Ovarian activity in the Booroola × Romney ewe possessing a major gene influencing fecundity

K. P. McNatty, K. M. Henderson, S. Lun, D. Heath, L. Kieboom, N. Hudson, J. Fannin, K. Ball, P. Smith

Wallaceville Animal Research Centre
Ministry of Agriculture and Fisheries, Upper Hutt

ABSTRACT

The pattern of ovarian follicular development in Booroola × Romney ewes (6-7 y) with an ovulation-rate ≥3 (F+ ewes) differed from that in Booroola × Romney ewes (6-7 y) with an ovulation-rate <3 (++). The difference was not due to variations in the number of ovarian follicles (≥1 mm diameter) or in the proportion of healthy follicles which were similar for both genotypes. It arose because follicles in F+ ewes produced oestradiol and reached maturity at a smaller diameter than in ++ ewes. During a prostaglandin induced follicular phase, the secretion rate of oestradiol from ovaries with 3 presumptive preovulatory follicles in F+ ewes was similar to that from ovaries with only 1 such follicle in ++ ewes. Moreover, the total number of granulosa cells (i.e., the oestrogen-secreting cells) in 3 preovulatory follicles from F+ ewes was similar to that in 1 such follicle in ++ ewes. In F+ ewes, the presumptive preovulatory follicles reached 4.6 ± 0.4 (s.e.m.) mm in diameter, whereas in ++ ewes, they reached 7.3 ± 0.3 mm in diameter.

It is hypothesised that the putative 'gene' effect is manifested in small ovarian follicles (≤2.5 mm diameter) which results in their maturation at a smaller diameter (i.e., 3 to 5 mm) than is the case for ++ ewes. As a consequence, the developement of at least 3 preovulatory follicles in F+ ewes may be obligatory to generate sufficient oestrogen-secreting cells to initiate oestrus and ovulation.

Keywords Booroola × Romney ewes; genotype; ovaries; follicles; corpora lutea; oestradiol; granulosa cells

INTRODUCTION

There is evidence to suggest that high fecundity Booroola ewes contain a major gene which influences their ovulation rate (Davis *et al.*, 1982; Piper and Bindon, 1982). Animals thought to be heterozygous (F+) and homozygous (FF) carriers of the putative gene have tentatively been segregated from non carriers (++) on the basis of at least 1 ovulation rate recording of 3 or 4 and ≥5 respectively (Davis *et al.*, 1982). Kelly *et al.* (1983) have shown that Booroola ewes with an ovulation-rate of ≥3 contain ovarian follicles which are more sensitive to PMSG than those with an ovulation-rate of ≤2. This finding suggests that ovarian follicular activity in F+ and FF Booroola ewes may be somewhat different from that in ++ ewes and raises the possibility that the Booroola gene may be operating an intra-ovarian mechanism, although extra-ovarian mechanisms cannot at this time be ruled out. The aim of this study was to investigate aspects of follicular and luteal activity in F+ and ++ Booroola × Romney ewes.

MATERIALS AND METHODS

Twenty-one Booroola × Romney ewes (6-7 yr) were classified as F+ on the basis of at least 1 ovulation-rate record of ≥3 but <5. A further 21 Booroola × Romney ewes of similar age were classified as ++ on the basis that the 4 annual recordings of ovulation and lambing rates were always <3. All the animals were injected with a prostaglandin (PG) derivative (cloprostenol, 125 μg s.c.) on the day 10 of the oestrous cycle to induce luteolysis. At varying time intervals after PG (0, 3, 6, 12, 24, 36 and 48 h) and under thiopentone anaesthesia, ovarian and peripheral venous blood was collected (i.e., 3 ++ and 3 F+ ewes at each time). The purpose of treating ewes with PG was to study preovulatory follicular development at precisely known stages of corpus luteum regression (McNatty *et al.*, 1982). During ovarian venous blood collection the rate of blood flow was also measured (McNatty *et al.*, 1981). Immediately after the blood collections had been completed, the ovaries of each animal were removed and all follicles (≥1 mm diameter) were isolated for further study. Individual follicles were classified as healthy on the basis of a visible thecal vasculature, ≥25% of the maximum granulosa-cell number for a given follicle diameter, a healthy-looking oocyte and the absence of debris in follicular fluid. Follicles were classified as atretic if 1 or more of these criteria were not satisfied (McNatty *et al.*, unpublished data).

The concentrations of oestradiol in ovarian venous plasma were measured by radioimmunoassay procedures described elsewhere (McNatty *et al.*, 1982). The ovarian secretion rates of oestradiol were calculated from a knowledge of haematocrit, blood flow and oestradiol concentration (McNatty *et al.*, 1981).

RESULTS

The numbers of antral follicles (mean ± s.e.m. per ewe ≥ 1 mm diameter) for each genotype did not change with respect to time after PG treatment. Irrespective of time after PG treatment, the mean numbers of follicles per ewe for each genotype were not significantly different from one another (+ + ewes, 39.0 ± 3.4; F+ ewes, 44.0 ± 2.6). Likewise, the proportions of healthy follicles for each genotype (21 ewes) were similar (+ + ewes, 38.7 ± 3.1% healthy follicles; F+ ewes, 39.7 ± 2.6%). The respective numbers (mean ± s.e.m.) per ewe of small (1 to 2.5 mm diameter), medium (3 to 4 mm diameter) and large (≥ 5 mm diameter) healthy follicles in the + + animals were 13.0 ± 1.1, 2.1 ± 0.4 and 1.1 ± 0.1, whereas the respective numbers in the F+ animals were 12.3 ± 1.2, 3.0 ± 0.3 and 0.3 ± 0.1 respectively. Compared to F+ ewes there were significantly more large ($P < 0.01$) and significantly fewer intermediate ($P < 0.05$) follicles in + + ewes but similar numbers of small follicles.

