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Season and Photoperiod Effects on Follicles and Atresia in the Sheep Ovary

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Abstract

Since follicles that ovulate during the breeding season in sheep begin their growth and development during the anoestrous season, the ovarian follicular population was investigated in late oestrus, mid-anoestrus and early oestrus. The effects of photoperiod (8 h light per day) and 2-bromo-α-ergocryptine (2 mg per day per sheep) throughout the anoestrous season on the follicular population were also studied.

The incidence of atresia of antral follicles was clearly associated with photoperiod such that in ewes that had experienced a period of short days there was a high incidence of atresia and vice versa. In addition those ewes investigated late in the oestrous season had a high incidence of follicular atresia but the number of non-atretic antral follicles was still elevated. These results suggest that the 'trigger' for ewes to go into or come out of anoestrus at the ovarian level is a change in the incidence of atresia.

The 2-bromo-α-ergocryptine treatment caused a significant reduction in the number of small pre-antral follicles entering the growth phase but had no effect on the larger follicles, which suggests that prolactin may be implicated in the initiation of follicular growth.

Introduction

Sheep in temperate latitudes are seasonal breeders with well-defined breeding and anoestrous seasons. The follicle that ovulates during the breeding season probably began its growth and development during the anoestrous period because the duration of follicular growth in the ewe has been estimated to be approximately 6 months (Cahill and Mauleon 1980). The pattern of development of follicles in the sheep throughout the anoestrous season remains unknown.

One hormone that varies closely with photoperiod in sheep is prolactin; during short-days peripheral concentrations are low and during long-days they are high (Thimonier *et al.* 1978). Specific binding sites for prolactin have been demonstrated on granulosa cells and corpora lutea (CL) in the pig (Rolland and Hammond 1975) and sheep (Salamonsen *et al.* 1982). Prolactin is implicated at all stages of folliculogenesis, as it has been found in the oocytes of primordial follicles in the rat (Nolin 1978) and in sheep its concentration is specifically lower in follicular fluid of large antral follicles than in plasma (McNatty *et al.* 1981). Furthermore, prolactin has been shown to be involved in follicular steroidogenesis (McNatty *et al.* 1974). The prolactin concentration during pro-oestrus in December (Northern Hemisphere, short-days) is correlated with the number of follicles in various classes, particularly with the number of pre-antral follicles (Cahill *et al.* 1981). The effects of prolactin and its seasonal fluctuations on the evolution of follicles from the primordial through to the pre-ovulatory stage remains unknown.

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The aim of the present study was to investigate the follicular population throughout the anoestrous season and examine how the population was influenced by treatments which are known to decrease the normal prolactin secretion, viz. short photoperiod and 2-bromo-α-ergocryptine.

Materials and Methods

During January 1979, 25 Ile-de-France ewes at Nouzilly, France, were divided at random into five groups (groups 1–5) each of five ewes. All ewes were primiparous, between $1\frac{1}{2}$ and 3 years old, in good condition and approximately 50–60 kg in liveweight. Throughout the experiment the ewes were housed indoors where the conditions of temperature and humidity were similar to those outdoors.

The five ewes in group 1 were ovariectomized in January to define the ovarian state at the beginning of the experiment which was at the end of the breeding season for this breed of ewe at Nouzilly (Table 1). From January until September, ewes in groups 2, 3 and 4 were housed together in a room and were subjected to the natural photoperiod experienced at Nouzilly (lat. 47°N.). Group 2 ewes underwent ovariectomy in April (mid-anoestrus) and ewes in groups 3 and 4 underwent ovariectomy in September (early breeding season). Group 4 ewes also received 2 mg/day of 2-bromo-α-ergocryptine (CB 154) dissolved in 2 ml of a mixture of tartaric acid and 70% (v/v) ethanol, from January until ovariectomy. Group 5 ewes were housed in a separate room and experienced 8 h light per day from January until September when they also underwent ovariectomy.

Ewes in groups 2, 3, 4 and 5 underwent endoscopy in February and every 14–16 days thereafter until mid-April when group 2 ewes underwent ovariectomy. Endoscopies recommenced on 12 July 1979 and continued every 14–16 days until ovariectomy for ewes in groups 3, 4 and 5. At endoscopy the numbers of CL or corpora albicantia were recorded.

