ROLE OF PROGESTERONE IN OVARIAN FOLLICULAR GROWTH AND DEVELOPMENT IN THE SHEEP

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SUMMARY

The role of progesterone in ovarian follicular growth and development was studied in female sheep. In Experiment 1, ovarian venous concentrations of oestradiol and ovarian follicular steroidogenesis were assessed during seasonal anoestrus and compared with those observed during the luteal phase of the breeding season. Ovarian venous blood was sampled for six hours, at the end of which time the ovaries were removed so the steroidogenic function of the follicles could be measured by the amount of oestradiol and total androgen produced *in vitro*. Ovarian venous concentrations of oestradiol during anoestrus were about half those during the luteal phase. The ovaries of anoestrous ewes contained equal numbers of small follicles (<2 mm in diameter) and more medium-sized follicles (2 to 4 mm) than those of ewes in the luteal phase. Ovaries contained several large follicles (> 4 mm) during the uteal phase but none during anoestrus. Small follicles produced equal amounts of oestradiol *in vitro* in the two seasons, but medium follicles of anoestrous ewes produced more than medium follicles from luteal phase ewes. The same pattern applied to total androgen production and thus the oestradiol:androgen (E:A) ratio. The large follicles from the luteal phase produced the most oestradiol and the least total androgen. Experiment 2 tested whether these differences were due to seasonal differences in progesterone concentrations. Follicles were collected from two groups of anoestrous ewes, one of which was pretreated with a single injection of exogenous progesterone. The four largest ovarian follicles were dissected and cultured *in vitro*. In all cases, oestradiol production from follicles >3 mm was greater than that from follicles <3 mm, but was not significantly affected by progesterone priming. However, androgen production by the >3 mm follicles was reduced by progesterone priming, so the E:A ratio was greatly increased. These observations suggest that progesterone facilitates follicular function during the breeding season, possibly by stimulating the aromatization of androgens, and that absence of this effect may be one of the factors limiting follicle development past the 4 mm stage during seasonal anoestrus.

Keywords: progesterone, ovarian follicles, steroidogenesis, sheep

INTRODUCTION

During seasonal anoestrus, the ovaries of ewes appear to be relatively inactive and contain less antral follicles than during the breeding season (Cahill and Mauléon 1980; Atkinson and Williamson 1985). The process of folliculogenesis takes up to six months (Turnbull *et al.* 1977) so much of this process precedes the final phase of the oestrous cycle during which the ovulatory destiny of the follicle is decided (Scaramuzzi *et al.* 1993). When ovulation is stimulated in seasonally or lactationally anoestrous ewes, the resultant corpora lutea are often short-lived and secrete small amounts of progesterone (Oldham and Martin 1978; McNeilly *et al.* 1981; Wright *et al.* 1983; Atkinson *et al.* 1986; Atkinson, 1988), perhaps because the ovulatory follicle does not have time to mature (Pearce *et al.* 1985; Roberts *et al.* 1985; Atkinson, 1988). However, a major hormone missing during seasonal anoestrus, progesterone, has been shown to ensure the development of functional luteal structures during seasonal anoestrus if it is given during the period when the ovulatory follicle is developing (review: Martin *et al.* 1986). At least part of this effect appears to be due to actions of progesterone at ovarian level (Hunter *et al.* 1986). The present study was designed to identify differences in follicular steroidogenic function between ewes during seasonal anoestrus and during the luteal phase of the oestrous cycle, and to test whether these differences are affected by pretreatment with progesterone.

MATERIAL AND METHODS

In the first experiment, we used Southdown ewes, a breed that undergoes a deep and prolonged anoestrus, so that ovarian venous oestrogen production, and ovarian follicular development and steroidogenesis could be reliably compared across seasonal extremes. The hypothesis that progesterone priming would enhance

steroidogenesis at the ovarian level, developed from the first study, was then tested in anoestrous Merino ewes, a breed for which the protocol for progesterone priming for ovulation induction has been well established (Martin *et al.* 1986).

