Ovarian follicular development in Booroola ewe lambs and in highly fecund Booroola ewes

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ABSTRACT

At 3 months of age, the ovaries of F+ Booroola lambs [i.e., those heterozygous for the fecundity (F)-gene] were lighter ($P<0.01$) than those of ++ Booroola lambs (i.e., those without the F-gene) which were of similar age and body weight. However, the number of follicles (≥ 1 mm diam.) in the above F+ lambs was 1.5 fold greater ($P<0.01$) than that in the ++ lambs. In lambs of both genotypes, ovarian follicles were active in synthesising testosterone and oestradiol and of reaching diameters which were similar to those of preovulatory follicles in mature ewes. However, none of the ovaries showed evidence of recent ovulations.

In Booroola ewes (4 to 8 years of age), the proportions of small ovarian follicles (0.5 to 1.0 mm diam.) that synthesised progesterone, androstenedione and oestradiol in culture were related ($P<0.05$) to genotype (homozygous, FF > F+ > ++ ewes). Also in the above ewes, follicles in the FF and F+ animals reached maturity at smaller diameters than in ++ animals, namely 3.4 ± 0.3, 4.1 ± 0.2 and 6.8 ± 0.3 mm (mean ± s.e.m.; n = 3 ewes/genotype) respectively. During a cloprostenol-induced follicular phase, the oestradiol secretion rates from FF ewes with 4.8 ± 0.3 'oestrogenic' follicles, F+ ewes with 3.2 'oestrogenic' follicles and ++ ewes with 1.5 ± 0.02 such follicles were similar as were the mean total numbers of oestradiol-secreting (i.e., granulosa) cells from these follicles, that is 5.4 x 10^6 cells in each genotype.

Collectively these findings show that the Booroola F-gene is expressed in the ovaries of ewes before puberty and during the earliest stages of follicular development. Moreover, as follicles in Booroola ewes with the F-gene mature at smaller diameters, the maturation of 5 or more follicles in FF ewes and 3 to 4 such follicles in F+ ewes may be necessary to produce the same number of oestrogen-producing (i.e. granulosa) cells as that from 1 or 2 mature follicles in ++ ewes.

Keywords Booroola; lambs; ewes; ovarian follicles; granulosa cells; follicle size; cell numbers.

INTRODUCTION

Highly fecund Booroola ewes contain a major gene which influences their ovulation rate (see Bindon (1984) for review). Homozygous (FF), heterozygous (F+) and non-carriers (++) of the putative gene have been segregated on the basis of at least one ovulation rate recording of ≥ 5, 3 or 4 and 1 or 2 respectively. We have previously demonstrated that ovarian granulosa cells from follicles ≥ 2.5 mm diameter were more responsive to follicle-stimulating hormone (FSH) and luteinising hormone (LH) compared to ++ ewes (Henderson *et al.*, 1985), and that follicles in F+ ewes secreted their maximum amounts of oestradiol-17 β and ovulated at a smaller diameter than in ++ ewes (F+, 3 to 4.5 mm; ++, 5 to 7 mm diam.; McNatty *et al.*, 1985).

This report extends the above findings to show that the Booroola F-gene is expressed in the ovaries of ewes before puberty and that its effects are also evident during the earliest stages of ovarian follicular development. Moreover, evidence is presented to support the notion that the maturation of 5 or more follicles in FF ewes, and 3 to 4 such follicles in F+ ewes may be necessary to produce similar numbers of oestrogen-producing (i.e., granulosa) cells to that from 1 to 2 mature follicles in ++ ewes.

MATERIALS AND METHODS

The Booroola Romney x Romney lambs (n = 13 F+, n = 16 ++) and Booroola x Merino ewes (n = 15 FF, n = 18 F+ and 18 ++ ewes 4 to 8 years of age) were kindly supplied by Mr G. Davis and his colleagues at the Invermay Agricultural Research Centre. The lambs were the progeny of either FF or ++ Booroola x Romney rams that had mated with Romney ewes. At 12 weeks of age, all the lambs were weighed and their ovaries removed for further study.

