Seasonal variation in oestrus, the secretion of oestrogen and progesterone and LH levels before ovulation in the ewe

A. G. Wheeler*, D. T. Baird†, R. B. Land and R. J. Scaramuzzi†‡

A.R.C. Animal Breeding Research Organisation, The King's Buildings, West Mains Road, Edinburgh EH9 3JQ, and †M.R.C. Unit of Reproductive Biology, Department of Obstetrics and Gynaecology, University of Edinburgh, 39 Chalmers Street, Edinburgh EH3 9ER, U.K.

Summary. Ewes with cervical ovarian autotransplants were studied after PGF-2 α -induced luteal regression in November and February (mid- and late-breeding season) and June (anoestrus). Progesterone and oestradiol-17 β secretion rates and LH concentrations were determined in serial ovarian venous blood samples and the ewes were frequently tested for oestrus. All 4 ewes in November and 3 of the 4 ewes in February exhibited oestrus and endocrine changes indicative of ovulation. The remaining ewe in February and the three ewes in June failed to show elevated oestradiol-17 β secretion rates after luteal regression, indicating the absence of follicles in the final stages of maturation. The preovulatory rise in the oestradiol-17 β secretion rate, the LH surge and the display of oestrus all occurred earlier, with respect to the PGF-2 α infusion, in November than in February, suggesting a greater stimulation of folliculogenesis, and therefore a greater availability of maturing follicles, in November than in February.

Introduction

The ewe exhibits marked variation in reproductive activity during the year, with loss of ovarian cyclicity during the summer anoestrus. Even within the breeding season there are variations in ovulation rate (Radford, 1959), duration of oestrus (McKenzie & Terrill, 1937) and oestrous cycle length (McKenzie & Terrill, 1937), suggesting that subtle changes in the hypothalamic–pituitary–ovarian axis probably occur continuously throughout the year. Although ovulation rates are also related to differences in genotype (Thimonier & Mauléon, 1969) and plane of nutrition (McKenzie & Terrill, 1937), very little is known about the physiological mechanisms involved. It has been suggested that the higher ovulation rates in certain breeds of sheep, e.g. Finnish Landrace, are associated with a relative insensitivity of the hypothalamic–pituitary system to the negative feedback effects of oestradiol (Land, Wheeler & Carr, 1976).

The present experiments were designed to investigate the variation in the endocrine changes associated with ovulation at three different times of the year in Finnish Landrace $\times$ Tasmanian Merino crossbreeds.

Materials and Methods

Four Finnish Landrace $\times$ Tasmanian Merino ewes (aged about 2½ or 3½ years in November 1972) with the left ovary autotransplanted to the neck (Goding, McCracken & Baird, 1967b) were allocated to this experiment. They were housed at the Animal Breeding Research Organisation, Dryden Field Laboratory, Roslin, Midlothian, under natural lighting conditions. Relocation of the ovary in the neck results in persistence of the corpus luteum, which fails to regress due to the absence of an

* Present address: Department of Anatomy, The Medical School, University of Birmingham, Birmingham B15 2TJ, U.K.

‡ Present address: C.S.I.R.O. Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, Australia.

adjacent uterine horn (Goding, Harrison, Heap & Linzell, 1967a), for up to 200 days before regression and ovulation occur spontaneously (Baird, Land, Scaramuzzi & Wheeler, 1976b). The endocrine events around ovulation were studied following the regression of the corpus luteum induced by the infusion of prostaglandin (PG) F-2 α via the ovarian artery (McCracken, Glew & Scaramuzzi, 1970).

Each ewe was studied on three occasions (November 1972, February 1973 and June 1973) which were chosen to represent the middle and end of the breeding season and anoestrus, as expected from the patterns of oestrous activity of the parent breeds (Wheeler & Land, 1977). The corpora lutea which were induced to regress in November were presumably those formed spontaneously at the first ovulation of the breeding season (about August–September), those regressed in February and June were presumably those formed from ovulations (after the previous induced regression) in November and February respectively. The experimental design was identical for each animal on the three occasions.