Twice weekly blood sampling commenced on 6 February and continued until the ewes underwent ovariectomy. At each sampling 5 ml of blood was taken by puncture of the jugular vein, centrifuged and the plasma collected and stored at −15°C until assayed. All samples were assayed for prolactin in a single assay according to the method described by Kann (1971) and the results of the assay are expressed as nanograms of NIH-P-S6 per millilitre; the sensitivity of the assay is 0·3 ng/ml and coefficient of variation of a reference sample of 6·28 ng/ml assayed in duplicate every 100 sample tubes in the present assay was 9%. Blood samples collected between 16 March and 12 July 1979 were assayed for progesterone by a rapid-assay technique (Terqui and Thimonier 1974) to determine whether the plasma concentration was greater or less than 1 ng/ml. Ovarian activity in ewes was defined as cyclic if there was a CL on the ovary at endoscopy or three consecutive blood samples had a plasma progesterone concentration of more than 1 ng/ml.

At ovariectomy the left ovary of each ewe was fixed in Bouin–Hollande's solution, serially sectioned at a thickness of 7 μm, mounted, stained with Feulgen's stain and inspected microscopically. All follicles with three or more layers of granulosa cells (approx. diam. 0·60 mm) were counted and measured on the section where the nucleolus of the nucleus of the oocyte was observed (Mariana *et al.* 1980) and then classified according to size, as previously described by Cahill *et al.* (1979). A follicle was defined as atretic when four or more pycnotic bodies were present on the section that was measured. This holds true for antral and pre-antral follicles (Cahill, unpublished data). Follicles where the oocyte was not present were not included in the analysis. Antrum formation was defined as commencing at a follicular diameter of 0·240 mm (Cahill *et al.* 1979). Follicle dimensions were taken from fixed ovaries and it was assumed that the follicles were spherical in shape.

Statistical Analyses

Comparisons of the numbers of atretic and non-atretic follicles and mean prolactin levels were made using analysis of variance. Linear regressions were used to test the correlations between prolactin concentrations and the follicular population.

Results

Cyclic Ovarian Activity

By 16 February, 9 of the 20 ewes in groups 2, 3, 4 and 5 had ceased cyclic ovarian activity, and by 13 April all of these were in anoestrus with no CL present. On 27 July, 4 of the 10 ewes in groups 3 and 4 had begun cyclic ovarian activity and by 7 September

all of these ewes had ovulated (Table 1). Group 5 ewes, which had 8 h light per day recommenced cyclic activity at the same time as groups 3 and 4 but had a very short period of cyclic ovarian activity as all ewes in this group had ovulated by 10 August but returned to a non-cyclic state by 7 September.

Table 1. Ovarian data at ovariectomy and mean prolactin concentrations from January until ovariectomy of the five ewes in treatment groups

Group	Treatment	Ovariectomy				Mean prolactin concn (ng/ml)
		Date	Season	No. ewes ovulating	Ovulation rate	
1	—	25 Jan.	Late oestrus	5	1·40	—
2	—	13 Apr.	Mid-anoestrus	0	—	77±24
3	—	14 Sept.	Early oestrus	5	1·40	206±26
4	Bromocriptine	14 Sept.	Early oestrus	4	1·50	1·0±0·2
5	Short days	14 Sept.	—[A]	0	—	46±6

[A] Ewes in group 5 were no longer 'seasonal' due to treatment imposed and were not showing cyclic activity at this time.

Follicular Populations

The mean number (±s.e.) of non-atretic follicles with three or more layers of granulosa cells per ovary was 145·4±7·2, 143·0±15·6, 181·6±29·7, 124·0±23·8 and 162·4±30·6 in groups 1, 2, 3, 4 and 5 respectively and these did not differ significantly.

Table 2. Mean numbers ±s.e. of follicles per ovary of five ewes ovariectomized in January (group 1), April (group 2) and September (groups 3, 4 and 5—group 3, normal photoperiod; group 4, 2 mg bromocriptine per day; and group 5, 8 h light per day from January to September)

Within columns, different superscripts a–d denote significant differences at $P<0·01$ and different superscripts e–f denote significant differences at $P<0·05$

