Differences in gonadotrophin-stimulated cyclic AMP production by granulosa cells from Booroola × Merino ewes which were homozygous, heterozygous or non-carriers of a fecundity gene influencing their ovulation rate

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Summary. Granulosa cells from follicles of different sizes from Booroola × Merino ewes which were homozygous (FF), heterozygous (F+) or non-carriers(++) of a fecundity gene were obtained 0–48 h after cloprostenol injection on Day 10 of the oestrous cycle. The highest mean amounts of cAMP produced by the cells did not differ between the genotypes. However, in the ++ ewes it was attained by cells from follicles ≥ 5 mm in diameter, whereas in F+ and FF ewes it was attained by cells from follicles 3–4.5 mm in diameter. Cells from 1–2.5-mm diameter follicles of FF ewes were more sensitive to FSH and LH than were corresponding cells from F+ or ++ ewes. Granulosa cells from ≥ 5 mm diameter follicles of ++ ewes 12–24 h after injection of cloprostenol had a lower mean response to FSH and LH than did cells obtained 0–6 or 36–48 h after cloprostenol. No such effect of time was evident for cells from any size of follicles obtained from F+ or FF ewes. In 1–2.5-mm diameter follicles, the mean aromatase activity of granulosa cells from ++ and F+ ewes was similar, but significantly lower than that of cells from FF ewes. In 3–4.5 mm diameter follicles, the mean aromatase activity of cells from F+ and FF ewes was similar, and significantly higher than that of cells from ++ ewes. For all 3 genotypes, there was a significant positive relationship between FSH or LH stimulation of granulosa cell cAMP production and cellular aromatase activity.

Introduction

The increased prolificacy of Booroola crossbred ewes endowed with the so-called F, or fecundity gene, is a consequence of their increased ovulation rate compared to ewes without the gene (see Bindon, 1984, for review). Sheep that are homozygous (FF), heterozygous (F+) and non-carriers (++) of the fecundity gene have been segregated on the basis of at least one ovulation rate recording of ≥ 5, 3 or 4, and 1 or 2 respectively (Davis *et al.*, 1982). In a previous study we compared the ability of FSH and LH to stimulate cAMP production by granulosa cells from different sized follicles obtained during the preovulatory period from F+ and ++ Booroola ewes (Henderson *et al.*, 1985). It was found that the highest mean amounts of cAMP produced by cells challenged with FSH or LH did not differ between the genotypes. However, they were achieved using cells from ≥ 5 mm diameter follicles in ++ ewes but only 3–4 mm diameter follicles in F+ ewes. On the basis of this and other studies (McNatty *et al.*, 1985) it has been suggested that follicles from F+ ewes mature at a smaller diameter than those from ++ ewes. In addition, it was found that in ++ but not F+ ewes the amount of cAMP produced by granulosa cells challenged with FSH was dependent upon the time the cells were collected during a cloprostenol-induced follicular

phase. FF ewes have an even higher ovulation rate than F+ ewes. The responsiveness to gonadotrophins of granulosa cells from FF ewes may therefore differ from that of cells from F+ ewes. The present study was therefore undertaken to compare the ability of FSH and LH to stimulate cAMP production by granulosa cells obtained from various sized follicles of FF, F+ and ++ Booroola ewes during a cloprostenol-induced follicular phase.

Materials and Methods

Sheep. This study was performed using 15 FF, 17 F+ and 18 ++ Booroola $\times$ Merino ewes aged 5–8 years. They were grazed on open pasture, and run with a vasectomized ram fitted with a marking harness to detect oestrous activity.