The FF, F+ and ++ ewes were classified as such on the basis of 2 to 4 previous annual ovulation rate recordings of ≥ 5, 3 to 4 or 1 to 2 respectively. At day 10 (time 0) of the oestrous cycle (oestrus = day 0) all but 3 ewes from each genotype were injected with cloprostenol (125 μ g s.c.; Coopers Animal Health Laboratories, Upper Hutt). At time 0 (uninjected ewes) and at intervals thereafter of 6, 12, 24, 36 and 48 h, ovarian (10 to 20 ml from both left and right ovarian veins) and peripheral (50 ml) venous blood was collected from 3 FF, 3 F+ and 3 ++ ewes anaesthetised with thiopentone sodium (Intraval; May & Baker, Wellington); the only exception to this was at 6 h after cloprostenol when no FF ewes were studied. During ovarian venous blood collection the rate of blood flow was also measured (McNatty *et al.*, 1981). Immediately after the blood

collections, the ovaries of each ewe were removed for further study.

The ovaries of each lamb and ewe were weighed and then all follicles (≥0.1 mm diam.) were isolated for further study as previously described (McNatty *et al.*, 1985).

The techniques for culturing small follicles (0.5 to 1 mm diam.) were identical to those described by McNatty *et al.* (1986a). Briefly, individual entire follicles were placed on a strip of sterile filter paper and supported on a stainless steel grid above 1.8 ml of culture medium consisting of Medium 199, Earle's salts, Hepes buffer (20 mM), 5% (v/v) calf serum, gentamycin sulphate (50 μ g/ml), LH (NIH-LH-S23, 1 μ g/ml) and FSH (NIH-FSH-S11, 1 μ g/ml) in a 35 x 10 mm Petri dish. Each dish was placed in a modified anaerobic jar which was then gassed with 50% O_2, 45% N_2 and 5% CO_2 to 70 kPa and placed in a 37°C incubator for 48 h.

The contents/concentrations of progesterone, androstenedione, testosterone and/or oestradiol in culture medium, follicular fluid and/or blood plasma were assayed by specific radioimmunoassays identical to those described elsewhere (McNatty *et al.*, 1985, 1986a). The ovarian secretion rates of oestradiol were calculated from a knowledge of haematocrit, blood flow and oestradiol concentration (McNatty *et al.*, 1981).

RESULTS

The mean ± s.e.m. weights of the F+ and ++ lambs at ovariectomy were 24.0 ± 0.9 (n = 13 lambs) and 22.4 ± 0.9 (n = 16) kg respectively. The mean weights of individual ovaries in the F+ and ++ lambs were 0.29 ± 0.01 and 0.41 ± 0.02 g respectively; ovaries in the F+ lambs were significantly lighter than in the ++ lambs ($P < 0.01$). There were more ovarian follicles (≥1 mm diam.) in the F+ than in the ++ lambs (30.2 ± 2.5 *v* 18.4 ± 1.8, $P < 0.01$). The mean ± s.e.m. numbers of 1 to 1.5, 2 to 2.5, 3 to 3.5, 4 to 4.5 and ≥ 5 mm diameter follicles in the F+ (n = 13) and ++ (n = 16) lambs were 23.5 ± 2.6, 5.3 ± 1.5, 1.0 ± 0.2, 0.4 ± 0.2, 0 and 10.1 ± 0.9, 6.3 ± 1.3, 1.3 ± 0.3, 0.4 ± 0.2 and 0.3 ± 0.1 respectively. No corpora lutea or corpora albicantia were observed in any of the ovaries in this study.

In the lambs, there was evidence of active steroid synthesis at the level of individual follicles. In the F+ lambs (n = 13) the geometric mean (and 95% confidence limits) concentrations of testosterone in 1 to 2.5 mm diam. and 3 to 4.5 mm diam. follicles were 44 (28,68) and 33 (19,56) ng/ml. In the aforementioned follicles the respective concentrations of oestradiol were 13 (7,22) and 21 (12,36) ng/ml. In the ++ lambs (n = 16) the geometric mean (and 95% confidence limits) concentrations of testosterone in 1 to 2.5 mm diam. and 3 to 4.5 mm diam. follicles were 86 (65,112) and 80 (50,129) ng/ml; in these follicles the respective concentrations of oestradiol were 16 (9,28) and 19 (9,38) ng/ml. For testosterone, the concentrations in 1 to 2.5 and 3 to 4.5 mm diam. follicles in F+ lambs were each significantly lower ($P < 0.025$) than the corresponding values in ++ lambs. There were no significant genotypic differences for oestradiol.

