How does the F gene influence ovulation rates in booroola ewes? A 1990 perspective

K.P. McNATTY, K.M. HENDERSON, J.S. FLEMING, C.A. PRICE AND I.J. CLARKE[1]

Wallaceville Animal Research Centre, MAF Technology,
P.O. Box 40063, Upper Hutt, New Zealand

ABSTRACT

The purpose of this paper is to summarise recent findings on the reproductive physiology of the Booroola ewe, with specific attention to factors influencing ovulation-rate (OR). The ovarian antral follicle populations, incidence of atresia, steroid secretion-rate and maximum OR potential in the different Booroola genotypes (FF, homozygotes; F+, heterozygotes; ++, non-carriers) are the same notwithstanding the fact that FF, F+ and ++ animals normally ovulate ≥5, 3-4 and 1-2 follicles respectively during each oestrous cycle. Moreover, the Booroola F gene does not appear to modify the characteristics of the follicle-stimulating hormone (FSH) or luteinizing hormone (LH) receptors, or the gonadotrophin-sensitive components of the cyclic AMP generating system or the steroid or inhibin biosynthetic functions in follicular cells. It seems that the F gene influences follicular growth before antrum formation resulting in fewer granulosa cells at all phases of antral growth and smaller sized follicles at ovulation. In essence, the ≥5, 3-4 or 1-2 presumptive preovulatory follicles in FF, F+ or ++ ewes are respectively 2.5-4.5 mm, 4-5 mm and >5 mm in diameter just before ovulation but the total populations of oestrogen-secreting follicular or progesterone-secreting luteal cells in the different genotypes are the same. These genotypic differences in ovarian characteristics have not been replicated by any of the known superovulation regimes.

At the level of the hypothalamus and pituitary no gene-associated differences have been noted in the concentrations of gonadotrophin-releasing hormone (GnRH) in the tissues or hypophyseal portal blood nor in the binding characteristics of GnRH to the pituitary gland. However F gene-specific differences have been noted in the plasma concentrations of FSH and LH in ovary-intact and ovariectomized (OVX) Booroola ewes (FF > ++; $P<0.05$). The F gene-specific differences in FSH but not LH in OVX ewes can be replicated by exogenous GnRH treatment of OVX ewes deficient in endogenous GnRH following surgical disconnection of the hypothalamus from the pituitary gland but with the superior hypophyseal arteries being left intact. The greater frequency of higher FSH values in ewes is also observed in both intact and castrate Booroola FF rams relative to ++ rams at $P\leq0.07$. When considered altogether the evidence suggests that Booroola sheep with the F gene have higher mean FSH concentrations in plasma relative to ++ sheep. However the differences are small with considerable overlap between animals of the different genotypes. It therefore remains to be established whether modest increases in plasma FSH concentration in ++ Booroola ewes are sufficient to modify follicular morphology and OR without altering steroid secretion.

Keywords Ovarian follicles, pituitary, hypothalamus, granulosa cells, FSH, LH, sheep, Booroola gene

INTRODUCTION

The Booroola Merino is one of the most prolific sheep breeds in the world (Bindon 1984). The exceptional prolificacy of the Booroola has been attributed to a single gene which influences its ovulation-rate (OR) as judged by the number of *corpora lutea* (CL) present during each oestrous cycle (Davis *et al.*,1982; Piper and Bindon, 1982). The putative gene is referred to as the fecundity or F gene. Homozygous (FF), heterozygous (F+) and non-carriers (++) have been distinguished on the basis of OR recordings of ≥5, 3 or 4 and 1 or 2 respectively (Davis *et al.*, 1982). In the Booroola ram no obvious physiological characteristics have been noted by which to distinguish the three genotypes.

The purpose of this paper is to summarise recent findings on the reproductive physiology of the Booroola ewe with specific attention to factors influencing OR.

