Plasma progesterone and LH concentrations in ewes after injection of an analogue of prostaglandin F-2 α

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Summary. Plasma progesterone and LH concentrations were monitored throughout a natural oestrous cycle in 12 Clun Forest ewes and compared to those following treatment with a single i.m. injection of 100 μ g ICI 80,996, an analogue of prostaglandin F-2 α , given during the luteal phase of the cycle. After injection of the analogue there was a high degree of synchrony in the return to oestrus (44.0 ± 1.9 h; mean $\pm$ S.E.M.) and the timing of the LH peak (48.5 ± 2.0 h) from injection. There were no significant differences in the plasma progesterone concentrations or in the height and duration of the preovulatory LH peak between control and treatment cycles. The technique offers the possibility of controlled ovulation in the ewe.

Introduction

Methods of controlled breeding in sheep involving the use of synthetic progestagens have led to reports of depression in fertility (Rahman & Kitts, 1967; Robinson, Salamon, Moore & Smith, 1967; Holst & Moore, 1970). It has been suggested from comparisons of patterns of cervical mucus that these methods of breeding control give rise to abnormal steroid hormone changes (Smith & Allison, 1971). In addition, the timing of the preovulatory LH peak relative to the onset of oestrus after use of synthetic progestagens is significantly different from that of a natural oestrus (Cumming *et al.*, 1970; Lintin & Lamming, 1973).

Artificial insemination (AI) at a fixed time interval from progestagen withdrawal has been partly successful in obtaining good fertility, but only when fresh semen less than 4 h old has been used. Fertility results from trials in the United Kingdom involving frozen semen have been particularly poor (H. B. Reed, personal communication), attributable at least in part to failure of sperm transport and poor sperm survival, presumably because of the abnormal endocrine environment (Quinlivan & Robinson, 1969; Hawk & Conley, 1971). A method of ovulation control which results in normal hormone changes is therefore needed before it is possible to determine the feasibility of carrying out AI in ewes with frozen semen.

Recent experimental evidence has suggested that prostaglandin (PG) F-2 α , released naturally from the uterus towards the end of the oestrous cycle, may be the agent responsible for initiating luteal regression (McCracken, Glew & Scaramuzzi, 1970; Caldwell & Moor, 1971; Wilson, Cenedella, Butcher & Inskeep, 1972). Chamley *et al.* (1972) infused PGF-2 α into the ovarian artery of ewes with ovarian transplants and demonstrated that it induced luteal regression and oestrus associated with the typical rises in oestradiol-17 β and LH.

The present study was therefore designed to study whether a single treatment of an analogue of PGF-2 α given at mid-cycle would routinely induce luteal regression and initiate a new oestrous cycle in ewes. The hormone changes associated with the induced oestrus were compared to those occurring at a natural oestrus with a view to assessing the application of this method of ovulation control in AI programmes for sheep.

Materials and Methods

Twelve 3–4-year-old Clun Forest ewes showing regular oestrous cycles were housed during October and November under conditions of natural daylength and temperature throughout the whole experimental period.

Blood samples were collected hourly from an indwelling jugular vein cannula for 24 h from the onset of a natural oestrus, determined by using a colour-marked vasectomized ram. Samples were then collected daily by jugular venepuncture throughout the ensuing oestrous cycle, and until the middle of the subsequent oestrous cycle, when all 12 ewes received a single i.m. injection of 100 µg ICI 80,996 (the PGF-2α analogue) in 2 ml saline. Preliminary studies had established this to be the minimum dose to induce oestrus in ewes. From 12 h before to 72 h after the injection blood samples were collected hourly from the jugular vein cannula, and then daily by jugular venepuncture throughout the following oestrous cycle. All blood samples were centrifuged at 1600 g for 30 min at 4°C and the plasma stored at –20°C until assayed.

Concentrations of plasma progesterone were measured by the radioimmunoassay described previously by Haresign, Foster, Haynes, Crighton & Lamming (1975). The degree of cross-reaction with the major steroids occurring in ovine plasma was <1% when the petroleum–ether extraction step was taken into consideration. The sensitivity of the assay was 26 pg/tube (0.08 ng/ml plasma), and the mean recovery $62.7 \pm 3.2\%$. Both the interassay coefficient of variation and the coefficient of variation of duplicate pairs in this study were <10%. Accuracy of the method was determined by adding known quantities of progesterone to 0.5 ml aliquots of plasma from a castrated ram: 0.1 ng added gave a measurement of 0.11 ± 0.01 (S.E.M.) ng, 0.2 ng added gave 0.19 ± 0.01 ng, 0.4 ng added gave 0.40 ± 0.02 ng, 1.0 ng added gave 0.96 ± 0.03 ng, 2.0 ng added gave 2.02 ± 0.03 ng.