In the Booroola ewes, the numbers of ovarian follicles (≥0.1 mm diam.) for each genotype did not change with respect to time after cloprostenol treatment. Irrespective of time after cloprostenol treatment, the mean numbers of follicles per ewe for each genotype were not significantly different from one another. The geometric mean (and 95% confidence limits, CL) numbers of follicles 0.1 to 1, 1 to 1.5, 2 to 2.5, 3 to 3.5, 4 to 4.5, ≥ 5 mm were 27 (17,43), 27 (21,35), 6 (4,8) 3 (1,4), 0.5 (0.1,1.1), 0 (0,0) for FF ewes (n = 15), 26 (16,46), 22 (17,30), 5 (4.7), 1 (1,2), 1 (1,2), 0 (0,0) for F+ ewes (n = 18) and 26 (30,24), 19 (16,23), 8 (6,12), 1 (1,2), 0.5 (0.2,1) 1 (1,1) for ++ ewes respectively (see McNatty *et al.*, 1986a,b).

In the ewes, the proportions of follicles (0.5 to 1 mm diam.) which produced ≥ 4 ng progesterone/48 h, ≥ 3 ng androstenedione/48 h and ≥ 0.8 ng oestradiol/48 h were significantly influenced by genotype ($P < 0.01$ for progesterone, $P < 0.05$ for androstenedione, and $P < 0.025$ for oestradiol). For progesterone, the respective proportions of FF, F+ and ++ follicles which produced ≥ 4 ng/48 h were 65% (n = 82 follicles overall), 47% (n = 58), and 38% (n = 55). For androstenedione, the respective proportions of FF, F+ and ++ follicles which produced ≥ 3 ng/48 h were 40% (n = 82), 26% (n = 58) and 22% (n = 55). For oestradiol, the respective proportions of FF, F+ and ++ follicles which produced ≥ 0.8 ng oestradiol/48 h were 30% (n = 82), 26% (n = 58) and 25% (n = 55).

Follicles of all genotypes were considered to be 'oestrogenic' structures if they contained ≥ 50 ng/ml oestradiol in follicular fluid. Previous studies have shown that 'oestrogenic' follicles are non-atretic follicles undergoing their final phases of maturation (McNatty, 1982). Moreover, several studies have shown that the number of oestradiol-enriched follicles between 12 and 36 h after cloprostenol treatment reflects the ovulation rate of the sheep breed in question (McNatty *et al.*, 1986b). The data summarising the number and diameter of 'oestrogenic' follicles and the number of granulosa cells in FF, F+ and ++ ewes together with the oestradiol secretion rate from ovaries containing 'oestrogenic' follicles at 12 to 36 h after cloprostenol treatment are shown in Table 1. The FF and F+ ewes contained 3.2 fold and 2.1 fold more 'oestrogenic' follicles respectively than did the ++ ewes. The respective diameters of 'oestrogenic' follicles in FF and F+ ewes were 2.2 mm, and 1.8 mm smaller (both $P < 0.01$) than those in ++ ewes. Moreover, the respective mean number of granulosa cells in 'oestrogenic' follicles of FF and F+ ewes were 2.7 x 10^6 and 2.1 x 10^6 fewer than in ++ ewes ($P < 0.01$ for FF and F+ ewes vs ++ ewes) (Table 1). However, the total number of granulosa cells in the

TABLE 1 Mean ± s.e.m. number and diameter of 'oestrogenic' follicles[1], number of granulosa cells in FF, F+ and ++ ewes and mean oestradiol secretion-rate[2] (geometric mean (95% confidence limits)) at 12 to 36 h after cloprostenol injection (from McNatty *et al.* (1986b))

Ewes Genotype	No.	No./ewe	'Oestrogenic' follicles Diameter (mm)	Granulosa cell no./follicle (x 10^6)	Foll. no. x granulosa cell no. (x 10^6)	Oestradiol secretion rate (ng/min)
++[3]	8	1.5 ± 0.2[a]	5.1 ± 0.3[a]	3.8 ± 0.4[a]	5.4 ± 0.4	3.2 (1.6,5.8)
F+	9	3.2 ± 0.2[b]	3.3 ± 0.2[b]	1.7 ± 0.1[b]	5.4 ± 0.4	4.5 (3.0,6.5)
FF	9	4.8 ± 0.3[c]	2.9 ± 0.1[b]	1.1 ± 0.05[c]	5.4 ± 0.5	3.0 (1.9,4.5)