E*xperiment 1*

Animals and blood sampling: A flock of 12 mature Southdown ewes was divided into two equal groups, one of which was studied during the middle of anoestrus (September) while the other was held until the breeding season (February). The cycles of the ewes studied during the breeding season were synchronized with intravaginal progestagen sponges (Repromap, Upjohn, Australia) for 14 days and were subjected to the sampling regime 11 days after sponge withdrawal, in the mid-luteal phase of the oestrous cycle. On the day of sampling all ewes were fitted with jugular cannulae through which anaesthetic (5% thiopentone sodium) and heparin were delivered. The ewes were anaesthetized for six hours during which time ovarian venous blood was sampled at 30 minute intervals to determine oestradiol-17ß secretion. Serial samples were taken but mean values for each ewe were used to test for treatment effects to overcome fluctuations caused by episodic secretion and short-term variations in blood flow.

Immediately after blood sampling, the ovaries were removed and placed in Minimum Essential Medium (MEM; Flow Laboratories, Australia) at 4°C. All follicles >1 mm in diameter were dissected, measured and classified as small (diameter <2 mm), medium (2 to 4 mm) or large (>4 mm) and counted. Opaque, poorly vascularized follicles were considered atretic and not incubated. All follicles were drained of antral fluid and incubated individually for assessment of steroid production (Atkinson *et al.* 1986). Draining the antral fluid ensured that the steroids measured in the media were those secreted during the incubation period. After 24 h of incubation, oestradiol was measured for all follicles, while total androgens were measured for all large follicles and randomly selected small and medium-sized follicles.

E*xperiment 2*

Mature Merino ewes were laparoscoped at the beginning of seasonal anoestrus (June) to ensure that they were anovulatory, and then randomly allocated to groups for im injection with 2 mL oil (n=10), or 2 mL oil containing 20 mg progesterone (n=6; Lindsay *et al.* 1982). After 48 hours, the ovaries were removed under barbiturate anaesthesia (5% thiopentone sodium) and placed in M199 culture medium with Earl's salt (Flow Laboratories, Ayrshire, Scotland).

To study ovarian morphology and steroid production, the four largest healthy follicles were dissected from each ovary and classified as healthy or atretic (as above). Healthy follicles were divided into those >3 mm in diameter and those <3 mm and incubated for 24 hours. The culture media were frozen until assayed for oestradiol and androstenedione.

A*ssay details*

Media from the follicle incubations were assayed for oestradiol-17ß without extraction in a radioimmunoassay (see Atkinson *et al.* 1986) for which the sensitivity was 10 pg/tube, intra-assay variation was <5% and interassay variation was <10%. The ovarian venous samples were extracted and assayed in the same assay (Atkinson *et al.* 1986; Atkinson 1988) and the values were corrected for extraction recovery (85%).

Total androgens (Experiment 1) were measured in a single radioimmunoassay (Atkinson *et al*. 1986) which showed significant cross-reaction with dihydrotestosterone (98%), 4-androstene-3ß,17ß-diol (47%) and androstenedione (4.7%). The sensitivity of the assay was 14.0 pg/tube and intra-assay variation was <5%. Androstenedione (Experiment 2) was assayed in media from the follicle incubations without extraction using a kit (bioMerieux, Marcy-l'Etoile, France) for which the sensitivity was 2 pg/tube and the intra-assay variation was <10%. Cross reactions given with the kit were: androsterone 11.6%, andrenosterone 2.7%, DHEA 2.25%, and all other steroids <1%.

Data Analysis

No transformations were needed for the ovarian venous oestradiol concentrations, but the *in vitro* oestradiol and androgen concentrations were logarithmically transformed to reduce variance heterogeneity. These data were subjected to ANOVA. Numbers of follicles were analysed using a Kruskal-Wallis non-parametric test. All results are presented as mean ± s.e. of untransformed data.

RESULTS

Experiment 1

Ovarian venous oestradiol concentrations were higher but more variable in the luteal phase group (322.1 ± 141.8 pg/ml) than in the anoestrous group (162.5 ± 38.5 pg/ml). Less healthy follicles were recovered from aoestrous ewes (8.3 ± 1.2) than from luteal phase ewes (9.9 ± 2.9; P<0.05). The anoestrous ewes produced equal numbers of small follicles (<2 mm diameter) and more medium follicles (2 to 4 mm) than the ewes in the luteal phase (Table 1). Follicle development during anoestrus stopped at 4 mm, whereas numerous large follicles (>4 mm) were evident during the luteal phase in the breeding season (Table 1).

In vitro, the small follicles of both groups produced similar amounts of oestradiol, but the medium follicles of the anoestrous ewes produced more oestradiol than similar-sized follicles from ewes in the luteal phase in the breeding season (Table 1). Large follicles were only collected during the luteal phase and they produced the most oestradiol. There were no differences in total androgen production between follicles in the various size classes, so the oestradiol:androgen ratios followed the same patterns as oestradiol concentrations (Table 1).