At the beginning of the experiment the ovarian artery and vein were cannulated via the right carotid artery and jugular vein (Collett, Land & Baird, 1973). Timed samples of ovarian venous blood (25 ml) were collected as previously described (Goding *et al.*, 1967b). Four control samples were collected at about 15-min intervals before the intra-arterial infusion of PGF-2 α , involving the use of a pressure cuff round the carotid, was started at the rate of 40 μ g/h for 4 h (Chamley *et al.*, 1972). Four further collections were made approximately every 15 min after the infusion to assess the acute effects of the infusion of PGF-2 α . From about 24 h after the start of PGF-2 α infusion until about 35 h after the onset of oestrus or, in the absence of oestrus, 80–100 h after the infusion, samples were collected at 3-h intervals and the ewe was tested for oestrus with a vasectomized ram.

The samples of blood were centrifuged at 1800 *g* at 4°C for 20 min and the plasma aspirated and stored at –20°C until analysis. The secretion rates of oestradiol-17 β and progesterone were calculated from a knowledge of plasma concentrations, haematocrit values and ovarian venous blood flows (Collett *et al.*, 1973); the haematocrits were determined at intervals of 9 h and the values for intermediate blood samples were calculated by interpolation.

Progesterone and oestradiol-17 β concentrations were measured by radioimmunoassays which have been validated for sheep ovarian venous blood (Scaramuzzi, Corker, Young & Baird, 1975). The progesterone assay had a mean minimum detectable dose of 0.214 ng/ml and a between-assay coefficient of variation of 14.2%. The oestradiol-17 β assay had a mean minimum detectable dose of less than 10 pg/ml and within- and between-assay coefficients of variation of 9.3 and 14.8% respectively. LH was measured by a double-antibody radioimmunoassay and expressed in ng NIH-LH-S17/ml (Carr & Land, 1975).

Differences between times of the year were assessed for statistical significance with Student's *t* test.

Results

The mean secretion of progesterone in the four control samples collected before infusion was > 0.75 μ g/min on each occasion (Table 1), indicating the presence of at least one corpus luteum (McCracken & Baird, 1969). The control secretion rates of progesterone (7.19 ± 1.19 (S.E.M.) μ g/min) and oestradiol-17 β (0.885 ± 0.217 ng/min) were similar at the three times of the year, indicating luteal and follicular activity before each induced luteal regression.

Luteal regression was induced on all occasions by the infusion of PGF-2 α . On all three occasions the values had reached <1 μ g/min by 24 h (see Text-fig. 1). After the infusion of PGF-2 α there was an immediate increase in the concentration of LH from a basal value of 1.24 ± 0.13 to 2.19 ± 0.29 ng/ml in the samples collected within 1 h of the completion of infusion ($P < 0.01$). During the next 24 h, before the LH peak, the basal value remained elevated at 39.4 ± 1.91 h (2.10 ± 0.19 ng/ml, $P < 0.001$). There were significant differences between occasions in the pattern of oestradiol-17 β secretion and LH concentration which followed luteal regression in November and February.

In November, the decline in progesterone secretion was accompanied by a rise in the secretion of oestradiol which, while variable and erratic, rose from a basal value of 1.54 ± 0.43 ng/min to reach a maximum value of 10.53 ± 2.19 ng/min. The concentration of LH rose significantly above the baseline

Table 1. Secretion rates of progesterone and oestradiol-17 β and the LH concentrations in the ewes before the infusion of PGF-2 α

Month	Ewe	Progesterone secretion rate ($\mu\text{g}/\text{min}$)	Oestradiol-17 β secretion rate (ng/min)	LH conc. (ng/ml)
November	9U6	6.426 $\pm$ 1.441	1.723 $\pm$ 0.586	1.673 $\pm$ 0.183
	OU57	9.611 $\pm$ 0.646	1.457 $\pm$ 0.179	1.175 $\pm$ 0.038
	OU75	5.794 $\pm$ 0.364	0.448 $\pm$ 0.029	1.163 $\pm$ 0.098
	OU77	4.110 $\pm$ 0.273	2.528 $\pm$ 0.561	1.853 $\pm$ 0.179
February	9U6	13.838 $\pm$ 0.682	0.590 $\pm$ 0.076	0.650 $\pm$ 0.034
	OU57	10.649 $\pm$ 2.392	0.463 $\pm$ 0.116	0.833 $\pm$ 0.010
	OU75	7.050 $\pm$ 0.528	0.595 $\pm$ 0.152	1.173 $\pm$ 0.027
	OU77	10.834 $\pm$ 1.819	0.121 $\pm$ 0.025	1.088 $\pm$ 0.033
June	9U6	8.041 $\pm$ 2.138	0.352 $\pm$ 0.023	0.568 $\pm$ 0.126
	OU75	1.489 $\pm$ 0.417	1.024 $\pm$ 0.275	1.523 $\pm$ 0.038
	OU77	0.808 $\pm$ 0.043	0.713 $\pm$ 0.106	1.573 $\pm$ 0.064

Each value is the mean $\pm$ S.E.M. of the four preinfusion blood samples.

