# OVULATION RATE IN THE EWE: MECHANISMS UNDERLYING GENETIC VARIATION.

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## INTRODUCTION

Marked genetic differences in ovulation rate provide a potentially powerful tool to elucidate the mechanisms which control ovulation rate. Many breeds of sheep have high ovulation rates and these can be divided into two groups. The first group includes the Romanov, Finnish Landrace and D'Man breeds, in which high ovulation rate reflects polygenic differences for genes with small effects. The second group reflects the action of a single gene, or a closely linked group of genes, such as the Booroola (*Fec*$^{B}$) Merino, Icelandic, Javanese Fat-Tail, Inverdale (*FecX* $^{I}$) Romney, Belclare, Cambridge and Olkuska. This paper reviews results from studies on gonadotrophin secretion, follicular growth and aspects of ovarian function in relation to genetic differences in ovulation rate in order to determine the likely underlying mechanisms. Are these operating primarily at the ovarian level or are the differences in ovarian function caused by differences at the level of the hypothalamus/pituitary gland?

### Control at the level of the hypothalamus/pituitary gland.

The possibility that ovulation rate may be causally related to differences in hypothalamic-/pituitary function, particularly concentrations of peripheral gonadotrophins, has been investigated. Studies in ewes with the *Fec*$^{B}$ gene found no differences in either GnRH concentrations in any area of the brain or GnRH receptor number in the pituitary gland (see reviews by McNatty *et al.* 1990; Montgomery *et al.* 1992).

*Luteinising hormone (intact ewes).* The general consensus is that peripheral LH concentration is not correlated with ovulation rate (Bindon *et al.* 1985). For example, a comparison of the patterns of LH in lines of Finn sheep selected on ovulation rate (high line 4.1, low line 2.7) found no consistent relationship between patterns of pulsatile LH release and ovulation rate (Haresign *et al.* 1995). Similarly, although LH pulses of greater amptitude have been measured in intact homozygous/heterozygous *Fec*$^{B}$ ewes, compared to non-carriers (McNatty *et al.* 1987), significantly higher LH concentrations were consistently observed (McNatty *et al.* 1989b). Studies of carrier and non-carrier Cambridge (ovulation rate: 8.7 vs 3.1) and Belclare (ovulation rate: 5.2 vs 2.2) ewes yielded no evidence for a significant association between carrier status and LH secretion. In support of this conclusion, that pattern of LH secretion is not related to ovulation rate, the mechanism controlling ovulation rate in Finnish Landrace, Romanov and Booroola Merino ewes are present throughout the reproductive cycle (Webb & Gauld, 1985; Driancourt *et al.* 1988; Webb *et al.* 1989; Driancourt *et al.* 1990). Oestrogen-active follicles can be found during both seasonal anoestrus and the breeding season (luteal and follicular phases), with their number being representative of the ovulation rate for the breed. Also ewes can be

induced to ovulate throughout the reproductive cycle following a challenge with an ovulatory dose of hCG (750 i.u.) and the number of induced ovulations is representative of the breed. Therefore, despite differences in the frequency of LH pulses during the breeding season versus seasonal anoestrus, the number of mature Graafian follicles is maintained.

*Follicle stimulating hormone (intact ewes).* Observations on serum FSH are equivocal. In some sheep breeds (D'Man, Finn), which are highly prolific due to polygenic effects, the concentrations of FSH during the preovulatory period, the gonadotrophin surge and the secondary FSH surge, are significantly increased compared to FSH concentrations in breeds of lower prolificacy (D'Man, Lahlou-Kassi *et al.* 1984; Finnish Landrace, Haresign *et al* 1995). In contrast, other studies (Finnish Landrace versus the less prolific Suffolk), during the preovulatory period, have failed to demonstrate a relationship between higher FSH concentrations and ovulation rate (Webb & England 1982). Further, in lines of Finn-Dorset ewes selected on testis size, in which no correlated difference in ovulation rate was observed, there was a significant difference in FSH concentration between the lines during the follicular phase (McNeilly *et al.* 1988). The physiological significance of FSH differences is further questioned by the recent finding of Fry & Driancourt (1996) that within the same ewes over three successive oestrous cycles, there was no relationship between ovulation rate and FSH concentration. Finally, Adams *et al,* (1988) found that FSH concentrations in two lines of Finnish Landrace sheep were lower than in the less prolific Galway ewes.