Recovery of granulosa cells. On Day 10 of the oestrous cycle (Day 0 = day of oestrus) the ewes were injected intramuscularly with 125 μ g cloprostenol (Coopers Animal Health, Upper Hutt, New Zealand) to induce luteolysis. Ovariectomy was performed 0, 6, 12, 24, 36 or 48 h later (3 ewes of each genotype at each time, except at 6 h when only 2 F+ and 0 FF ewes were studied, due to the limited number of ewes available). Immediately after ovariectomy, the corpora lutea and all antral follicles ≥ 1.0 mm in diameter were individually dissected into sterile Medium 199 with Earle's salts (Eagle, 1959), supplemented with Hepes buffer (20 mM), gentamicin (50 μ g/ml; Gibco, Grand Island, NY, U.S.A.), sodium heparin (50 i.u./ml; Weddel Pharmaceuticals Ltd, London, U.K.) and 1.0% bovine serum albumin (Fraction V; Sigma Chemical Co., St Louis, MO, U.S.A.) (Medium A). After recording the follicular diameter and external appearance, and removing the follicular fluid, the granulosa cells were recovered from each follicle by washing the internal face of the follicle wall repeatedly with Medium A. After removing the oocyte, released clumps of cells were dispersed by pipetting several times through a finely drawn Pasteur pipette. The total number of granulosa cells recovered from each follicle was determined by counting using a haemocytometer. Each follicle was classified as non-atretic or atretic on the basis of its morphological characteristics and number of granulosa cells as described previously (Henderson *et al.*, 1985). The influence of follicular health on steroid production by these granulosa cells has been reported previously (McNatty *et al.*, 1986b). However, because of the relatively few cells in small and/or atretic follicles, and the large number of cells required to perform replicate dose-response studies, cells were pooled according to follicle size irrespective of health. For each ewe, cells were pooled from follicles of 1–2.5 mm, 3–4.5 mm and, in ++ ewes, ≥ 5 mm diameter.

Determination of cellular cAMP production in response to LH and FSH, and oestradiol-17 β synthetase (aromatase activity). Each pool of granulosa cells was split into 2 fractions, one to study cAMP production in response to challenge with LH or FSH, the other to study aromatase activity. The cells were centrifuged at 200 *g* for 10 min and resuspended in Dulbecco's phosphate-buffered saline containing 0.1% bovine serum albumin (DBS-BSA) for determining the cAMP response to gonadotrophins, or in Medium A devoid of sodium heparin (Medium B) for determination of aromatase activity. Aliquants of each were taken for determination of cell number by haemocytometer counts. To determine responsiveness to gonadotrophins, aliquants of granulosa cells (1.5×10^5 cells) in 0.5 ml DBS-BSA were dispensed into a series of 10 $\times$ 75 mm plastic test-tubes. Ovine FSH (NIADDK-oFSH-16) or ovine LH (NIADDK-oLH-24) was added in 0.5 ml DBS-BSA to give a final concentration of gonadotrophin of 0, 0.1, 1, 10, 100 or 1000 ng/ml. In some instances, 3-isobutyl-1-methylxanthine was also included in the incubations at final concentration of 500 μ M. Duplicate or triplicate tubes were established for each dose. The tubes were capped and incubated at 37°C for 1 h in a shaking water bath before being transferred to an 80°C water bath for 15 min. Some tubes were transferred directly to the 80°C bath so that the endogenous cAMP content of the cells at zero time could be determined. All the tubes were frozen (–20°C) until assayed for cAMP by radioimmunoassay. Preliminary studies showed that the rate of cAMP production by granulosa cells incubated for 2 h at 37°C was constant.

For determination of aromatase activity, aliquants of cells ($6\text{--}60 \times 10^4$) in 0.5 ml Medium B were pipetted into duplicate or triplicate tubes containing 0.5 ml of a solution of testosterone (2 μ g/ml) in Medium B. The tubes were gassed with 5% CO₂ in air, capped and incubated for 3 h at 37°C in a shaking water bath. At the end of the incubation, the tubes were snap frozen to –70°C. Subsequently, the contents of the tubes were thawed, centrifuged and the supernatants assayed for oestradiol-17 β . Under these conditions, the rate of oestradiol-17 β production is constant for 3 h.

Radioimmunoassays. Concentrations of cAMP and oestradiol-17 β in incubation media were measured by specific, direct radioimmunoassays described previously (McNatty *et al.*, 1984, 1986a). The limit of sensitivity of the assays (per tube) was 5 fmol for cAMP and 5 pg for oestradiol-17 β . The intra- and inter-assay coefficients of variation were <12%. Values for cAMP and oestradiol-17 β were normalized with respect to granulosa cell number, and the results were expressed as pmol cAMP or ng oestradiol-17 β per 10^6 cells.

Statistics. The data were analysed statistically using analysis of variance in conjunction with Newman-Keuls multiple range test, Student's *t* test or χ^2 as appropriate. When heterogeneity of variance was indicated by Bartlett's test (data for Table 1 and Figs 1 & 2), the variances were equalized by transforming the data to logarithms ($\ln(x + 1)$). In these instances the data have been presented as geometric means together with 95% confidence limits, except in Fig. 1 where for clarity means and the s.e.m. have been presented. The level of significance was set at $P < 0.05$.