Follicles of both genotypes were considered to be 'oestrogenic' structures if they contained ≥ 50 ng/ml in follicular fluid; basal values of oestradiol in follicular fluid were < 10 ng/ml. Using this criterion it was evident that follicles from F+ ewes produced oestradiol earlier in their development than in + + ewes. For example, from all animals studied (i.e., 21 per genotype, 17 large, 4 intermediate and 0 small healthy follicles were 'oestrogenic' (i.e., on average 1 oestrogenic follicle per ewe) in + + ewes whereas 3 large, 37 intermediate and 8 small healthy follicles were 'oestrogenic' (i.e., 2.3 follicles/ewe in F+ ewes. For both genotypes, at 12, 24 and 36 h after PG treatment, at least 1 ovary per animal contained an 'oestrogenic' follicle. The mean ovarian secretion rates of oestradiol from these 'oestrogenic' ovaries were not different between genotypes but there were 3.8-fold more oestrogenic follicles in the F+ ewes than in the + + ewes (Table 1). Moreover, the mean follicular diameter of the 'oestrogenic' follicle in the F+ ewes was significantly smaller ($P < 0.01$) than in the + + ewes.

At 48 h after PG injection the mean (± s.e.m.) follicular diameter of the presumptive preovulatory follicles in F+ and + + ewes was 4.6 ± 0.4 mm (9 follicles, 3 ewes) and 7.3 ± 0.3 mm (3 follicles, 3 ewes) respectively. The mean (± s.e.m.) number of granulosa cells in the above follicles of F+ ewes was 2.3 ± 0.3 million whereas in the + + ewes it was 6.5 ± 0.8 million.

DISCUSSION

These findings suggest that the pattern of ovarian follicular development in Booroola × Romney ewes with an ovulation-rate of ≥ 3 (F+ ewes) differs from that in animals with an ovulation-rate of < 3 (+ + ewes). But it was not due to a difference in the number of antral follicles (≥ 1 mm diameter) or in the number of healthy follicles. Instead, the difference between the 2 genotypes appeared to be the result of an earlier maturation of follicles in the F+ ewes. Ovarian follicles in F+ ewes developed a capacity to synthesise oestradiol earlier and they also reached preovulatory size at a smaller diameter than in + + ewes. Presumably, the maturation of follicles in F+ ewes at a smaller follicular diameter is directly related to the earlier synthesis of oestradiol (see Peters and McNatty, 1980). In sheep ovaries, granulosa cells are the major source of oestradiol (McNatty *et al.*, 1984). Induction of oestrogen synthesising enzyme activity in granulosa cells in critically dependent on FSH stimulation (Hiller *et al.*, 1982). Perhaps therefore, the greater sensitivity of F+ ewes to PMSG (Kelly *et al.*, 1984) is evidence to support the notion that granulosa cells in F+ ewes are more sensitive to FSH than are cells from + + ewes.

In preovulatory follicles from F+ ewes there were about one-third as many granulosa cells as in follicles from + + ewes. However, the *in vitro* output of oestradiol (per cell) from granulosa cells of F+ ewes was similar to that from cells of + + ewes (unpublished data). Thus, the finding of a similar oestradiol secretion rate from ovaries of F+ ewes with ~3 'oestrogenic' follicles compared to that from ovaries of + + ewes is consistent with the hypothesis that a follicular-cell number from 3 F+ follicles is needed to generate the same quantity of oestradiol to that produced by 1 + + follicle. Whether this particular

TABLE 1 Number and diameter of 'oestrogenic'[a] follicles in F+ and + + ewes and oestradiol secretion-rate from ovaries containing 'oestrogenic' follicles at 12 to 36 h after induction of luteolysis.

Genotype (No. of ewes)	No. of ovaries with 'oestrogenic' follicles	'Oestrogenic' follicles Number	'Oestrogenic' follicles Diameter (mean ± s.e.m.; mm)	Oestradiol[b] secretion-rate (ng/min)
+ + (9)	10	11	5.0 ± 0.3	3.5 ± 0.8
F+ (9)	13	31	3.5** ± 0.1	2.7 ± 0.7

[a] An 'oestrogenic' follicle contains ≥ 50 ng/ml oestradiol in follicular fluid.
[b] The oestradiol secretion-rate is that from ovaries containing an oestrogenic follicle.

quantity of oestradiol (i.e., 4 μg/24 h) is rate-limiting with respect to oestrus and ovulation in both genotypes is unknown.

In conclusion, these data suggest that the putative 'gene' effect is manifested in the ovary in small (≤2.5 mm diameter) follicles which results in their maturation at a smaller follicle diameter (i.e., 3 to 5 mm) than for those in + +ewes (i.e., >5 mm diameter). As a consequence the development of 3 preovulatory follicles in F+ewes may be obligatory in order to generate sufficient oestrogen-secretion cells to initiate oestrus and ovulation.

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