequent blood sampling the ewes were checked for oestrus at hourly intervals by using a vasectomized ram.

Treatments and blood sampling. The 6 treated ewes each received 3 progesterone implants (Sil-Estrus: Ceva, Paris, France), containing 375 mg progesterone in a silicone-elastomer matrix, in the subcutaneous tissue of the axilla region on Day 8 of the oestrous cycle, and these were left *in situ* for 12 days. Control ewes received no treatment.

Daily blood samples were collected by jugular venepuncture from the day of oestrus (Day 0) of the cycle in which treatment was given, and continued for the duration of the cycle after the synchronized oestrus (treated ewes), or for the duration of the cycles immediately preceding and following the oestrus at which frequent blood samples were collected (control ewes). In addition, hourly blood samples were collected via the catheter from 24 h before withdrawal of progesterone implants, or from Day 13 of the cycle for control ewes, and continued until 24 h after the onset of oestrus. Blood samples were centrifuged immediately after collection, and the plasma stored at -20°C until assayed for LH and progesterone. All hourly blood samples were analysed for LH, and all daily samples and alternate hourly samples for progesterone. Laparoscopy was performed on all ewes under barbiturate anaesthesia 7 days after the period of intensive sampling to determine ovulation rates.

Hormone analyses. Progesterone concentrations were measured by the radioimmunoassay described by Haresign, Foster, Haynes, Crighton & Lamming (1975). This assay shows negligible ($< 1\%$) cross-reaction with other major steroids in sheep plasma, and within this study the inter- and intra-assay coefficients of variation were 11.4% and 6.6% respectively. The limit of sensitivity was 0.1 ng/ml plasma with a mean extraction efficiency of $80.0 \pm 1.3\%$.

Plasma LH concentrations were measured by the specific double-antibody technique of Foster & Crighton (1974), as modified by McLeod, Haresign & Lamming (1982). The limit of sensitivity within this study was 0.5 ng NIH-LH-S18 equiv./ml plasma, and the inter- and intra-assay coefficients of variation were 11.8% and 10.7% respectively.

Analysis of results. Characteristics of preovulatory LH release, the timing of the LH peak, the timing of oestrus and ovulation rates were all compared using Student's *t* test. The onset of the LH peak was defined as that time when LH concentrations first exceeded 10 ng/ml, and its duration was calculated as the time over which LH concentrations remained elevated above this value.

The mean rate of change in hormone concentrations during the follicular phase for control and treated ewes was calculated by regressing hormone concentrations for individual ewes against time over the period from either the onset of natural luteal regression or the time of progesterone implant withdrawal until mean progesterone concentrations attained values of ≤ 0.5 ng/ml (for progesterone) or the onset of the preovulatory LH surge (for LH). The significance of the difference in these values between the two groups was determined by comparing the slopes of the linear regression lines, using Student's *t* test. A single linear regression provided the best fit to the LH data derived from control ewes, but a two-component regression was used for describing the LH data from synchronized ewes, the first representing the 4-h period immediately after progesterone implant withdrawal, and the second the period thereafter until the onset of the preovulatory LH surge.

Results

All ewes came into oestrus during the period of intensive blood sampling, but the interval from plasma progesterone reaching basal values (≤ 0.5 ng/ml) to the onset of oestrus was significantly shorter ($P < 0.05$) in the synchronized ewes (Table 1). However, treatment had no significant effect on ovulation rate.

There was no significant difference in mean maximum plasma progesterone concentrations over the luteal phase of the cycle immediately before the period of frequent sampling between control (4.6 ± 0.8 ng/ml) and treated ewes (5.4 ± 0.8 ng/ml), although insertion of progesterone implants on Day 8 of the cycle did extend oestrous cycle length by 6 days. There was evidence of a small, though non-significant, rise in plasma progesterone concentrations in treated ewes as a result of progesterone implant insertion which persisted until about Day 15, the time of the onset of natural luteal regression in control ewes. However, the implants did maintain plasma progesterone concentrations at normal luteal-phase values until the time of their removal on Day 20. The length of