Results

Influence of genotype on the number of corpora lutea and antral follicles

The number of corpora lutea recovered from each genotype was 5.1 ± 0.4 (mean $\pm$ s.e.m.) for FF ewes ($N = 15$), 2.8 ± 0.1 for F+ ewes ($N = 17$) and 1.2 ± 0.1 for ++ ewes ($N = 18$) ($FF > F+ > ++$, $P < 0.01$). There was no influence of genotype on the mean numbers of follicles of 1–2.5 or 3–4.5 mm diameter, or the percentage of these follicles which were considered non-atretic. FF, F+ and ++ ewes had 37 ± 4 , 32 ± 3 and 30 ± 2 follicles respectively of 1–2.5 mm diameter, of which $44 \pm 4\%$, $45 \pm 4\%$ and $53 \pm 3\%$ respectively were non-atretic. There were 4.1 ± 0.7 , 3.5 ± 0.3 and 2.5 ± 0.5 follicles of 3–4.5 mm diameter in FF, F+ and ++ ewes respectively, of which $86 \pm 5\%$, $80 \pm 5\%$ and $72 \pm 7\%$ respectively were non-atretic (all values are mean $\pm$ s.e.m.). No follicles of ≥ 5 mm diameter were recovered from the FF ewes while only 2 (both atretic) were recovered from one F+ ewe. Of the 18 ++ ewes, 15 had one follicle ≥ 5 mm in diameter (all non-atretic), one ewe had 2 follicles of this size (1 atretic and 1 non-atretic) and 2 ewes had no follicles ≥ 5 mm in diameter. The number of follicles of each diameter recovered was independent of the time after cloprostenol treatment for all genotypes.

Influence of genotype and follicular diameter on LH and FSH stimulation of cAMP production by granulosa cells

As follicular diameter increased there was a shift to the left in the dose–response curves for FSH and LH stimulation of cAMP production for all genotypes (Fig. 1). With increasing follicular diameter, lower doses of gonadotrophin were able to stimulate cAMP production above basal levels, while higher amounts of cAMP were produced in response to the top doses of gonadotrophin. The minimum doses able to stimulate cAMP production were 10 ng FSH/ml and 0.1 ng LH/ml. At each dose of FSH ≥ 10 ng/ml and of LH ≥ 0.1 ng/ml there was no significant difference between the amounts of cAMP produced by cells from ≥ 5 mm diameter follicles of ++ ewes and cells from 3–4.5 mm diameter follicles of FF or F+ ewes. However, cells from 3–4.5 mm diameter follicles of FF and F+ ewes produced significantly more cAMP than did cells from similar sized follicles of ++ ewes at each dose of FSH ≥ 10 ng/ml or LH ≥ 0.1 ng/ml. There was little difference between FF and F+ ewes except that 100 ng FSH or LH/ml caused a slight, but significant, increase in cAMP production (relative to 0 ng FSH or LH/ml) with cells from 1–2.5 mm diameter follicles in FF, but not F+ ewes. Cells of 1–2.5 mm diameter follicles of FF ewes were therefore on average more sensitive to gonadotrophins than were cells of similar sized follicles of F+ or ++ ewes.

Inclusion of 3-isobutyl-1-methylxanthine (500 μ M) in the incubation medium to inhibit granulosa cell phosphodiesterase activity increased gonadotrophin stimulation of cAMP production by approximately 1.8- to 3-fold, regardless of ewe genotype or follicular diameter.

Influence of time after cloprostenol treatment on FSH and LH stimulation of cAMP production by granulosa cells

Granulosa cells collected from large follicles (≥ 5 mm diam.) of ++ ewes 12–24 h after cloprostenol treatment produced a significantly lower mean amount of cAMP when challenged with FSH or LH (100 ng/ml) than did cells obtained 0–6 h or 36–48 h after cloprostenol (Fig. 2). In F+ and FF ewes, the response of granulosa cells to FSH and LH 12–24 h after cloprostenol was not consistently low, in contrast to the ++ ewes, but was much more variable, as indicated by the large 95% confidence limits. No significant effect of time on the mean response of granulosa cells to FSH or LH was thus evident for the F+ and FF ewes. Mean cyclic AMP production in response to FSH or LH stimulation by cells from follicles < 5 mm in diameter in ++ ewes or 1–2.5 mm in diameter in F+ or FF ewes was also independent of time of collection of the cells.