[1] Follicle containing < 50 ng oestradiol/ml follicular fluid
[2] Oestradiol secretion rate = total output from left and right ovaries
[3] One ewe excluded because no 'oestrogenic' follicles were present
For each column a *v* b, $P<0.01$; a *v* c, $P<0.01$; b *v* c, $P<0.025$

'oestrogenic' follicles from each genotype (i.e., number of granulosa cells x number of 'oestrogenic' follicles/genotype) was identical. In addition, the oestradiol secretion rates from either the ovaries with the 'oestrogenic' follicles or from both ovaries from each ewe with respect to genotype were not significantly different.

At 48 h after cloprostenol treatment, the respective mean ± s.e.m. diameters of the presumptive preovulatory follicles in FF, F+ and ++ ewes (n = 3 genotype) were 3.4 ± 0.3 mm, 4.1 ± 0.2 mm and 6.8 ± 0.3 mm (FF *v* ++, $P<0.01$; FF *v* F+, $P<0.05$; F+ *v* ++, $P<0.05$). Moreover, the respective mean number of granulosa cells in these follicles were (1.8 ± 0.3) x 10^6, (2.2 ± 0.3) x 10^6, and (6.6 ± 0.3) x 10^6 (FF *v* ++, $P<0.01$; FF *v* F+, $P<0.05$; F+ *v* ++, $P<0.01$).

DISCUSSION

These data show that the Booroola F-gene is being expressed in the ovaries of lambs. The ovaries of the F+ Booroola lambs were characterised by a high level of antral follicular activity, and a minimal amount of interstitial tissue. This was in contrast to the ovaries of the ++ lambs which had a lower level of antral follicular activity than in F+ lambs as well as a much greater amount of interstitial tissue. Of interest was the finding that some of the ++ but none of the F+ lambs had follicles ≥ 5 mm diameter; this was also a common characteristic in mature Booroola ewes (see McNatty *et al.*, (1985) and this paper). These gross differences in follicle numbers, distribution and amount of interstitial tissue were the major reasons why the ovaries of ++ ewes were 1.4 times heavier than those of F+ ewes. The ovarian follicles (≥1 mm diam.) in F+ lambs contained higher testosterone concentrations than did follicles in ++ lambs; these findings were consistent with the view that the ovaries of lambs with the F-gene are both structurally and functionally different from those in non-carriers. It was also of interest to note that the ovaries of both F+ and ++ lambs contained many antral follicles actively secreting oestradiol. The level of follicular oestradiol activity is similar to that observed in mature ewes during anoestrus or during the luteal phase of the oestrous cycle (McNatty *et al.*, 1984).

In contrast to the findings for Booroola lambs these data show that the Booroola F-gene does not have a major influence on the total numbers of follicles (≥1 mm diam.) in mature ewes; in each of the genotypes the follicle numbers were similar. However, the patterns of follicle growth were clearly different since the large follicles (≥5 mm diam.) which were routinely observed in ++ ewes were conspicuously absent from the FF and F+ ewes.

These data also show that the Booroola F-gene has an influence on the maturation of ovarian follicles from an early stage of growth. Proportionately more of the small follicles in FF ewes have the capacity to synthesise high levels of steroid relative to those from F+ and ++ ewes. In the study on the larger follicles (i.e., ≥1 mm diam.) in the 3 genotypes, those in FF, F+ and ++ ewes reached maturity at 2 to 4.5 mm, 3 to 4.5 mm and ≥ 5 mm diam. respectively. Also each mature follicle in the FF ewes contained fewer oestrogen-secreting (i.e., granulosa) cells than in those from F+ ewes which in turn contained less than in ++ ewes. Collectively, however, the total number of granulosa cells in the 'oestrogenic' follicles in each of the 3 genotypes was similar. Since the oestradiol secretion rates at 12 to 36 h after cloprostenol were similar between the genotypes, it could be argued that the maturation of ≥5 preovulatory follicles in FF ewes and 3 to 4 such follicles in F+ ewes may be necessary to provide a cell mass capable of producing the same quantity of oestradiol as that from 1 or 2 such follicles in ++ ewes.

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