Experiment 2

The production of oestradiol and androstenedione *in vitro* did not differ significantly between the control and progesterone-primed ewes for any follicle size. However, E:A was significantly higher in follicles > 3 mm from progesterone-primed ewes than in follicles from controls (Figure 1).

Table 1. Numbers of healthy (non-atretic) ovarian follicles and their *in-vitro* steroid production after dissection from the ovaries of ewes during the luteal and anoestrous phases.

*** P<0.05 ** P<0.01, compared to values for same-sized follicles during the luteal phase**

Follicle diameter		Luteal	Anoestrous
<2 mm	Number of follicles	5.9 ± 2.4	5.5 ± 0.9
	Oestradiol (pg/mL)	216.2 ± 89.9	149.3 ± 26.6
	Androgen (ng/mL)	8.0 ± 7.6	4.5 ± 1.5
	E:A Ratio	0.48 ± 0.2	0.66 ± 0.1*
2 to 4 mm	Number of follicles	1.6 ± 0.4	3.4 ± 0.6*
	Oestradiol (pg/mL)	102.6 ± 59.3	2549.8 ± 131.8**
	Androgen (ng/mL)	12.8 ± 11.6	22.8 ± 15.4
	E:A Ratio	0.35 ± 0.2	0.82 ± 0.1*
>4 mm	Number of follicles	1.0 ± 0.3	0.0 ± 0.0*
	Oestradiol (ng/mL)	22.6 ± 14.6	——
	Androgen (ng/mL)	6.4 ± 6.0	——
	E:A Ratio	1.69 ± 0.5	——

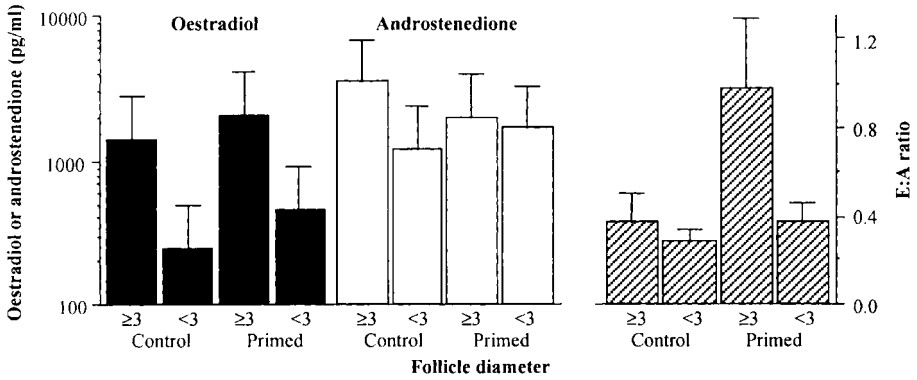

Figure 1. Oestrogen:androgen ratios from healthy ovarian follicles cultured *in vitro*. The follicles were taken from seasonally anoestrous ewes treated with a single injection of 20 mg progesterone ('Primed') or with vehicle ('Control')

DISCUSSION

Taken together, these studies strongly suggest that progesterone plays a role in determining the steroidogenic capacity of developing follicles, and that the absence of progesterone in the hormonal milieu of follicles as they approach the final stages of development is one of the reasons for reproductive inefficiencies during the anoestrous season. Amongst the unfavourable outcomes implicated here are the frequent failure for preovulatory follicles to develop, and the frequent early demise of corpora lutea following induction of ovulation by hormonal therapy or the ram effect (Oldham and Martin 1978; Hunter *et al.* 1986; Atkinson and Williamson, 1985). Many other endocrine and metabolic factors also change with the seasons, including gonadotrophin patterns, so further work is required to verify the details of the role of progesterone. This approach is justified, however, because we do know that progesterone does have a direct effect on developing follicles in the ovary of the sheep (Hunter *et al.* 1986).

The progesterone supplement altered steroid production by increasing oestrogen production rather androgen production, and the oestrogen:androgen ratio of the large follicles was improved. This suggests that progesterone enhances the aromatization of androgen to oestrogen, especially in large (>4 mm) follicles, because such recruitment of such follicles would have been initiated by the ram effect within a few hours of the progesterone injection in our second experiment.

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