Group	No. of pre-antral follicles		No. of antral follicles		
	Total	<0·07 mm diam.	Total non-atretic	Non-atretic >2 mm	No. atretic
1	89·0±4·3	11·8±3·2[a]	56·4±11·0	1·4±0·2[e]	10·2±1·5[d]
2	91·8±17·3	9·2±1·9[a]	51·2±4·0	0·6±0·4[f]	11·6±1·3[d]
3	121·0±23·5	31·0±6·7[b]	60·6±8·4	2·2±0·4[e]	6·2±1·0[e]
4	70·6±10·5	19·0±1·0[d]	53·4±15·3	1·2±0·4[e]	3·8±1·1[e]
5	95·2±21·6	25·2±6·6[b]	67·2±14·1	0·4±0·4[f]	15·8±2·8[d]

Pre-antral follicles

The mean number of pre-antral follicles tended to increase after mid-anoestrus (Table 2); this increase was due mainly to more follicles entering the growth phase as shown by the increase in the number of small pre-antral follicles of less than 0·070 mm diameter. Bromocriptine also caused a large decrease in the number of follicles entering the growth phase as indicated by the decrease in the mean number of pre-antral follicles of less than 0·070 mm diameter in group 4 compared to group 3. Group 5 ewes were not significantly different from ewes in group 3, in the number of pre-antral follicles of any size.

Antral follicles (non-atretic)

The number of antral follicles per ovary varied greatly, from 34 to 110. Unlike the pre-antral follicles there were no significant differences between any groups in the mean number of antral follicles (Table 2). The mean number of large antral follicles (>2·0 mm diam.) was significantly lower ($P<0·05$) in groups 2 and 5, where the ewes were in anoestrus at the time of slaughter, than in groups 1, 3 and 4 where the ewes were undergoing cyclic ovarian activity.

Atresia

Atresia as defined was observed to occur in antral follicles only. Both the mean number of atretic follicles and the proportion of antral follicles that were atretic did not differ between January and April (groups 1 and 2) but were significantly ($P<0·01$) decreased in September (group 3; Table 2). Ovaries from ewes treated with bromocriptine (group 4) also had a significantly lower ($P<0·01$) number and proportion of atretic follicles compared with groups 1, 2 and 5, but not with group 3. There was a highly significant increase ($P<0·01$) in the number and proportion of atretic follicles in group 5, ewes receiving 8 h light per day, compared with group 3 ewes receiving normal light. Between groups 1 and 5, which were both experiencing short-days, there was no significant difference in the number or proportion of atretic follicles.

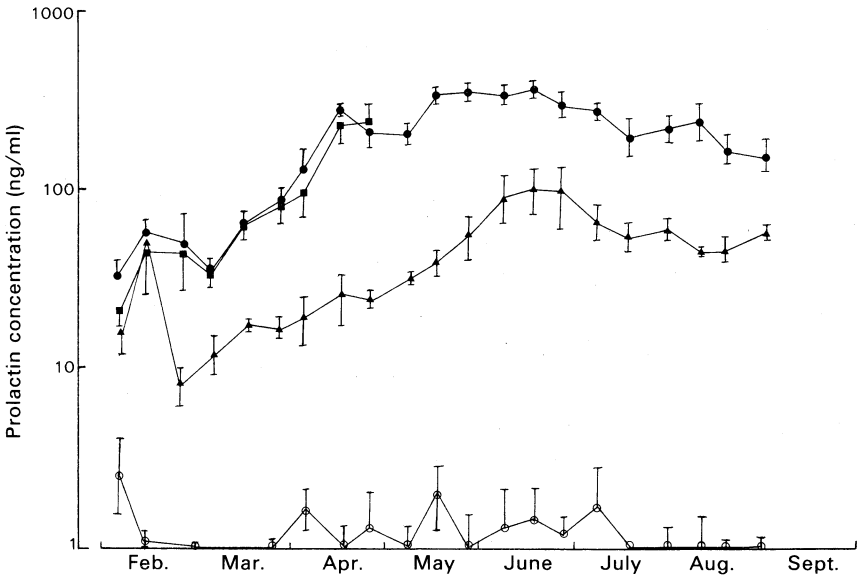

Fig. 1. Mean prolactin concentration per ewe in groups 2, 3, 4 and 5. ■ Group 2, normal photoperiod, ovariectomized in April. ● Group 3, normal photoperiod, ovariectomized in September. ○ Group 4, normal photoperiod and 2 mg bromocriptine per day. ▲ Group 5, 8 h light per day.

Prolactin Concentrations

The prolactin profiles were very different (Fig. 1) between groups 2, 3, 4 and 5 and the mean concentrations per ewe per group over the experimental period were significantly different (Table 1).