Table 1. Comparison of the timing of oestrus, features of preovulatory LH release and ovulation rate in ewes at a natural oestrus and after synchronization of oestrus by using progesterone implants

	Natural oestrus	Synchronized oestrus	Significance level
Rate of decline in progesterone conc. (ng/ml h ⁻¹)	0.2 ± 0.1	1.8 ± 0.2	$P < 0.001$ †
Rate of increase in tonic LH conc. (ng/ml h ⁻¹)	0.06 ± 0.01	0.85 ± 0.07*	$P < 0.001$ †
Basal progesterone to onset of oestrus (h)	30.4 ± 2.1	25.8 ± 1.3	$P < 0.05$
Basal progesterone to onset of LH surge (h)	37.2 ± 2.0	29.6 ± 1.0	$P < 0.01$
Onset of oestrus to onset of LH surge (h)	6.8 ± 2.3	3.8 ± 1.5	NS
Peak LH conc. (ng/ml)	205 ± 23	185 ± 26	NS
Duration of LH surge (h)	12.8 ± 0.4	11.2 ± 0.4	NS
Ovulation rate	2.0 ± 0.3	1.8 ± 0.3	NS

Values are mean ± s.e.m. for 6 ewes per group.

* During the first 4 h after implant removal (see text).

† Estimated from the differences in mean slopes of the linear regression lines (see text).

Text-fig. 1. Mean ($\pm$ s.e.m.) plasma progesterone (Δ , $\circ$) and LH ($\blacktriangle$, $\bullet$) concentrations at a natural oestrus (-----) or after progesterone implant withdrawal (—) in cyclic ewes. The data are derived from 6 ewes per treatment group and have been normalized about the time of basal progesterone and the time of preovulatory peak LH concentrations. $\downarrow$ denotes the time of implant removal.

the oestrous cycle immediately after the period of intensive blood sampling ranged from 16 to 18 days for individual ewes and did not differ significantly between treated and control ewes. In addition, neither the patterns of change in progesterone concentrations nor the maximum concentrations attained in either group during this post-treatment cycle differed significantly from those observed in control ewes during the cycle immediately preceding the period of intensive blood sampling.

Removal of progesterone implants resulted in an abrupt decline in plasma progesterone concentrations to ≤ 0.5 ng/ml within 8 h in all treated ewes (Text-fig. 1). The mean rate of decline in progesterone concentrations in these ewes was 9-fold greater ($P < 0.001$) than that during natural luteolysis (Text-fig. 1; Table 1).

The mean patterns of change in LH concentrations for both groups of ewes, based on 4-h block means and normalized about the time of basal progesterone concentrations or preovulatory LH peak concentrations, are depicted in Text-fig. 1. In the control ewes, there was a gradual but progressive increase in tonic LH concentrations as the progesterone concentrations declined. This occurred at a mean rate of 0.06 ± 0.01 ng/ml h^{-1} and continued until the onset of the preovulatory

LH surge. By way of contrast, tonic LH concentrations in progesterone-synchronized ewes increased abruptly, from a mean value of 1.7 ± 0.1 ng/ml up to a value of 5.1 ± 0.2 ng/ml ($P < 0.001$) within the first 4 h after progesterone implant removal. Thereafter the slope of the linear regression line between tonic LH concentrations and time up to the onset of the LH surge did not differ significantly from zero, indicating no further significant change in concentrations until the onset of the preovulatory LH surge. The initial rate of change in tonic LH concentrations in synchronized ewes was significantly ($P < 0.001$) greater than that observed over the natural follicular phase of the cycle in control ewes (Table 1).

The interval between progesterone attaining basal values (≤ 0.5 ng/ml) and the onset of the preovulatory LH peak was significantly shorter ($P < 0.001$) in the synchronized ewes (Table 1). Although there was also a tendency for the interval between the onset of oestrus and the onset of the LH peak to be reduced in the synchronized ewes, this was not significant. None of the other characteristics of preovulatory LH release was significantly influenced by treatment (Table 1).

