Response of ewes to a single injection of an analogue of PGF-2 α given at different stages of the oestrous cycle*

Stella Acritopoulou† and W. Haresign‡

Department of Physiology and Environmental Studies and ‡Department of Agriculture and Horticulture, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD, U.K.

Summary. Six groups of 4 ewes were given a single i.m. injection of 100 μ g ICI 80,996 on Days 3, 5, 8, 11, 14 or 16 of the oestrous cycle. On Day 3 treatment was only partly effective in causing luteal regression, with only 2 of the 4 treated ewes returning to oestrus. Injection had no apparent effect when given on Day 16, at a time when luteal regression had already started naturally. However, treatment between Days 5 and 14 inclusive of the oestrous cycle evoked a rapid fall in progesterone concentrations to basal values of <0.5 ng/ml within 24 h with a high degree of synchrony in the return of oestrus (37.7 ± 1.6 h) and the timing of the LH peak (45.3 ± 3.2 h) after injection.

We conclude that the corpus luteum of the ewe is responsive to ICI 80,996 only between Days 5 and 14 of the oestrous cycle.

Introduction

It is now well established that injection of prostaglandin (PG) F-2 α or one of its analogues at any time between Days 5 and 16 of the bovine oestrous cycle will result in luteal regression with a consequent return to oestrus within 2–3 days (Rowson, Tervit & Brand, 1972; Roche, 1974). However, PG treatment has no effect when administered before Day 5 or after Day 16 of the cycle (Louis, Hafs & Seguin, 1973). Consequently, in order to ensure that all cattle respond when treatment starts without reference to the stage of the oestrous cycle it is necessary to give two injections 10–12 days apart. With such a regimen all animals are in the sensitive mid-luteal phase of the cycle at the time of the second injection, and will show synchronized oestrus and ovulation (Hafs, Manns & Lamming, 1975).

A single i.m. injection of 100 μ g of the PGF-2 α analogue, ICI 80,996, given to ewes between Days 5 and 8 of the oestrous cycle, caused rapid luteolysis and resulted in well synchronized behavioural oestrus (Acritopoulou, Haresign, Foster & Lamming, 1977), but response to treatment at other stages of the cycle was not studied. The objective of the present study was to determine the period within the sheep oestrous cycle during which treatment with the PG analogue is effective, with the ultimate aim of developing a suitable double-injection treatment regimen for sheep.

Materials and Methods

The 24 Clun Forest ewes were 3–4 years old and all were showing regular recurrent oestrous cycles. At the height of the breeding season in November, the ewes were randomly allocated to 6

* Reprint requests to Dr W. Haresign.

† Present address: Department of Animal Husbandry, School of Agriculture and Forestry, Aristotelian University of Thessaloniki, Thessaloniki, Greece.

groups of 4 animals, and were given a single i.m. injection of ICI 80,996 on Day 3, 5, 8, 11, 14 or 16 of the oestrous cycle (oestrus = Day 0).

All ewes were housed under conditions of natural daylength and temperature, and a jugular vein cannula was inserted 2 days before the start of the experiment to facilitate collection of frequent blood samples. Blood samples (alternate 10 ml and 2 ml samples) for progesterone and LH or LH determinations alone respectively were collected from 12 h before treatment until 24 h after the onset of the induced oestrus or 72 h after treatment if the ewes did not show oestrus. Thereafter daily blood samples (10 ml) for progesterone determination were collected throughout the ensuing oestrous cycle. All blood samples were centrifuged at 1600 *g* and 4°C for 30 min and the plasma was stored at -20°C until assayed.

Treatment consisted of single i.m. injection of 100 µg ICI 80,996 in 2 ml saline (9 g NaCl/l) on the appropriate day of the oestrous cycle. Tests for oestrus with a vasectomized ram were carried out every hour over the frequent sampling period and all ewes were housed continually with a colour-marked vasectomized ram during the post-treatment cycle.

Plasma progesterone concentrations were measured in the 2 h (10 ml) and daily samples by the radioimmunoassay described by Haresign, Foster, Haynes, Crighton & Lamming (1975). The degree of cross-reaction with other major steroids in sheep plasma was <1%, the sensitivity of the assay was 0.08 ng/ml plasma and the inter- and intra-assay coefficients of variation in this study were both <10%. Plasma LH concentrations were measured in the hourly samples with a specific double-antibody radioimmunoassay described by Foster & Crighton (1974). Cross-reaction with ovine FSH, GH, TSH and prolactin were negligible and the sensitivity of the assay was 0.1 ng NIH-LH-S18 equivalents/ml plasma. Inter- and intra-assay coefficients of variation were both <10%.

Results

Table 1 shows the individual time intervals from injection of ICI 80,996 to the onset of oestrus, together with the characteristics of LH release for all 24 ewes, the mean values for each group and the overall mean values.