Fig. 1. Effect of increasing concentrations of (a) FSH and (b) LH on in-vitro cAMP production by granulosa cells from 1-2.5 mm (Δ — Δ), 3-4.5 mm ($\square$ — $\square$) and $\geq$ 5 mm ($\circ$ — $\circ$) diameter follicles of ++, F+ and FF Booroola $\times$ Merino ewes. Values are means of (N) ewes. Vertical lines show the s.e.m. when larger than the size of the symbol. *Lowest concentration of gonadotrophin to stimulate cAMP production significantly (relative to 0 ng/ml) for that follicle size range ($P < 0.05$). Mean values with different letter superscripts for the same dose of FSH or LH are significantly different ($P < 0.05$). Groups of mean values without superscripts are not significantly different.

Fig. 2. Effect of time after cloprostenol treatment on (a) FSH and (b) LH (100 ng/ml for each) stimulation of cAMP production by granulosa cells of large follicles from ++, F+ and FF ewes. Large follicles in ++ ewes are those ≥ 5 mm in diameter and in FF and F+ ewes those 3–4.5 mm in diameter. Values are geometric means with 95% confidence limits indicated by the vertical lines for (N) ewes. Mean values with different letters within the same genotype are significantly different ($P < 0.05$).

Influence of follicular diameter and ewe genotype on granulosa cell aromatase activity

There was a significant increase in granulosa cell aromatase activity as follicular diameter increased in all 3 genotypes (Table 1). The aromatase activity of cells from 1–2.5 mm diameter follicles of F+ and ++ ewes did not differ significantly, but was significantly lower than that of similar sized follicles from FF ewes. The aromatase activity of cells from 3–4.5 mm diameter follicles of F+ and FF ewes was significantly higher ($P < 0.01$) than that of cells from similar sized follicles of ++ ewes, but not significantly different ($P > 0.05$) from the aromatase activity of cells from ≥ 5 mm diameter follicles of ++ ewes.

Table 1. Influence of follicular diameter and genotype on granulosa cell aromatase activity (ng oestradiol-17 β /10⁶ cells)

Genotype	Follicular diameter (mm)		
	1-2.5	3-4.5	≥ 5
++	0.20 ^a (18) 0.15-0.27	1.36 ^c (14) 0.56- 2.57	3.89 ^d (16) 2.13-6.63
F+	0.40 ^a (17) 0.27-0.54	4.43 ^d (16) 2.71- 6.95	—
FF	0.73 ^b (15) 0.33-1.26	6.67 ^d (14) 3.78-11.54	—

Values are geometric means with 95% confidence limits below for (N) sheep.

Values not sharing a common superscript in the same row or column are significantly different ($P < 0.05$).

Table 2. Frequency table showing the relationship between FSH (100 ng/ml) or LH (100 ng/ml) stimulation of cAMP production and cellular aromatase activity

Genotype	Aromatase (ng oestradiol-17 β per 10 ⁶ cells)	cAMP production (pmol/10 ⁶ cells) in response to stimulation with:					
		FSH			LH		
		<1	1-5	>5	<1	1-5	>5
++	<1	22	8	0	27	10	0
	1-5	5	3	2	0	1	2
	>5	0	2	6	0	2	6
F+	<1	16	1	0	17	3	0
	1-5	4	3	2	2	1	2
	>5	0	2	5	0	1	7
FF	<1	9	4	0	8	4	0
	1-5	3	2	2	3	3	1
	>5	0	2	7	0	2	8

Relationship between FSH and LH stimulation of granulosa cell cAMP production, and cellular aromatase activity

For all 3 genotypes, there was a significant positive relationship ($P < 0.01$, χ^2 analyses) between the ability of granulosa cells to metabolize testosterone to oestradiol-17 β (aromatase activity) and the amount of cAMP produced by the cells in response to challenge with FSH or LH (Table 2).

Discussion

In our previous study, we demonstrated differences in LH and FSH stimulation of cAMP production between granulosa cells obtained from F+ and ++ Booroola $\times$ Romney ewes (Henderson *et al.*, 1985). The present study of Booroola $\times$ Merino ewes has confirmed these findings, and in addition shown that the aromatase activity and the responsiveness to LH and FSH

of granulosa cells from FF ewes in turn differs from that of F+ and ++ ewes. In this study, granulosa cells were pooled according to follicular diameter, irrespective of follicle health, whereas in our previous study cells from non-atretic and atretic follicles were studied separately. However, the finding that cells from ++ and F+ ewes responded to LH and FSH in a similar fashion in both studies indicates that, provided follicles are of the same diameter, it makes little difference to the final results whether cells from non-atretic and atretic follicles are pooled or not. This is further supported in that the effects of follicular diameter on granulosa cell aromatase activity, for all 3 genotypes, are similar to findings of the previous study (McNatty *et al.*, 1986b), in which cells from non-atretic and atretic follicles were studied separately.