Plasma LH concentrations were measured by a specific double-antibody radioimmunoassay described previously (Foster & Crighton, 1974). The antiserum used had negligible cross-reactions with ovine FSH, growth hormone and prolactin. There were no differences between the levels of LH detected by the assay in plasma samples obtained before and after treatment of ewes with 300 µg TSH-RH, suggesting that cross-reaction with TSH was low. The sensitivity of the assay was 0.05 ng NIH-LH-S18 equivalents/tube (0.1 ng/ml). The intra- and interassay coefficients of variation were both <10%.

Analysis of all data was by *t* test.

Results

Duration of the oestrous cycle. The mean ($\pm$ S.E.M.) length of the pretreatment oestrous cycle for all ewes was 16.8 ± 0.3 days and ranged from 15 to 18 days, whilst that of the cycle after treatment was 17.4 ± 0.2 days (range 16–18 days), this difference being insignificant.

Plasma progesterone concentrations. The general pattern of progesterone changes was similar for all ewes although considerable variation was observed between animals in the maximum concentrations measured, ranging from 2.8 to 7.6 ng/ml and 2.5 to 9.1 ng/ml in the pre- and post-treatment cycles, respectively. The mean daily plasma progesterone concentrations for all 12 ewes throughout the sampling period are shown in Text-fig. 1. No significant differences existed between the pre- and post-treatment cycles.

The single injection of ICI 80,996 in mid-cycle resulted in a fall in plasma progesterone concentration (Text-fig. 1) from a mean preinjection value of 3.1 ± 0.3 ng/ml to 0.9 ± 0.2 ng/ml within 6 h of treatment. By 24 h the mean concentration was 0.4 ± 0.1 ng/ml and all animals came into oestrus at a mean time interval of 44.0 ± 1.9 h from injection.

Plasma LH concentrations. An elevation of plasma LH concentration was measured in all 12 ewes at the time of oestrus after injection of the prostaglandin analogue (169.2 ± 12.1 ng NIH-LH-S18 equiv/ml) and in 11 ewes at the natural oestrus (174.6 ± 7.5 ng NIH-LH-S18 equiv/ml). Marked variations were observed between ewes in the height of the LH peak, but the difference between the mean peak heights was not significant. The mean durations of the LH peaks were 12.3 ± 0.6 h ($N = 11$) and

Text-fig. 1. Mean $\pm$ S.E.M. peripheral plasma progesterone concentrations in 12 ewes throughout a natural oestrous cycle and following a single i.m. injection (arrow) of 100 μ g ICI 80,996 during the luteal phase of the subsequent cycle. H indicates the day of oestrus.

12.3 ± 0.7 h ($N = 12$) at the natural and induced oestrus, respectively, with the mean time intervals from the onset of oestrus of 3.9 ± 1.4 h and 4.9 ± 1.1 h. None of these differences was significant. The mean time interval from injection of the prostaglandin analogue to the LH peak was 48.5 ± 2.0 h ($N = 12$).

Discussion

The concentrations of plasma progesterone recorded during the oestrous cycle tended to be higher than those reported by Stabenfeldt, Holt & Ewing (1969) and Thorburn, Bassett & Smith (1969), but were of the same order of magnitude as those previously reported from this laboratory for the same breed of ewe (Haresign *et al.*, 1975). The very rapid decrease in progesterone concentrations following injection of the prostaglandin analogue clearly indicates the potent luteolytic activity of ICI 80,996 in the ewe, and the induced luteal regression was followed by a highly synchronized return to oestrus.

One ewe failed to show a rise in LH concentration at the natural oestrus and the reason for this is not clear. The progesterone concentration at the recorded onset of oestrus for this ewe was relatively high (0.8 ng/ml) and there was an unexpected rise to 1.6 ng/ml the following day. The results suggest that there may have been a false detection of oestrus in this ewe and that the onset may have occurred before the period of frequent sampling.

Unlike the findings with synthetic progestagens (see 'Introduction'), no significant differences in the LH response between a natural oestrus and that induced with the prostaglandin analogue could be demonstrated in the present study. As in cattle (Louis, Hafs & Morrow, 1974), there was no evidence of any effect of treatment on subsequent luteal function as assessed from the progesterone profiles in the pre- and post-treatment cycles.