Two of the ewes treated on Day 3 of the oestrous cycle (Nos 12 and 17) showed oestrus 29 and 31 h respectively after injection of ICI 80,996, whilst the other 2 ewes (Nos 14 and 15) did not respond to treatment. The plasma progesterone concentration in all 4 ewes in this group at the time of the injection was below 1 ng/ml. Daily peripheral plasma progesterone concentrations of Ewes 14 and 15, which did not respond to treatment, continued to increase after injection, reaching maximum concentrations between Days 12 and 13 and then falling to basal levels of <0.5 ng/ml before the natural oestrus which occurred on Days 16 and 17 respectively. Plasma progesterone concentrations in Ewes 12 and 17 fell to very low levels (<0.5 ng/ml) within 24 h of injection and remained low throughout the induced oestrus. Thereafter, progesterone concentrations followed the pattern typical of a natural oestrous cycle, and a natural oestrus was exhibited 16 and 17 days after the induced oestrus.

Peripheral plasma LH concentrations of Ewes 14 and 15 remained basal (<5 ng/ml) while those of Ewes 12 and 17 became elevated and reached peak values within 24 and 42 h of treatment respectively.

Of the 16 ewes treated between Days 5 and 14 of the cycle only 2 (No. 6, Day 11, and No. 9, Day 14) failed to respond to treatment. Ewe 6 appeared to have a silent heat because luteolysis, as judged by the fall in plasma progesterone concentration, did occur in response to treatment. Thereafter, her progesterone concentration followed the pattern typical of a normal oestrous cycle and the ewe showed oestrus 18 days after treatment. Natural luteolysis had already taken place in Ewe 9 at the time of treatment and this ewe exhibited oestrus only 18 h after injection.

In all ewes responding to treatment plasma progesterone concentrations fell to basal values

Table 1. Characteristics of oestrus, LH release and length of the post-treatment oestrous cycle in ewes treated with 100 μ g ICI 80,996 at different stages of the oestrous cycle

Group	Day of treatment	Ewe	ICI 80,996 injection to:		Onset of oestrus to onset of LH peak (h)	LH peak duration (h)	LH peak height (ng/ml)	Post-treatment oestrous cycle (days)
			Onset of oestrus (h)	Peak LH (h)				
1	3	12	29	24	-9	12	54	16
		14*	—	—	—	—	—	—
		15*	—	—	—	—	—	—
		17	31	42	7	12	102	17
2	5	1	30	30	-3	12	88	15
		2	30	25	-8	11	88	—‡
		11	40	50	7	11	75	16
		16	31	29	-2	10	79	17
Mean ± s.e.m.			32.8 ± 2.4	33.5 ± 5.6	-1.5 ± 3.1	11.0 ± 0.4	82.5 ± 3.3	16.0 ± 0.6
3	8	10	42	48	5	12	160	24
		21	40	40	-3	13	93	17
		22	43	52	6	11	95	18
		23	34	50	10	12	66	—‡
Mean ± s.e.m.			39.8 ± 2.0	47.5 ± 2.6	4.5 ± 2.7	12.0 ± 0.4	103.5 ± 20.0	19.7 ± 2.2
4	11	3	43	58	5	11	63	16
		4	46	65	14	11	55	18
		5	48	65	13	11	158	16
		6*	—	—	—	—	—	—
Mean ± s.e.m.			45.7 ± 1.5	62.7 ± 2.3	10.7 ± 2.8	11.0 ± 0.0	92.0 ± 33.0	16.7 ± 0.7
5	14	7	38	49	4	13	92	16
		9	18†	19†	-3	12	160	17
		18	34	49	11	12	175	17
		24	39	49	4	12	116	18
Mean ± s.e.m.			37.0 ± 1.5	49.0 ± 0.0	4.0 ± 2.9	12.3 ± 0.3	135.8 ± 19.2	17.0 ± 0.4
6	16	8*	—	—	—	—	—	—
		13*	—	—	—	—	—	—
		19	13†	15†	-2	11	93	19
		20	13†	21†	5	11	94	17
Overall mean ± s.e.m. (N)			37.4 ± 1.5 (16)	45.3 ± 3.2 (16)	3.2 ± 1.5 (19)	11.6 ± 0.2 (19)	100.3 ± 8.5 (19)	17.3 ± 0.5 (17)

* Did not show oestrus in response to injection of ICI 80,996 (see text).

† Values eliminated from Mean of the group and the overall Mean (see text).

‡ Did not show overt oestrus at the end of the post-treatment cycle (see text).

(<0.5 ng/ml) by 24 h after injection, and there was a pronounced LH release at the induced oestrus (see Table 1). There was considerable variation between ewes in the size of the LH peak, perhaps related to the collections of blood being only hourly. The changes in plasma progesterone concentration in the post-treatment oestrous cycle of ewes responding to treatment were typical of those recorded for a natural oestrous cycle. One ewe (No. 23, Day 8) became ill during the post-treatment oestrous cycle and data for this animal are therefore incomplete.

Of the 4 ewes treated on Day 16 of the cycle, 2 were already in oestrus when treatment was given (Nos 8 and 13) and in the other 2 (Nos 19 and 20) natural luteolysis had already occurred

when the injection was given and both animals showed oestrus 14 h later. There were no observed endocrine changes in response to the PG injection in ewes which were treated after natural luteolysis had already occurred.