Follicles of FF, F+ and ++ Booroola × Merino ewes reach preovulatory maturity when 2–4.5 mm, 3–4.5 mm and ≥5 mm in diameter, respectively (McNatty *et al.*, 1986b). This is reflected in the follicular diameters at which granulosa cells show their highest mean response (in terms of cAMP production) to FSH and LH (Fig. 1), and highest mean aromatase activity (Table 1). Although cells from 3–4.5 mm diameter follicles of FF and F+ ewes responded in a similar fashion to both FSH and LH, and had similar aromatase activities, cells from 1–2.5 mm diameter follicles of FF ewes were slightly more sensitive to FSH and LH than were corresponding cells of F+ ewes (Fig. 1), and had a higher mean aromatase activity (Table 1). This is consistent with some follicles of FF ewes attaining preovulatory maturity earlier (i.e. at a smaller diameter) than follicles of F+ ewes. It cannot be ascertained from the present study whether it is an increase in the rate of follicular maturation and/or a decrease in the rate of follicular atresia which allows follicles of F gene-bearing ewes to attain preovulatory maturity at smaller diameters than in ++ ewes.

In all 3 genotypes, granulosa cells became more sensitive to gonadotrophins as follicular diameter increased, as indicated by the shifts in the dose–response curves to FSH and LH (Fig. 1). Although FSH is essential for antral follicle development, the later stages of follicular development in sheep occur in the face of declining plasma concentrations of FSH, brought about by the negative feedback effects of steroids and inhibin produced by the developing follicle(s) (Baird *et al.*, 1981; Miller *et al.*, 1981; Henderson *et al.*, 1986a). Falling plasma FSH concentrations may therefore be compensated for to some extent by granulosa cells becoming more sensitive to FSH, as follicle size increases.

At 12–24 h after cloprostenol treatment, the mean response to FSH and LH of granulosa cells from ≥5 mm diameter follicles of ++ ewes was significantly less than at other times (Fig. 2). Therefore, despite the increased sensitivity of large follicles to FSH, the low plasma FSH concentrations occurring at this time may result in the gonadotrophic environment being inadequate to support fully proper functioning of such large follicles. Previous studies with low fecundity Romney ewes have also shown that when plasma FSH concentrations were depressed, after treatment with follicular fluid, granulosa cells from large (≥5 mm diam.) but not medium or small follicles had a reduced ability to produce cAMP when challenged *in vitro* with FSH or LH (Henderson *et al.*, 1986b). None of the large follicles from ++ ewes examined 12–24 h after cloprostenol treatment were judged atretic by our morphological criteria, despite the reduced responsiveness of the granulosa cells to FSH and LH. However, a reduction in the ability of gonadotrophins to stimulate cAMP production by granulosa cells may represent a very early, possibly reversible, stage of atresia. Morphological signs of atresia first appear relatively late in the atretic process, some considerable time after the initial biochemical lesions have occurred. In F+ and FF ewes there was no significant effect of time on FSH or LH stimulation of cAMP production by granulosa cells from large follicles (Fig. 2). Cells from a lower proportion of the follicles examined from F+ and FF ewes, than from ++ ewes, showed a low responsiveness to FSH and LH 12–24 h after cloprostenol treatment. Perhaps in F gene-bearing ewes large follicles may be more resistant to the deleterious effects of low plasma FSH concentrations, and/or plasma FSH concentrations are higher than those of ++ ewes (Bindon *et al.*, 1984; McNatty *et al.*, 1987).

There was a significant positive relationship between granulosa cell aromatase activity and FSH or LH stimulation of cAMP production in all genotypes (Table 2). There were no instances of the

cells having a high responsiveness to FSH or LH but a low aromatase activity, or *vice versa*. In sheep, as in the rat (Wang *et al.*, 1982), FSH stimulation of cAMP production may be an essential early event necessary for full functioning of the aromatase enzyme complex. Whether production of large amounts of cAMP in response to LH is a cause or consequence of high cellular aromatase activity is uncertain.