THE OVARY

Follicular Characteristics

The population of antral follicles and incidence of follicular atresia in Booroola ewes of the different genotypes are similar to one another and to those found

[1] Medical Research Centre, Prince Henry's Hospital, St. Kilda Road, Melbourne, Australia

in other breeds/strains such as the Romney [Table 1; McNatty *et al.* (1986a)]. However during the oestrous cycle or anoestrus, follicles in FF ewes grow to a maximum of 4.5 mm diameter whereas in ++ or Romney ewes, at least one follicle grows beyond 5 mm diameter (Table 1). Irrespective of breed or genotype follicular maturation is associated with an increasing capacity to synthesize oestradiol (McNatty, 1982). A close correlation exists between the number of oestrogen-enriched follicles and OR (McNatty *et al.*, 1983, 1986a). In FF ewes, oestrogen-enriched follicles have diameters of 2-4 mm whereas in ++ or Romney ewes such follicles have diameters exceeding 4 mm (Table 1; McNatty *et al.*, 1986a). In Booroolas, as in other strains of Merino or Romney, granulosa cells are the major source of oestradiol since they are the only follicular cell type capable of metabolising (i.e. aromatising) androgen to oestradiol (McNatty *et al.*, 1986a). Follicles containing granulosa cells with aromatase activity are present on most days of the oestrous cycle and also during anoestrus. In FF ewes between 3 and 8 follicles have a capacity for oestrogen synthesis, whereas in ++ or Romney ewes only 1 or 2 such follicles have this capacity. This difference between the genotypes may arise because of the stimulation of aromatase enzyme activity in smaller sized FF or F+ follicles which are more frequent in numbers relative to the numbers of follicles of larger diameter (Table 1).

The effects of short-term exogenous hormone therapy to superovulate sheep are limited, at least in part, by the number of antral follicles present. Since the different Booroola genotypes have similar populations of antral follicles, one might expect similar OR when all are exposed to saturating amounts of superovulating hormones (e.g. pregnant mare's serum gonadotrophin, PMSG). This notion is supported by the results of Kelly *et al.* (1983/84) who found a mean OR of ~7 in both F+ (N=17) and ++ (N=19) ewes after 1000 iu PMSG had all been administered to all animals.

A significant feature of ovaries of Booroola ewes is that non-atretic antral follicles in FF or F+ ewes contain fewer granulosa cells compared to similar sized antral follicles from ++ or Romney ewes (McNatty *et al.*, 1986a). This difference in the population of granulosa cells also occurs before puberty (McNatty *et al.*, 1987a). In FF, F+ and ++ Booroola ewes with corresponding ovulation-rates of ≥5, 3 or 4 and 1-2, the total number of oestrogen-secreting granulosa cells per ewe was similar (i.e. $\approx 5.4 \times 10^6$), as were the ovarian secretion-rates of androstenedione, testosterone and oestradiol.

Thus, although the total numbers of antral follicles, proportions of non-atretic follicles, and maximum OR potential in the different Booroola genotypes are the same, the F gene results in the maturation of a larger number of smaller preovulatory follicles, each containing fewer granulosa cells. However, the total complement of granulosa cells per ewe and the ovarian steroid secretion-rates appear similar between the genotypes.

TABLE 1 Ovarian follicle numbers in FF and ++ Booroola x Merino ewes and in Romney ewes

Breed	Genotype (N)	Follicular diameter (mm) >0.1 -0.4	>0.5 -0.9	>1.0 -1.4	>1.5 -1.9	>2.0 -2.4	>2.5 -2.9	>3.0 -3.4	>3.5 -4.0	>4.0 -4.4	>4.5 -4.9	>5.0	Total
Booroola	FF (6)	15 (9,27)	32 (18,56)	10 (6,19)	16 (8,33)	4 (1,10)	1 (0,3)	2 (1,4)	1 (0,3)	0 (0,1)	-	-	87 (53,140)
	++ (8)	12 (6,21)	24 (13,45)	9 (5,14)	13 (7,24)	8 (5,13)	4 (2,8)	3 (1,5)	1 (0,3)	1 (0,1)	0 (0,1)	1 (0,2)	84 (57,124)
Romney	- (8)	16 (11,24)	25 (16,40)	7 (5,11)	9 (6,12)	6 (4,10)	6 (3,9)	3 (1,8)	1 (0,2)	1 (0,2)	0 (0,1)	1 (0,1)	86 (66, 112)

Values are geometric means (and 95% confidence limits). N = number of ewes. The follicles enclosed by [ ] are those which have a potential for peak oestradiol synthesis (see McNatty *et al.* 1986a). None of the values in any of the columns are significantly different from one another.