Discussion

These results indicate that a single i.m. injection of ICI 80,996 given between Days 5 and 14 of the oestrous cycle inclusive will induce rapid luteal regression followed by oestrus and ovulation in the ewe. The CL formed following such treatment results in normal plasma progesterone concentrations during the ensuing oestrous cycle, demonstrating absence of any treatment effects on subsequent luteal function. This is in agreement with previous results (Acritopoulou *et al.*, 1977) and with similar findings in cattle (Louis, Hafs & Morrow, 1974). The length of the post-treatment oestrous cycle fell within the normal range expected for this breed of sheep with the exception of Ewe 10, and the reasons for this abnormality are not clear.

By contrast, ICI 80,996 injection on Day 3 was only partly effective in causing luteolysis, and treatment given on Day 16 of the cycle when natural luteal regression had already occurred was without any apparent effect. The variability in the response of ewes to ICI 80,996 treatment on Day 3 of the cycle may be due to error in determination of the stage of the cycle; with once daily detection of oestrus it is only possible to identify the day of oestrus to within 1 day. It is therefore possible that those ewes responding to treatment on Day 3 of the cycle were at a more advanced stage of luteal development than those which failed to respond.

However, the reasons for the absence of oestrus in Ewe 6 treated on Day 11 of the oestrous cycle are not clear. The progesterone profile indicated that this ewe had a silent heat but the LH concentrations were atypical because in the 72 h after PG injection only a small increase up to 20 ng/ml was recorded and there was no pronounced peak. It is possible that this small elevation was sufficient to induce ovulation, although the possibility that an LH peak occurred outside of the frequent sampling period cannot be ruled out.

With the exception of ewes which failed to respond to treatment, well defined LH peaks were observed at the induced oestrus comparable to those recorded during both a natural oestrus and following a single mid-cycle injection of ICI 80,996 (Acritopoulou *et al.*, 1977).

The time intervals from injection of ICI 80,996 to onset of oestrus and the LH peak in the 6 groups (see Table 1) would appear to be longer for the groups treated at mid-cycle (i.e. Days 8 or 11) than in those treated early (Days 3 or 5) or late (Day 14) in the oestrous cycle. However, the small group sizes preclude firm conclusions being drawn from the limited data available.

The overall mean interval of 37.7 ± 1.6 h between injection of ICI 80,996 and onset of oestrus recorded in this study was significantly ($P < 0.05$) shorter than that of 44.0 ± 1.9 h observed in a previous study (Acritopoulou *et al.*, 1977). However, no significant difference could be demonstrated between the two studies in the timing of the LH peak in relation to injection of ICI 80,996 (45.3 ± 3.2 h and 48.5 ± 2.0 h respectively). The apparent discrepancy between trials in these two factors may be associated with the problems of accurate detection of the onset of oestrus, and LH release would appear to be a more reliable index of synchronization than onset of oestrus.

It is clear from these data that a single injection of the prostaglandin analogue given to a randomly cycling flock of ewes during the breeding season will result in synchronization of ovulation in about 66% of them. To ensure that all ewes respond to treatment, the two PG injections must be given 9–10 days apart so that all ewes will be in the sensitive stage of the cycle at the time of the second injection, irrespective of stage of the cycle at the time of the first injection.

We thank the Meat and Livestock Commission for financial support and I.C.I. Ltd for supplies of ICI 80,996. The work was carried out whilst S.A. was in receipt of a Scholarship for postgraduate study from the Republic of Greece State Scholarship Foundation.

References

- Acritopoulou, S., Haresign, W., Foster, J.P. & Lamming, G.E. (1977) Plasma progesterone and LH concentrations in ewes after injection of an analogue of prostaglandin F-2 α . *J. Reprod. Fert.* **49**, 337–340.
- Foster, J. P. & Crighton, D.B. (1974) Luteinizing hormone (LH) release after single injections of a synthetic LH-releasing hormone (LH-RH) at three different reproductive stages and comparison with the natural LH release at oestrus. *Theriogenology* **2**, 87–100.
- Hafs, H.D., Manns, J.G. & Lamming, G.E. (1975) Fertility of cattle from AI after PGF-2 α . *J. Anim. Sci.* **41**, 355.
- 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.
- Louis, T.M., Hafs, H.D. & Seguin, B.E. (1973) Progesterone, LH, estrus and ovulation after prostaglandin F-2 α in heifers. *Proc. Soc. exp. Biol. Med.* **143**, 152–155.
- Louis, T.M., Hafs, H.D. & Morrow, D.A. (1974) Intra-uterine administration of prostaglandin F-2 α in cows: progesterone, estrogen, LH, estrus and ovulation. *J. Anim. Sci.* **38**, 347–353.
- Roche, J.F. (1974) Synchronization of oestrus and fertility following artificial insemination in heifers given PGF-2 α . *J. Reprod. Fert.* **37**, 135–138.
- Rowson, L.E.A., Tervit, R. & Brand, A. (1972) The use of prostaglandins for synchronization of oestrus in cattle. *J. Reprod. Fert.* **29**, 145.

Received 14 May 1979