There were no significant linear correlations, even after pooling results from groups 2, 3, 4 and 5, between the number of follicles less than 0·070, 0·090 and 0·240 mm in diameter and the mean concentration of prolactin, 1 week, 1 month and 2 months

prior to slaughter. The only two ewes within group 4 that had no detectable prolactin also had far fewer pre-antral follicles (40 and 52 follicles) than the other three ewes which had 0·5–5 ng/ml prolactin (80, 87 and 94 follicles; $P<0·01$).

Discussion

The important features of this study concern firstly, the relationship between photoperiod and the incidence of atresia and secondly, the reduction in the number of pre-antral follicles following bromocriptine treatment.

The incidence of atresia was clearly associated with photoperiod such that after a period of long days (groups 3 and 4) there was a marked reduction in the incidence of atresia, whilst after experiencing a period of short days (groups 1, 2 and 5, ovariectomized in January and April and after 8 h light per day) ewes had higher levels of atresia. A seasonal influence on the incidence of atresia has previously been demonstrated in the cow (Rajakoski 1960). Furthermore, the incidence of atresia and also the number of large non-atretic follicles appeared to be associated with the cyclic ovarian activity of the ewe. Non-cyclic ewes which had no functional CL (groups 2 and 5) had few large antral follicles and a high incidence of atresia. These results suggest that the 'trigger' at the ovarian level which determines that a ewe goes into anoestrus is firstly an increase in the incidence of atresia, and the ewe remains in anoestrus owing to this high level of atresia. The ewes in group 1 in the present study were on their last or second-last cycle before anoestrus and already had a high incidence of atresia but still had quite a few large antral follicles (>2·0 mm diam.). However, by mid-anoestrus (group 2) the incidence of atresia, which was still high, had reduced the number of large follicles. Conversely when ewes returned to cyclic activity (group 3) the incidence of atresia was decreased and there was a build-up in the number of large follicles. Hormonal mechanisms are probably responsible for these changes in the incidence of atresia. In the present study, prolactin concentration varied according to the photoperiod, supporting the results of Thimonier *et al.* (1978). However, groups 3 and 4, which both had low levels of atresia, had high and very low prolactin concentrations, respectively, which suggests that the mechanism whereby photoperiod changes the incidence of atresia does not operate via prolactin activity at the ovarian level. Photoperiod has been shown to affect the secretion of gonadotrophin, i.e. LH pulse frequency (Lincoln and Peet 1977; Scaramuzzi and Baird 1977), and thus it is probable that the photoperiod effects on atresia observed in this study have been mediated via the gonadotrophins.

There were fewer large non-atretic antral follicles (>2·0 mm) observed in ovaries at mid-anoestrus which agrees with the results of Kammerlade *et al.* (1952) and Cahill and Mauleon (1980). Gherardi and Lindsay (1980) observed that ewes stimulated to ovulate with pregnant mare serum gonadotrophin (PMSG) showed a poorer response in ovulation rate in anoestrus (spring) than in the oestrous (autumn) season. This observation is supported by the present study, since in spring there probably would have been fewer large antral follicles and thus the number to ovulate would also have been fewer.

That bromocriptine treatment reduced the rate at which primordial follicles entered the growth phase is indicated by the significant reduction in the number of small pre-antral follicles. Despite this, the number of large pre-antral and antral follicles was unchanged. The concentrations of LH and FSH do not appear to be greatly affected by bromocriptine (Wright *et al.* 1982; Cognie and Oldham, unpublished data) thus it is unlikely that its effect on pre-antral follicles was mediated via the gonadotrophins. Of the five animals treated with bromocriptine (group 4), the two ewes which had no detectable prolactin also had a very reduced number of pre-antral follicles, which suggests that some prolactin, even though very small in quantity (0·5–5 ng/ml), is required for the initiation of follicular growth; however, the number of animals used was too small for the results to be conclusive. These results are supported by the work of Nolin (1978) who found

prolactin in the oocytes of primordial follicles in the rat. It is unlikely that normal secretion of prolactin in ewes during short-day photoperiods ever falls as low as 0·5–5 ng/ml (Thimonier *et al.* 1978), i.e. low enough to influence the rate that follicles enter the growth phase. Certainly in the present study, the short-day photoperiod treatment did not affect any category of pre-antral follicles.