Earlier reports for Booroola ewes have also been equivocal. For example, Bindon *et al.* (1988) found no significant difference between ewes with the $Fec^B$ gene, compared to ewes lacking the gene, for the period from 96 h to 24 h prior to the preovulatory gonadotrophin surge, the period when selection of ovulatory follicles occurs. In contrast, in another study around the time of luteolysis, Booroola ewes ($Fec^BFec^B$ ) had significantly higher FSH concentrations than controls ($Fec^+Fec^+$), while $Fec^BFec^+$ ewes had intermediate FSH concentrations (McNatty *et al.* 1987). Similar results have also been obtained first by Boulton *et al.* (1995), in a $F_2$ population in which the background genotype of all sheep was on average 50% Booroola Merino and 50% Scottish Blackface and second by Fry & Driancourt (1996), when comparing $Fec^BFec^+$ versus $Fec^+Fec^+$ Merino d'Arles ewes with 2 ovulations.

The situation is further complicated by the finding that a number of FSH isoforms have been identified that may differ in biological potency. However, Robertson *et al,* (1984) reported no quantitative difference in pituitary FSH between Booroola and control Merinos on day 3 of the oestrous cycle. Phillips *et al.* (1993) confirmed that there are no overall differences in FSH isoforms between Booroola genotypes. However, these authors did report differences between days 13-16 of the oestrous cycle, so this explanation cannot be ignored completely.

*Gonadotrophins in ovariectomized ewes.* Increased ovulation rate in some breeds may reflect a lower sensitivity of the hypothalamus/pituitary gland to the negative feedback effects of ovarian hormones. Oestradiol can reduce ovulation rate in Booroola Merinos (O'Shea & Hillard, 1996), with a possible suggestion of differences in response/sensitivity between carriers and non-carriers. Higher concentrations of ovarian hormones need not therefore result in a greater

reduction in the release of gonadotrophin (Land, 1976). Hence, there could be differences in ovulation rate, without any differences in peripheral gonadotrophins concentrations. If this hypothesis is correct, although this could not be confirmed in Finn lines (Webb *et al.* 1992), differences in FSH concentrations should be observed between high and low prolific breeds or strains after removal of the inhibitory actions of ovarian hormones.

Examination of FSH concentrations in both High and Low Finn lines and Belclare carriers and non-carriers found no evidence for a difference following ovariectomy (Hanrahan, unpublished observations). McNatty *et al.* (1989b) have also presented data for long-term ovariectomized Booroola ewes which show no differences between $Fec^{B}Fec^{+}$ and $Fec^{+}Fec^{+}$ ewes. In contrast, long-term ovariectomized Finnish Landrace ewes were found to have higher FSH concentrations than the less prolific Scottish Blackface ewes (Webb *et al.* 1985), although interpretation is limited by the between breed comparison. Workers in New Zealand subsequently suggested that Booroola ewes carrying the $Fec^{B}$ gene do have higher concentrations following ovariectomy (McNatty *et al.* 1990; Phillips *et al,* 1993) and this has been confirmed by Boulton *et al.* (1995). Interestingly a within breed, between line comparison of Finn-Dorset ewes, selected on testis size, but with no between line difference in ovulation rate, did exhibit a significant between line difference in FSH concentrations 2-15 days following ovariectomy (McNeilly *et al.* 1988). Importantly, even though there was no significant difference in FSH concentrations between heterozygous Booroola ewes and non-carriers, a significant difference in ovulation rate was maintained (Boulton *et al.* 1995). Overall these results suggest that the $Fec^{B}$ gene may be operating at the level of the pituitary gland to account for these differences in peripheral concentrations. It appears not to be operating within the hypothalamus since no $Fec^{B}$ gene-specific differences were noted for GnRH with respect to pulse frequency, pulse amptitude or overall secretion rate (McNatty *et al.* 1993). Although the $Fec^{B}$ gene may cause increased peripheral concentrations of FSH, recently Fleming *et al.* (1995) failed to find differences either in pituitary gonadotrophin content or gonadotrophin subunit gene transcription. It was suggested that differences in FSH secretion caused by the $Fec^{B}$ gene are more likely to arise from differences in post-translational modification or secretion of the FSH protein in $Fec^{B}$ carriers, although as already discussed, there appear to be no overall differences in FSH isoforms between Booroola genotypes.