Discussion

The inverse relationship between the patterns of change in progesterone and tonic LH secretion over the follicular phase of the oestrous cycle in control ewes is in general agreement with that previously reported. It is apparent from the studies of Baird & Scaramuzzi (1976) and Karsch *et al.* (1978) that progesterone is a major component of the negative feedback system regulating tonic LH secretion. However, one striking feature of the data from the current experiment is that removal of progesterone implants resulted in a 9-fold increase in the mean rate of decline in plasma progesterone concentrations relative to the rate of fall of this hormone at luteolysis at the end of the natural cycle. This was mirrored by a similarly dramatic increase in tonic LH secretion, an observation consistent with the proposed role for progesterone in the control of LH secretion. Although the present trial was conducted using implants containing natural progesterone, the same conclusions can be extrapolated to intravaginal pessaries containing synthetic progestagen, because removal of such pessaries also produces a similar change in the pattern of tonic LH secretion (W. Haresign, unpublished observations). However, such alterations in the pattern of hormone secretion had no significant effect on ovulation rate or the pattern of progesterone secretion in the subsequent cycle.

Since the gradual rise in tonic LH secretion over the follicular phase of the oestrous cycle in the ewe is responsible for driving the gradual pro-oestrous rise in oestradiol production (Karsch *et al.*, 1978), and there is a very close temporal association between individual pulses of LH and oestradiol (Baird *et al.*, 1976; Scaramuzzi & Baird, 1979), it is highly probable that the faster rate of increase in tonic LH secretion observed at the synchronized oestrus would produce a more marked initial stimulation of oestradiol secretion. Unfortunately, plasma oestradiol concentrations were not measured in this study to confirm whether this was in fact the case. However, the significant shortening of the intervals from the attainment of basal progesterone concentrations and both the onset of oestrus and the onset of the preovulatory LH surge are consistent with an earlier increase to the threshold levels of oestradiol required for the induction of oestrous behaviour and positive feedback responses. Furthermore, Echternkamp *et al.* (1976) have indeed observed that the synchronization of oestrus in the ewe with pessaries containing medroxyprogesterone acetate is associated with an initial marked increase in plasma oestradiol concentrations, with little change thereafter until the onset of the preovulatory LH peak. In contrast, oestradiol concentrations at a natural oestrus increased gradually and consistently over the 48 h before the onset of the LH peak.

The rate and pattern of cervical mucus secretion at oestrus are determined not only in a dose-dependent manner by the levels of oestradiol, but also by the interactive effects of this hormone with declining progesterone titres (Allison & Robinson, 1972). The more dramatic changes in plasma progesterone concentrations of treated ewes in this trial are indicative of a more rapid

switch in the oestrogen:progesterone ratio, whether or not there was any difference in the rate of oestradiol secretion, and this may well give rise to the changed patterns of cervical mucus secretion which have been reported to occur at a synchronized oestrus (Smith & Robinson, 1970; Smith & Allison, 1971).

It has been recognized for some time that the synchronization of oestrus in the ewe with synthetic progestagens reduces sperm transport to levels below that observed at a natural oestrus (Quinlivan & Robinson, 1969; Hawk & Conley, 1971a) and that this is a function of changes in the pattern of cervical mucus secretion (Allison & Robinson, 1972). Similar problems of sperm transport occur when oestrus is synchronized with progesterone (Pearce & Robinson, 1985). Indeed, Pearce & Robinson (1985) concluded that the major endocrine factor responsible for this reduction in sperm transport was the abnormally rapid decline in plasma progesterone concentrations compared to that observed at a natural oestrus. No attempt was made in the present study to relate the changes in hormone secretion to cervical mucus production and sperm transport, and it is also recognized that extrapolation from one study to another is always dangerous. Nevertheless, the possibility that it is the interactive effects of an abnormally high rate of removal of progestational activity on tonic LH secretion which results in subfertility at a synchronized oestrus would seem to justify further investigation.

I thank the National Institutes of Health, Bethesda, MD, U.S.A., for supplies of purified ovine LH.

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Received 28 January 1985