A large reduction was observed in the rate follicles entered the growth phase following bromocriptine treatment (group 4) and yet the incidence of atresia and ovulation rate remain unchanged from the untreated ewes (group 3). This demonstrates that the ovary has been able to overcome the problem of having few pre-antral follicles, probably by increasing the rate at which follicles enter the antral phase.

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References

Cahill, L. P., Mariana, J. C., and Mauleon, P. (1979). Total follicular populations in ewes of high and low ovulation rates. *J. Reprod. Fertil.* **55**, 27–36.

Cahill, L. P., and Mauleon, P. (1980). Influence of season, cycle and breed on follicular growth rates in sheep. *J. Reprod. Fertil.* **58**, 321–8.

Cahill, L. P., Saumande, J., Ravault, J. P., Blanc, M., Thimonier, J., Mariana, J. C., and Mauleon, P. (1981). Hormonal and follicular relationships in ewes of high and low ovulation rates. *J. Reprod. Fertil.* **62**, 141–50.

Gherardi, P. B., and Lindsay, D. R. (1980). The effect of season on the ovulatory response of Merino ewes to pregnant mare's serum. *J. Reprod. Fertil.* **60**, 425–9.

Lincoln, G. A., and Peet, M. J. (1977). Photoperiodic control of gonadotrophic secretion in the ram: a detailed study of the temporal changes in plasma levels of follicle-stimulating hormone, luteinizing hormone and testosterone following an abrupt switch from long to short days. *J. Endocrinol.* **74**, 355–67.

Kammerlade, W. G., Welch, J. A., Nalbandov, A. V. and Norton, H. W. (1952). Pituitary activity of sheep in relation to the breeding season. *J. Anim. Sci.* **11**, 646–55.

Kann, G. (1971). Dosage radioimmunologique de la prolactine plasmatique chez les ovins. *C.R. Acad. Sci., Paris* (D. Ser.) **272**, 2808–11.

Mariana, J. C., Bachacou, J., and Terriot, M. (1980). Realisation d'un reaseau permettant la classification des structures histologiques de forme ellipitique en fonction de leur taille. *Reprod. Nutr. Develop.* **20**, 1855–8.

McNatty, K. P., Sawers, R. S., and McNeilly, A. S. (1974). A possible role for prolactin in the control of steroid secretion by the human Graafian follicle. *Nature (London)* **250**, 653.

McNatty, K. P., Gibb, M., Dobson, C., Thurley, D. C., and Findlay, J. K. (1981). Changes in the concentration of gonadotrophic and steroidal hormones in the antral fluid of ovarian follicles throughout the oestrous cycle of the sheep. *Aust. J. Biol. Sci.* **34**, 67–80.

Nolin, J. M. (1978). Apparent incorporation of endogenous prolactin by dictyate mammalian oocytes. *J. Cell. Biol.* **79**, 177a.

Rajakoski, E. (1960). The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclic and left–right variations. *Acta Endocrinol.* **52**, 1–68.

Rolland, R., and Hammond, J. M. (1975). Demonstration of a specific receptor for prolactin in porcine granulosa cells. *Endocrinol. Res. Commun.* **2**, 281–98.

Salamonsen, L., Herington, A. C., Gunsalus, G., Waters, M. J., and Hammond, J. M. (1982). Lactogenic hormone binding in ovine corpus luteum. *Proc. Aust. Soc. Reprod. Biol.* **14**, 25 (Abstr.).

Scaramuzzi, R. J., and Baird, D. T. (1977). Pulsatile release of luteinizing hormone and the secretion of ovarian steroids in sheep during anoestrus. *Endocrinology* **101**, 1801–6.

Terqui, M., and Thimonier, J. (1974). Nouvelle methode radioimmunologique rapide pour l'estimation du niveau de progesterone plasmatique. Application pour la diagnostic precoce de la gestation chez la brebis et la chèvre. *C.R. Acad. Sci. Paris* (D. Ser.) **279**, 1109–12.

Thimonier, J., Ravault, J. P., and Ortavant, R. (1978). Plasma prolactin variations and cyclic ovarian activity in ewes submitted to different light regimens. *Ann. Biol. Anim. Biochem. Biophys.* **18**, 1229–35.

Wright, P. J., Findlay, J. K., and Anderson, G. A. (1982). The failure of prolactin to enhance the inhibitory effect on oestradiol-17β and LH synthesis and release in ewes. *J. Reprod. Fertil.* **62**, 537–42.

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