Text-fig. 1. Changes in the LH concentration (■—), progesterone (●·····) and oestradiol-17 β (○—) secretion rates and the duration of oestrus (bar) after luteal regression induced by PGF-2 α (arrows) in (a) November (mid-breeding season), (b) February (late breeding season) and (c) June (mid-anoestrum) in one Finnish Landrace $\times$ Tasmanian Merino ewe with an ovarian autotransplant.

Table 2. Endocrine changes, time intervals between, and durations of endocrinological features and the characteristics of oestrus in various ewes after luteolysis induced by PGF-2 α in November and February

Month	Ewe	Interval (h) from start of infusion to:						Maximum LH secretion rate (ng/min)
		Maximum oestrogen secretion	Onset of LH peak	Maximum LH conc.	Onset of oestrus	End of oestrus	Duration of LH peak (h)	
November	9U6	49.2	45.8	48.8	35.5	74.5	11.9	9.30
	OU57	38.8	41.0	44.6	32.0	69.7	15.2	13.85
	OU75	48.1	47.0	52.8	38.1	69.3	14.8	7.84
	OU77	46.5	43.5	52.0	35.6	68.3	17.2	14.61
	Mean	45.7 $\pm$ 2.3	44.3 $\pm$ 1.3	49.6 $\pm$ 1.9	35.3 $\pm$ 1.3	70.5 $\pm$ 1.4	14.8 $\pm$ 1.1	11.40 $\pm$ 1.67
February	9U6	60.4	54.5	67.5	53.7	94.4	5.7	9.17
	OU57	56.1	56.3	59.0	42.8	81.7	11.9	9.11
	OU75	43.5	52.6	58.4	42.0	87.7	11.8	6.03
	Mean	53.3 $\pm$ 5.1	54.5 $\pm$ 1.1	61.6 $\pm$ 2.9	46.2 $\pm$ 3.8	87.9 $\pm$ 3.7	9.8 $\pm$ 2.1	8.10 $\pm$ 1.04
Student's <i>t</i>		1.51	5.62	3.66	3.12	5.03	2.32	1.53
<i>P</i>		N.S.	<0.01	<0.02	<0.05	<0.01	N.S.	N.S.

at about the time of the maximum oestradiol-17 β secretion rate and reached a peak 6 h later (Table 2). The manifestation of oestrus and these endocrine changes were taken as presumptive evidence of ovulation.

In February, 3 of the 4 ewes showed behavioural and endocrine changes that were characteristic of ovulation. The time of onset of oestrus and of the LH surge, of maximum secretion of oestradiol-17 β and of LH all occurred significantly later in February than in November (Table 2). Although the maximum rate of oestradiol-17 β secretion was less in February (8.10 ± 1.04 ng/min) than in November (10.53 ± 2.18 ng/min), the difference was not statistically significant ($P > 0.05$). The duration of the LH surge and the maximum height of the peak showed a similar trend, but again the differences were not statistically significant. The remaining ewe failed to show oestrus after PGF-2 α -induced luteolysis: the secretion of oestradiol-17 β remained low and there was no LH peak. It seems reasonable to assume that terminal development of a Graafian follicle and ovulation did not occur.

In the three ewes studied during anoestrus in June 1973, regression of the corpus luteum occurred as on previous occasions, but none of the ewes subsequently showed oestrus or the endocrine changes suggestive of follicle maturation and ovulation.

Discussion

The changes in the secretion of progesterone and oestradiol and the concentration of LH on the seven occasions when luteal regression was considered to have been followed by ovulation are similar to those described following luteal regression induced in normal animals with PGF-2 α (Barrett *et al.*, 1971; Chamley *et al.*, 1972) or with a synthetic analogue (Baird & Scaramuzzi, 1976). In the ewe, progesterone and oestradiol-17 β are derived exclusively from the corpus luteum and Graafian follicle(s) respectively so that their secretion reflects the activity of these ovarian compartments (Baird & Scaramuzzi, 1976). During the luteal phase in the ewe follicular development is reflected by the secretion of oestradiol-17 β which fluctuates up to values of about 3 ng/min (Baird *et al.*, 1976a). The Graafian follicle is extremely sensitive to minor changes in the basal concentration of LH (Baird, Swanston & Scaramuzzi, 1976c) and the observed variation in the secretion of oestradiol-17 β from one collection to the next probably reflects the response to small pulses of LH.