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References

- Baird, D.T., Swanston, I.A. & McNeilly, A.S. (1981) Relationship between LH, FSH and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe. *Biol. Reprod.* **24**, 1013–1025.
- Bindon, B.M. (1984) Reproductive biology of the Booroola Merino sheep. *Aust. J. biol. Sci.* **37**, 163–189.
- Bindon, B.M., Piper, L.R., Cummins, L.J., O'Shea, T., Hillard, M.A., Findlay, J.K. & Roberston, D.M. (1984) Reproductive endocrinology of prolific sheep: studies of the Booroola Merino. In *Genetics of Reproduction in Sheep*, pp. 217–236. Eds R. B. Land & D. W. Robinson. Butterworths, London.
- Davis, G.H., Montgomery, G.W., Allison, A.J., Kelly, R.W. & Bray, A.R. (1982) Segregation of a major gene influencing fecundity in progeny of the Booroola sheep in New Zealand. *N.Z. J. agric. Res.* **25**, 525–529.
- Eagle, A. (1959) Amino acid metabolism in mammalian cell cultures. *Science, N.Y.* **130**, 432–437.
- Henderson, K.M., Kieboom, L.E., McNatty, K.P., Lun, S. & Heath, D. (1985) Gonadotrophin-stimulated cyclic AMP production by granulosa cells from Booroola × Romney ewes with and without a fecundity gene. *J. Reprod. Fert.* **75**, 111–120.
- Henderson, K.M., Ellen, R.L., Savage, L.C., Ball, K. & McNatty, K.P. (1986a) Effect on ovulation rate of increasing or decreasing ovarian exposure to follicle stimulating hormone during the preovulatory period in ewes. *Proc. N.Z. Soc. Anim. Prod.* **46**, 157–160.
- Henderson, K.M., Prisk, M.D., Hudson, N., Ball, K., McNatty, K.P., Lun, S., Heath, D., Kieboom, L.E. & McDiarmid, J. (1986b) Use of bovine follicular fluid to increase ovulation rate or prevent ovulation in sheep. *J. Reprod. Fert.* **76**, 623–635.
- McNatty, K.P., Hudson, N.L., Henderson, K.M., Lun, S., Heath, D.A., Gibb, M., Ball, K., McDiarmid, J.M. & Thurley, D.C. (1984) Changes in gonadotrophin secretion and ovarian antral follicular activity in seasonally breeding sheep throughout the year. *J. Reprod. Fert.* **70**, 309–321.
- McNatty, K.P., Henderson, K.M., Lun, S., Heath, D.A., Ball, K., Hudson, N.L., Fanning, J., Gibb, M., Kieboom, L.E. & Smith, P. (1985) Ovarian activity in Booroola × Romney ewes which have a major gene influencing their ovulation rate. *J. Reprod. Fert.* **73**, 109–120.
- McNatty, K.P., Kieboom, L.E., McDiarmid, J., Heath, D.A. & Lun, S. (1986a) Adenosine cyclic 3',5'-monophosphate and steroid production by small ovarian follicles from Booroola ewes with and without a fecundity gene. *J. Reprod. Fert.* **76**, 471–480.
- McNatty, K.P., Lun, S., Heath, D.A., Ball, K., Smith, P., Hudson, N.L., McDiarmid, J., Gibb, M. & Henderson, K.M. (1986b) Differences in ovarian activity between Booroola × Merino ewes which were homozygous, heterozygous and non-carriers of a major gene influencing their ovulation rate. *J. Reprod. Fert.* **77**, 193–205.
- McNatty, K.P., Hudson, N., Henderson, K.M., Gibb, M., Morrison, L., Ball, K. & Smith, P. (1987) Differences in gonadotrophin concentrations and pituitary responsiveness to GnRH between Booroola ewes which were homozygous (FF), heterozygous (F+) and non-carriers (++) of a major gene influencing their ovulation rate. *J. Reprod. Fert.* **80**, 577–588.
- Miller, K.F., Nordheim, E.V. & Ginther, O.J. (1981) Periodic fluctuations in FSH concentrations during the ovine estrous cycle. *Theriogenology* **16**, 669–679.
- Wang, C., Hsueh, A.J.W. & Erickson, G.F. (1982) The role of cyclic AMP in the induction of estrogen and progesterin synthesis in cultured granulosa cells. *Molec. cell. Endocr.* **25**, 73–83.

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