Luteal Characteristics

After ovulation, the plasma progesterone concentrations, total luteal weight and cell composition in FF and ++ ewes on Days 4, 10 and 12 of the oestrous cycle were found to be the same, notwithstanding differences in the number of CL (Niswender *et al.*, 1990). As the OR increases in ++ ewes from 1 to 2 or in superovulated ewes from 1 to >5, there is a significant but relatively small decline in the mean weight of individual CL (Table 2). By contrast in FF ewes, as the OR increases from 3-5 to >5, there is no significant change in the mean weight of individual CL and moreover within sheep, the CL are much smaller overall and tend to be more uniform in weight (i.e. 0.1-0.25 mg) compared to those in superovulated ewes (i.e. 0.1-0.60 mg) (Kelly *et al.*, 1983, 1984, Henderson *et al.*, 1988). As the OR increases in FF ewes there is no major increase in plasma progesterone or total weight of luteal cell tissue, both of which remain similar to ++ ewes or Romney ewes with an OR of 1-2. However, in superovulated ++ or Romney ewes there is both a corresponding increase in plasma progesterone and total CL mass.

TABLE 2 Mean weight (g) of individual corpora lutea (CL) per ewe with respect to ovulation-rate (OR) in Booroola x Merino ++ and FF ewes and in superovulated Romney ewes

OR	Booroola Genotype ++	Booroola Genotype FF	Superovulated Romney Ewes
1	0.66±0.02[a] [25]	-	0.73±0.02[a] [25]
2	0.56±0.02[b] [16]	-	0.55±0.01[b] [46]
3-5	-	0.18±0.02[a] [14]	0.41±0.02[c] [11]
>5	-	0.14±0.01[a] [10]	0.34±0.01[d] [13]

Values are means ± s.e.m. Numbers in square brackets = number of ewes; values in columns with different alphabetical superscripts are significantly different (P<0.05; ANOVA). CL from all animals were recovered between Days 8-12 of the oestrous cycle. Data from K.M. Henderson and K.P. McNatty (unpublished data).

Gonadotrophin Responsiveness, Inhibin and Steroid Synthesis

The gonadotrophins' follicle stimulating hormone (FSH) and luteinizing hormone (LH) are determinants of follicular maturation and ovulation-rate (McNatty, 1982; Henderson and McNatty, 1987). FSH and LH exert their effects by first binding to specific cell types in the ovary, namely the theca and/or granulosa cells. The subsequent cellular response to gonadotrophin binding is mediated, at least in part, by the intracellular chemical messenger adenosine 3',5'-cyclic monophosphate (cAMP). In turn, increased cAMP synthesis may lead to an increase in follicular steroid and/or inhibin synthesis.

Studies with ^{125}I-labelled human FSH or ^{125}I-human chorionic gonadotrophin (hCG; as a surrogate for LH) revealed no F gene-specific differences in the characteristics of FSH binding to granulosa cells or LH binding to theca interna, luteal or granulosa cells (McNatty *et al.*, 1986b, 1989a).

If it is assumed that 1-2.5 mm and 3-4.5 mm diameter follicles in FF ewes are respectively at similar phases of development as 3-4.5 mm and ≥5 mm diameter follicles in ++ ewes, no significant genotypic effects were found with respect to FSH or LH induced cAMP synthesis or catabolism or with respect to the ability of various pharmacological agents (i.e. cholera toxin, forskolin or pertussis toxin) to stimulate the membrane components of the cAMP generating system (McNatty *et al.*, 1990). Likewise, no gene-specific differences were observed in the ability of granulosa cells to synthesize bio- or immunoreactive inhibin or oestradiol or that of theca interna cells to synthesize androgen (K.M. Henderson, unpublished data; McNatty *et al.*, 1986a). Thus the Booroola F gene seems to result in the earlier maturation (or differentiation) of follicles without altering gonadotrophin receptor - cAMP - steroid or inhibin biosynthetic functions in ovarian follicular cells. It seems that the F gene influences follicle maturation before antrum development, but precisely how or when remains obscure. *In vitro* studies with mouse ovaries show that the growth of primary (small

preantral) follicles is influenced profoundly by FSH and other serum factors which are presumed to be growth factors (Ryle, 1969; Quist *et al.*, 1990). Whether these or other hormones are regulated/influenced by the F gene remains to be determined.