The high degree of synchrony in the return to oestrus and the timing of the LH peak in relation to injection of the prostaglandin analogue suggest that it may be possible to use this compound in controlled breeding programmes during the breeding season in conjunction with AI. Furthermore, the fact that the prostaglandin analogue does not produce the abnormal endocrine changes associated with the use of synthetic progestagens may allow the use of frozen rather than fresh semen.

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References

- CALDWELL, B.V. & MOOR, R.M. (1971) Further studies on the role of the uterus in the regulation of corpus luteum function in sheep. *J. Reprod. Fert.* **26**, 133–135.
- CHAMLEY, W.A., BUCKMASTER, J.M., CAIN, M.D., CERINI, J., CERINI, M.E., CUMMING, I.A. & GODING, J.R. (1972) The effect of prostaglandin $F_{2\alpha}$ on progesterone, oestradiol and luteinizing hormone secretion in sheep with ovarian transplants. *J. Endocr.* **55**, 253–263.
- CUMMING, I.A., BLOCKEY, M.A., BROWN, J.M., CATT, K.J., GODING, J.R. & KALTENBACH, C.C. (1970) The release of luteinizing hormone in ewes following the withdrawal of intravaginal sponges containing progestagen. *Proc. Aust. Soc. Anim. Prod.* **8**, 383–387.
- FOSTER, J.P. & CRIGHTON, D.B. (1974) Luteinizing hormone (LH) release after single injection of a synthetic LH-releasing hormone (LH-RH) at three different reproductive stages and comparison with the natural LH release of oestrus. *Theriogenology* **2**, 87–100.
- HARESIGN, W., FOSTER, J.P., HAYNES, N.B., CRIGHTON, D.B. & LAMMING, G.E. (1975) Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod. Fert.* **43**, 269–279.
- HAWK, H.W. & CONLEY, H.H. (1971) Loss of spermatozoa from the reproductive tract of the ewe and intensification of sperm breakage by progestagen. *J. Reprod. Fert.* **27**, 339–347.
- HOLST, P.J. & MOORE, N.W. (1970) Control of oestrus and ovulation by progesterone and cronolone administered either intramuscularly or intravaginally and subsequent fertility. *Aust. J. agric. Res.* **21**, 371–382.
- LINTIN, K. & LAMMING, G.E. (1973) The effect of progestagens and PMSG on peripheral plasma LH levels in the Clun Forest ewe. *J. Reprod. Fert.* **35**, 607–608.
- LOUIS, T.M., HAFS, H.D. & MORROW, D.A. (1974) Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: progesterone, estrogen, LH, estrus and ovulation. *J. Anim. Sci.* **38**, 347–353.
- MCCRACKEN, J.A., GLEW, M.E. & SCARAMUZZI, R.J. (1970) Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. *J. clin. Endocr. Metab.* **30**, 544–546.
- QUINLIVAN, T.D. & ROBINSON, T.J. (1969) Numbers of spermatozoa in the genital tract after artificial insemination of progestagen-treated ewes. *J. Reprod. Fert.* **19**, 73–86.
- RAHMAN, S.S. & KITTS, W.D. (1967) Hormonal control of reproduction in lactating and non-lactating anestrus ewes. *Can. J. Anim. Sci.* **47**, 65–69.
- ROBINSON, T.J., SALAMON, S., MOORE, N.W. & SMITH, J.F. (1967) The evaluation of SC-9880-impregnated intravaginal sponges for the synchronisation of oestrus for large scale artificial insemination of Merino ewes in summer and autumn. In *The Control of the Ovarian Cycle in Sheep*, pp. 208–236. Ed. T. J. Robinson. Sydney University Press.
- SMITH, J.F. & ALLISON, A.J. (1971) The effect of exogenous progestagen on the production of cervical mucus in the ewe. *J. Reprod. Fert.* **24**, 279–282.
- STABENFELDT, G.H., HOLT, J.A. & EWING, L.L. (1969) Peripheral plasma progesterone levels during the ovine oestrous cycle. *Endocrinology* **85**, 11–16.
- THORBURN, G.D., BASSETT, J.M. & SMITH, I.D. (1969) Progesterone concentration in the peripheral plasma of sheep during the oestrous cycle. *J. Endocr.* **45**, 459–469.
- WILSON, L., CENEDELLA, R.J., BUTCHER, R.L. & INSKEEP, E.K. (1972) Levels of prostaglandins in the uterine endometrium during the ovine estrous cycle. *J. Anim. Sci.* **34**, 93–99.

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