*Conclusions on the importance of peripheral gonadotrophin concentrations.* Results fail to provide consistent evidence for differences in peripheral FSH concentrations being the primary cause of high ovulation rate in ewes carrying either a major gene or multiple genes. However, interpretation can be problematical when comparisons are made between breeds, or even within breeds, when inappropriate controls are used. There is substantial genetic variation between individuals, within a population, for physiological traits such as peripheral gonadotrophin concentration. Also this variation is not necessarily directly related to genetic variation in prolificacy. For example, the heritability of LH release after a GnRH challenge is 0.44 (Haley *et al.* 1989) and similarly 0.44 for FSH concentration in prepubertal lambs (Bodin *et al.* 1988). This demonstrates that two breeds or two strains within a breed may well differ in traits such as gonadotrophin concentrations simply due to chance variation in the genes that were sampled in their foundation. Even for within breed comparisons, care must be exercised because the

presence of genetic variation means that differences between families will exist which may be independent of the trait of interest. Therefore two groups of progeny, from two different sires, may well differ in peripheral hormone concentrations for this reason. The presence of such 'sire effects' means that trials should include progeny from several or many sires and even then the data should be analysed taking account of its genetic structure (Patterson & Thompson, 1973).

**Control at the level of the ovary.**

Gonadotrophins are required for the growth of follicles, particularly those >2 mm diameter (see Campbell *et al.* 1995). Three studies in hypophysectomized or hypothalamic-pituitary disconnected ewes, two in Booroola Merinos (Fry *et al.* 1988: McNatty *et al.* 1993) and the other in Romanov and Ile de France ewes (Driancourt *et al.* 1988), have demonstrated that the differences in ovulation rate could be maintained with exogenous gonadotrophins. These results indicate that high prolificacy, due either to a single gene or a number of genes, is due primarily to ovarian rather than pituitary factors. This conclusion has recently been confirmed in Scottish Blackface $Fec^B$ gene carriers and non-carriers, where ovarian autotransplants were used to facilitate the collection of ovarian venous blood and detailed scanning of ovarian responses. Ewes were treated with GnRH-antagonist plus progestagen sponges for 3 weeks to suppress endogenous FSH and LH and then stimulated with a physiologically standardized gonadotrophin regime (Campbell *et al.* 1996a). Despite the same FSH and LH treatment, $Fec^B$ gene carriers had significantly more (3.5±0.2) preovulatory follicles/corpora lutea than non-carriers (1.7 ± 0.3).

Using a similar model, with GnRH antagonist treatment, (Hanrahan, unpublished observations) differences in ovarian response between carrier and non-carrier Cambridge ewes was also observed. However, in this case, administration of exogenous ovine FSH evoked follicle growth in a significantly greater proportion of the non-carriers, suggesting reduced ovarian response/sensitivity in carriers. In contrast, using physiologically relevant granulosa and theca culture systems (Campbell *et al.* 1996b), it was demonstrated that the $Fec^B$ gene acts by enhancing ovarian sensitivity and/or response to gonadotrophins (Webb *et al.* 1995), as assessed by increased steroid production. These results, while not definitive, suggest that the genetic differences in ovulation rate are due to ovarian differences rather than differences in pituitary function.