In November, the preovulatory rise of oestradiol-17 β , the onset of oestrus, the LH discharge and the presumed ovulation all occurred more quickly following luteal regression than later in the breeding season. The time taken for these events will presumably depend on the availability of Graafian follicle(s) at a stage of development suitable for preovulatory maturation. The greater the number of developing antral follicles, the greater the chance of one or more being ready to respond immediately to the rise of LH associated with the fall of progesterone. Thus, the advancement of these endocrine events together with the increased ovulation rate (Wheeler & Land, 1977) are indicative of a relatively greater level of folliculogenesis in the middle than at the end of the breeding season.

The mechanisms responsible for this change in follicular activity are probably related to a change in the control of gonadotrophin secretion. In castrated ewes, the interval between injection of oestradiol-17 β benzoate and the beginning of the LH discharge is independent of the time of year (Land *et al.*, 1976), and the earlier discharge of LH in November may therefore simply be related to the earlier rise in oestrogen secretion. The lack of a preovulatory rise of oestradiol-17 β and LH in June presumably represents a failure of the hypothalamic-pituitary system to respond to withdrawal of luteal progesterone by secretion of FSH and LH in amounts sufficient to support the final maturation of the Graafian follicle. There may also be quantitative changes in sensitivity of the hypothalamic-pituitary system to both the negative (Legan, Hauger, Foster & Karsch, 1976) and positive (Land *et al.*, 1976) feedback effects of oestrogen.

It is unlikely that the differences in endocrine relationships between times of the year were a result of differences in length of exposure to progesterone because the corpora lutea were probably all of a similar age (90–110 days). The fact that the secretion of progesterone and oestradiol-17 β during the control periods were similar on each occasion support this view.

The significant rise in the basal concentration of LH following the infusion of PGF-2 α is due to the coincidental fall in the concentration of progesterone (Baird & Scaramuzzi, 1976). In most

species progesterone influences the secretion of LH by enhancing the negative feedback effect of oestrogen (Harris & Naftolin, 1970), although in the ewe lamb progesterone alone appears to inhibit the secretion of LH (Foster & Karsch, 1976). In the adult ewe the concentration of LH declines progressively as the secretion of progesterone increases throughout the luteal phase of the cycle (Land, Pelletier, Thimonier & Mauléon, 1973). Whether progesterone can inhibit LH in the absence of oestrogen in the adult ewe remains to be determined.

We are grateful to Marjorie Fordyce, W. G. Davidson and R. D. Preece for assistance with the surgery and the collection of samples. The LH assays were performed in Mr W. R. Carr's laboratory with equine (anti-bovine LH) antiserum provided by Dr R. B. Snook and purified ovine LH provided by Dr M. Jutisz. Mr I. Swanston assisted with the oestradiol-17 β assays. A.G.W. is grateful to the A.R.C. for a post-graduate studentship.