THE HYPOTHALAMIC PITUITARY AXIS

F gene-specific differences have been found in the plasma concentrations of FSH and LH in both ovary intact and ovariectomized (OVX) Booroola ewes and in concentrations of FSH but not LH in Booroola ewe lambs (Bindon, 1984; McNatty *et al.*, 1987b; Braw-Tal and Gootwine, 1989; McNatty *et al.*, 1989b; Montgomery *et al.*, 1989). The FSH differences between the genotypes are small and only observed by serial sampling over long periods of time. In general, the FSH values are more frequently higher in FF compared to ++ ewes ($P<0.05$; McNatty *et al.*, 1987b, 1989b,c). In some but not all experiments, the FF ewes also have higher amplitude LH pulses compared to ++ ewes (McNatty *et al.*, 1989b,c). In both gonad-intact and castrated Booroola rams, the FSH but not LH values were also higher in FF compared to ++ genotypes at $P\leq0.07$ (Price *et al.*, 1990). The differences in gonadotrophin secretion do not seem to be due to differences in the negative feedback effects of gonadal hormones. For example, no evidence from studies in gonad-intact or castrated ewes or rams has been found for gene-specific differences in the sensitivity of the hypothalamic-pituitary axis to progesterone (ewes), oestradiol (ewes), testosterone (rams) or steroid-free bovine follicular fluid as a source of inhibin (ewes and rams) (McNatty *et al.*, 1989c; Price *et al.*, 1990).

However in both gonad-intact ewes and rams, differences have been noted in the pituitary FSH and/or LH responses to GnRH (McNatty *et al.*, 1987b; Price *et al.*, 1990). In ewes, the FSH and LH response to a bolus GnRH injection was lower in the FF compared to the ++ genotype (McNatty *et al.*, 1987b). In rams, the FSH and LH responses to GnRH were higher in FF than in ++ rams (Price *et al.*, 1990). The reasons for the sex differences are not clear but may relate to time of the year and endogenous hormonal milieu at the time of testing. In any event, these studies when considered together suggest that F gene-specific differences may exist at the level of the pituitary gland and in the responsiveness of this gland to GnRH. Additional studies on the hypothalamic-pituitary axis have not demonstrated any other gene-specific differences. For example, studies on brain contents of GnRH, hypothalamic-portal blood concentrations of GnRH or its secretion-rates, or the pituitary binding characteristics for GnRH have not revealed F gene-specific differences (Gale *et al.*, 1988; Fleming *et al.*, 1990; K.P. McNatty and I.J. Clarke, unpublished data). Furthermore, no differences have been found from FSH half-life clearance studies or FSH isoforms to suggest that the F gene is influencing these processes (Robertson *et al.*, 1984; Fry *et al.*, 1987). However further studies on rates of pro-GnRH synthesis and on FSH isoforms are needed before these possible sources of F gene difference can be discounted.

In order to test pituitary sensitivity to GnRH *in vivo* it is desirable to have an animal devoid of endogenous GnRH and gonadal hormones but with a functionally intact pituitary gland. The hypothalamic-pituitary disconnected ovariectomized ewe (HPD-OVX) as described by Clarke *et al.* (1983) is perhaps the most suitable experimental animal. Recently, mixed aged (3-8 yr) Booroola Merino or Romney ewes (N=21/genotype; FF or ++) which had been OVX 2-4 months previously were subjected to HPD surgery and blood sampled once weekly for 6 weeks to establish basal FSH and LH concentrations. Thereafter these animals were blood sampled every 2nd or 3rd day for 5 weeks during which time the animals were pulsed intravenously with 250 ng GnRH once every 2 h for the duration of the experiment. Before GnRH treatment, no gene-specific differences in FSH and LH concentrations were noted on any sampling day. In the GnRH-HPD-OVX ewes the mean (± s.e.m.) FSH/LH concentrations before GnRH treatment were 1.0(±0.1)/0.4(±0.04) and 1.1(±0.1)/0.5(±0.1) ng/ml respectively for FF (N=21) and ++ ewes (N=21). However, after GnRH treatment began, the plasma FSH but not LH concentrations were significantly higher in FF compared to ++ ewes (Figure 1), while no gene differences were noted in either pituitary weights or pituitary FSH or LH contents. In contrast OVX ewes the mean (± s.e.m.) FSH/LH concentrations before OVX were 1.8(±0.1)/0.3(±0.1) and 1.2(±0.1)/0.3(±0.1) ng/ml respectively for FF (N=21) and ++ ewes (N=21); the FSH but not LH concentrations were significantly higher in the FF compared to ++ ewes ($P<0.01$). After OVX the plasma FSH but not LH