**Ovarian follicle growth and maturation.**

If the site of action of either a major gene or a number of genes regulating ovulation rate is within the ovaries, have factors been identified that may be involved? A variety of studies have shown that the large antral follicle population is not correlated to ovulation rate (see Webb & Gauld 1985). However, ovarian follicles in Merinos, carrying the $Fec^B$ gene, mature and ovulate at a significantly smaller diameter and have significantly fewer granulosa cells than non-carriers (see McNatty *et al.* 1990). This has recently been confirmed with ultrasound scanning of autotransplanted ovaries in Scottish Blackface $Fec^B$ carrier and non-carrier ewes. In addition, in $Fec^B$ ewes during the luteal phase, the diameter of corpora lutea and the largest follicles in the first follicular wave were smaller than those in non-carrier ewes (Souza, Campbell, Webb & Baird, unpublished observations). Booroola ewes also appear to have an extended period of time during which recruitment of ovulatory follicles takes place (Driancourt *et al.* 1985).

In the majority of prolific breeds, preovulatory follicles are smaller, suggesting that this is a characteristic associated with high ovulation rate, rather than the cause of high ovulation rate. For example, this characteristic has been observed in Finnish Landrace ewes (Webb *et al.* 1989). Also the difference in preovulatory size appears to be most limited in Romanov ewes (approximately 1 mm smaller) and largest in $Fec^{B}Fec^{B}$ versus $Fec^{+}Fec^{+}$ (up to 4 mm; Driancourt *et al.* 1991) ewes. Although there appears to be no difference between Finnish Landrace and Scottish Blackface for *in vitro* oestradiol production by individual follicles (Webb *et al.* 1989), granulosa cells from Finnish Landrace ewes produced more oestradiol *in vitro* compared to Scottish Blackface ewes. Also, oestradiol output per granulosa cell and testosterone output per thecal cell were greater for High line than Low line Finns (Driancourt *et al.* 1996b). As stated previously, on a per cell basis, for the same diameter follicles, theca and granulosa cells from $Fec^{B}$ carriers produce more steroids in culture, after gonadotrophin challenge, compared to non-carrier cells (Webb *et al.* 1995).

If differences in ovarian sensitivity/response to gonadotrophins are important, how are these effects manifest? No differences have been found in FSH or LH binding characteristics in granulosa cells, in LH receptor binding characteristics to thecal cells or in LH induced cAMP or steroid synthesis. One difference that has been correlated with the presence of the $Fec^{B}$ gene is reduced follicular fluid inhibin concentrations (see McNatty *et al.* 1990). Both Booroola and control Merinos had significantly lower mean bio-active inhibin concentrations in follicular fluid than a 'T' Merino line selected for litter size. Importantly, however, despite a twofold difference in ovulation rate between $Fec^{B}Fec^{B}$ ewes (ovulation rate : 6.65) compared to the heterozygotes (ovulation rate : 3.25), inhibin concentrations were not significantly different. The reason for these differences in respect to the mechanism of action of the major gene is not known, particularly since $Fec^{B}$ ewes are more responsive than control Merinos to immunization against either native bovine inhibin or a synthetic fragment of the α-subunit of porcine inhibin (see McNatty *et al.* 1990). Recently no differences were found between $Fec^{B}$ carriers and non-carriers in response to passive inhibin immunization (Wheaton *et al.* 1996). Furthermore, even though inhibin plays an important role in the regulation of FSH secretion in sheep (Martin *et al.* 1988), the pattern of secretion of biologically active dimeric inhibin in blood is less clear due to limitations in the assays previously employed because of the interference from free ∝ inhibin subunits. For example, in Finn lines selected on ovulation rate, inhibin output expressed either per follicle or per granulosa cell, did not differ between lines (Driancourt *et al.* 1996b). However, using an assay where this problem has been overcome, no difference in secretion rate of inhibin A during either the follicular or luteal phases, between ewes with and without the $Fec^{B}$ gene, was found (Souza, Campbell, Webb and Baird, unpublished observations).