References

- BAIRD, D.T. & SCARAMUZZI, R.J. (1976) Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the pre-ovulatory period in the ewe: the effect of progesterone. *J. Endocr.* **70**, 237–245.
- BAIRD, D.T., LAND, R.B., SCARAMUZZI, R.J. & WHEELER, A.G. (1976a) Endocrine changes associated with luteal regression in the ewe: the secretion of ovarian oestradiol, progesterone and androstenedione and uterine prostaglandin F_{2 α} throughout the oestrous cycle. *J. Endocr.* **69**, 275–286.
- BAIRD, D.T., LAND, R.B., SCARAMUZZI, R.J. & WHEELER, A.G. (1976b) Functional assessment of the auto-transplanted uterus and ovary in the ewe. *Proc. R. Soc. B* **192**, 463–474.
- BAIRD, D.T., SWANSTON, I. & SCARAMUZZI, R.J. (1976c) Pulsatile release of LH and secretion of ovarian steroids in sheep during the luteal phase of the estrous cycle. *Endocrinology* **98**, 1490–1496.
- BARRETT, S., BLOCKEY, M.A. DEB., BROWN, J.M., CUMMING, I.A., GODING, J.R., MOLE, B.J. & OBST, J.M. (1971) Initiation of the oestrous cycle in the ewe by infusions of prostaglandin F_{2 α} to the autotransplanted ovary. *J. Reprod. Fert.* **24**, 136–137.
- CARR, W.R. & LAND, R.B. (1975) Plasma luteinizing hormone levels and testis diameters of ram lambs of different breeds. *J. Reprod. Fert.* **42**, 325–333.
- 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 α} on progesterone, oestradiol and luteinizing hormone secretion in sheep with ovarian transplants. *J. Endocr.* **55**, 253–263.
- COLLETT, R.A., LAND, R.B. & BAIRD, D.T. (1973) The pattern of progesterone secretion by the auto-transplanted ovary of the ewe in response to ovine luteinizing hormone. *J. Endocr.* **56**, 403–411.
- FOSTER, D.L. & KARSCH, F.J. (1976) Inhibition of tonic secretion of luteinizing hormone by progesterone in immature female sheep. *Endocrinology* **99**, 1–6.
- GODING, J.R., HARRISON, F.A., HEAP, R.B. & LINZELL, J.L. (1967a) Ovarian activity in the ewe after auto-transplantation of the ovary or uterus to the neck. *J. Physiol., Lond.* **191**, 129–130P.
- GODING, J.R., MCCracken, J.A. & BAIRD, D.T. (1967b) The study of ovarian function in the ewe by means of a vascular autotransplantation technique. *J. Endocr.* **39**, 37–52.
- HARRIS, G.W. & NAFTOLIN, F. (1970) The hypothalamus and control of ovulation. *Br. med. Bull.* **26**, 3–9.
- LAND, R.B., PELLETIER, J., THIMONIER, J. & MAULÉON, P. (1973) A quantitative study of genetic differences in the incidence of oestrus, ovulation and plasma luteinizing hormone concentration in the sheep. *J. Endocr.* **58**, 305–317.
- LAND, R.B., WHEELER, A.G. & CARR, W.R. (1976) Seasonal variation in the oestrogen induced LH discharge of ovariectomised Finnish Landrace and Scottish Blackface ewes. *Annls Biol. anim. Biochim. Biophys.* **16**, 521–528.
- LEGAN, S.J., HAUGER, R.L., FOSTER, D.L. & KARSCH, F.J. (1976) Marked seasonal variation in the mechanism controlling tonic LH secretion in ewes. *Fedn Proc. Fedn Am. Socs exp. Biol.* **35**, 615.
- MCCracken, J.A. & BAIRD, D.T. (1969) The study of ovarian function by means of transplantation of the ovary in the ewe. In *The Gonads*, pp. 175–209. Ed. K.W. McKerns. Appleton-Century-Crofts, New York.
- MCCracken, J.A., GLEW, M.E. & SCARAMUZZI, R.J. (1970) Corpus luteum regression induced by prostaglandin F_{2 α} . *J. clin. Endocr. Metab.* **30**, 544–546.
- McKENZIE, F.F. & TERRILL, C.E. (1937) Estrus, ovulation and related phenomena in the ewe. *Bull. Univ. Mo. agric. Exp. Stn* No. 264.
- RADFORD, H.M. (1959) Variation in the incidence of twin ovulation in Merino ewes on a constant plane of nutrition. *Aust. J. agric. Res.* **10**, 377–386.
- SCARAMUZZI, R.J., CORKER, C.S., YOUNG, G. & BAIRD, D.T. (1975) Production of antisera to steroid hormones in sheep. In *Steroid Immunoassay*, pp. 111–122. Eds R. H. D. Cameron, S. G. Hillier & K. Griffiths. Alpha Omega Alpha, Cardiff.
- THIMONIER, J. & MAULÉON, P. (1969) Variations saisonnières du comportement d'oestrus et des activités ovariennes et hypophysaires chez les ovins. *Annls Biol. anim. Biochim. Biophys.* **9**, 233–250.
- WHEELER, A.G. & LAND, R.B. (1977) Seasonal variation in oestrus and ovarian activity of Finnish Landrace, Tasmanian Merino and Scottish Blackface ewes. *Anim. Prod.* **24**, 363–376.