concentrations were also significantly higher in FF compared to ++ ewes ($P<0.05$) (Figure 1).

In summary, the studies on HPD-OVX Booroola ewes suggest that FF animals are more sensitive to GnRH with respect to FSH but not LH secretion, but that this difference in sensitivity is not expressed at the level of the GnRH receptor.

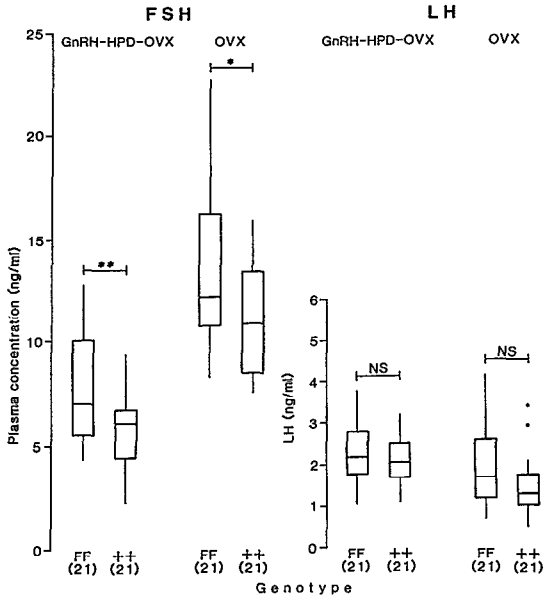

FIG 1 Box and Whisker plots of mean FSH and LH plasma concentrations from hypothalamic-pituitary-disconnected OVX Booroola FF and ++ ewes treated intravenously with 250 ng GnRH in saline with 0.1% ovine serum albumin once every two hours for 36 days (i.e., GnRH-HPD-OVX ewes) or from a different flock of OVX Booroola FF and ++ ewes.

CONCLUSIONS

The OR differences between the FF, F+ and ++ genotypes appear to involve a factor(s) influencing ovarian follicle maturation/differentiation either before or during antrum formation. It is by no means certain as to whether this factor(s) is of ovarian or extraovarian origin. If the factor is hormonal it has not yet been measured in any intensive way (e.g. inhibin, IGF_1, IGF_2 or IGF binding proteins) or if it has been measured (e.g. FSH) then it is operating at a level of subtlety that hasn't been fully appreciated hitherto. For example, the difference in the mean concentrations of plasma FSH between FF and ++ ewes and rams is small (i.e. <2 ng/ml) and there is a considerable overlap between the genotypes. Nevertheless, the finding that the gene difference in FSH secretion in ovary-intact and OVX ewes can be replicated by GnRH therapy to HPD-OVX ewes suggests that some association between the F gene and GnRH-induced pituitary FSH secretion exists. Moreover, FSH has been found to promote preantral follicular growth (Ryle, 1969; Quist *et al.*, 1990), stimulate oestrogen biosynthetic activity in small antral follicles (2-4.5 mm diameter; McNatty *et al.*, 1985) and lead to the formation of CL at smaller diameters (Henderson *et al.*, 1988). Thus, in many respects FSH is a suitable hormone to be linked to the F gene. However it remains to be established whether modest increases in plasma FSH concentration in ++ Booroola ewes would be sufficient to modify the population of granulosa cells in individual follicles, stimulate the formation of 3-8 'oestrogenic' follicles and subsequently 3-8 CL of uniform size without significantly changing the plasma steroid concentrations. In any event a more detailed understanding of how OR is regulated in Booroola ewes offers new possibilities for the controlled superovulation of livestock.

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