**Growth factors and novel ovarian proteins.**

These foregoing results suggest that the key mechanisms involved in the determination of ovulation rate in breeds where both polygenic and major genes are involved have yet to be clarified. It may be that other growth factors need to be identified and novel factors isolated. The locus of the Booroola gene has been established (Montgomery *et al.* 1993; Lanneluc *et al.* 1994) and because the loci of numerous growth factors are also known, it is unlikely that the Booroola gene is directly related to growth factors already known to affect ovarian function such

as IGF-1, EGF and FGF. However, this does not preclude the possibility that the $Fec^B$ gene can influence the response of follicular cells to growth factors in synergy with gonadotrophins. The cellular proteins contained in the follicular wall (theca and granulosa cells) of ovulatory follicles was compared in carrier and non-carrier Belclare ewes by two dimensional gel electrophoresis (PAGE) and image analysis. 138 spots were identified as being present at least once. Of these, 2 spots (78 kD, pI 5.6 and 49 kD, pI 5.8) were always absent in the Belclare non-carriers, (Reynaud, Hanrahan & Driancourt, unpublished observations). The hypothesis that a high MW spot is related to the FSH receptor is presently being evaluated. Regarding secreted proteins, which were studied both in $Fec^BFec^B$ versus $Fec^+Fec^+$ and Belclare (carriers vs non-carriers) ewes, a high MW spot (96 kD, pI 7.5) was always present in $Fec^+Fec^+$ and Belclare non-carriers (Driancourt *et al.* 1996a; Driancourt & Hanrahan, unpublished observations). The low amounts of this protein have hindered its characterisation, but its absence may be a good marker for the presence of a major gene in both Booroola and Belclare gene carriers.

**Abnormal ovarian development.**

The identification of ovarian sterility in association with major gene effects on ovulation rate provides further support for the concept that variation in ovulation rate is primarily at the ovarian level. The x-linked Inverdale gene ($FecX^I$) has been shown to increase ovulation by one egg in heterozygous carriers whereas homozygous carriers are sterile, with streak ovaries which contain only primary follicles (Braw-Tal *et al.* 1993). In Cambridge and Belclare flocks in which a gene with a large effect on ovulation rate has been shown to be segregating, ewes with non-functional ovaries have also been observed. In contrast with Inverdale ewes, some sterile Belclare and Cambridge ewes contain small growing follicles and some ewes occasionally have follicles visible on the surface of the ovary. The available evidence shows that the gene causing sterility in these two breeds is inherited as an autosomal recessive. It is also suggested that while the gene involved is associated with ovulation rate, it does not account for all of the major gene effects on ovulation rate in these breeds (Hanrahan, unpublished observations). Thus ewes with unexceptional ovulation rate records have been shown to be carriers of the "sterility" gene. Likewise rams identified as non-carriers of the major gene for ovulation rate have sired sterile daughters. Plasma gonadotrophin levels are elevated in sterile ewes, however the hypothalmic/pituitary system responds normally to exogenous oestradiol and progesterone. Thus, the abnormal follicular development seems to be due to changes at the ovarian level. A further point to be noted is that there has been no evidence for abnormal ovarian development in association with the Booroola gene, suggesting that it acts at a different site in the control system.

## CONCLUSIONS

While some association has been observed between serum FSH concentrations and genetic differences in ovulation rate, these are not consistent among breeds or strains and do not provide convincing evidence that differences in gonadotrophin secretion is the fundamental determinant in the variation in ovulation rate. However, FSH has been shown to act synergistically with insulin to stimulate granulosa cell proliferation (Webb & McBride 1991; Campbell *et al.* 1996). Growth hormone also stimulates follicle growth in sheep (Gong *et al.* 1996), presumably acting through insulin, since systemic IGF-I concentration is not associated with ovulation rate (Spicer *et al.* 1993). Interestingly, follicular fluid IGF-II concentrations were found to be higher in

Booroola Merino crosses (Spicer *et al.* 1995) and this may be significant since IGF-II appears to have a more significant role at the ovarian level. Overall, current evidence suggests that the ovaries have a more important role in the control of ovulation rate than was originally thought. This hypothesis is strongly supported by the phenomenon of sterile ewes. However, analysis of follicular growth patterns in Booroola, Romanov and Finnish Landrace ewes suggests that high ovulation rates are achieved through different pathways (Driancourt *et al.* 1986). This is consistent with a number of different loci, with large effects on ovulation rate, acting through a variety of causal mechanisms. In conclusion, despite the finding of genetic markers for the $Fec^B$ gene, there is still much to be learned about the primary mechanisms which underlie the genetic differences in ovulation rate.

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