Induction of ovulation in chronically hypophysectomized Booroola ewes

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Summary. Booroola Merino ewes, with (F+; N = 17) and without (++; N = 13) a copy of the fecundity gene were hypophysectomized and 6 weeks later were given an i.m. injection of PMSG (high, medium or low dose) followed by hCG. The induced ovulation rates were observed laparoscopically. Ovulation rates were significantly higher ($P < 0.01$) in Booroola F+ ewes than in ++ ewes (8.00 ± 1.66 s.e.m. vs 3.62 ± 1.10 respectively). This suggests that the high fecundity of the Booroola ewe may be due primarily to ovarian rather than pituitary factors.

Keywords: Booroola; hypophysectomy; PMSG; hCG; ovulation rate

Introduction

The exceptional prolificacy of the Booroola Merino can be attributed largely to a single major gene (F) which increases ovulation rate (Piper & Bindon, 1982). The physiological mechanism involved in the expression of the fecundity of the Booroola ewe is still unclear. The increased number of follicles that mature and ovulate in each oestrous cycle in the Booroola ewe may result from differences in gonadotrophin secretion or from differences within the follicle itself (Bindon, 1984).

The gonadotrophins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are responsible for the normal growth and development of ovarian follicles through to ovulation (McNatty *et al.*, 1975; Baird & McNeilly, 1981; Baird, 1983; Driancourt *et al.*, 1984). Most studies of the reproductive endocrine status of the Booroola ewe have indicated a lack of correlation between peripheral concentrations of the gonadotrophins and ovulation rate both between strains of sheep (Booroola FF vs F+ vs ++: Bindon *et al.*, 1982; Booroola vs control: Scaramuzzi & Radford, 1983) and between individuals of the same strain (Brien *et al.*, 1976; Findlay & Cumming, 1976; Bindon *et al.*, 1979, 1982). This suggests that there are qualitative differences in the hormones (Wide, 1985) or that the number of follicles ovulating is mediated through influences other than the gonadotrophins. However, the radioimmunoassays (RIAs) used to measure FSH and LH may have been insensitive to the relatively small increases in gonadotrophin concentrations required to cause an increase in ovulation rate (Brown, 1978). More sensitive FSH RIAs have indicated that Booroola ewes may have slightly elevated plasma FSH concentrations during specific periods of the oestrous cycle (Bindon *et al.*, 1985; McNatty *et al.*, 1987). Robertson *et al.* (1984) have found that Booroola ewes have a significantly greater pituitary FSH content (per gland or per g wet weight) than do control ewes, which is consistent with the lower inhibin content of their ovaries (Cummins *et al.*, 1983).

The high ovulation rate of the Booroola ewe may also be due to expression of the F gene at the ovarian level. This study investigated the contribution of the ovary in determining the high ovulation rate characteristic of the Booroola ewe.

Materials and Methods

Animals. Mature (4–5 years) Booroola Merino ewes were bred at C.S.I.R.O. at Armidale, N.S.W., and had been diagnosed, after repeated laparoscopic determination of ovulation rate, as heterozygous carriers (F+) or non-carriers (++) of the putative fecundity gene (F). The F+ ewes had an ovulation rate of >3 and the ++ ewes an ovulation rate of <3 (Davis *et al.*, 1982).

Procedure. All ewes were hypophysectomized (Fry *et al.*, 1987) and received daily glucocorticoid replacement therapy (0.28 mg Dexadrenon: Intervet, Lane Cove, Australia). These ewes were challenged with 10 μ g gonadotrophin-releasing hormone (GnRH) (LH-RH: Peninsula Laboratories, Belmont, CA, U.S.A.) 1 week after hypophysectomy and serially bled at 15-min intervals for 2 h to check for residual gonadotrophin release (Lee *et al.*, 1976; Bremner *et al.*, 1980). After 1 month, an intravaginal progestagen pessary (Repromap: Upjohn, Rydalmere, Australia) was inserted into each ewe for 12 days. At pessary removal ewes were injected (i.m.) with 500 i.u. (low), 1000 i.u. (medium) or 1500 i.u. (high) PMSG (Folligon: Intervet) and 24 h later received an additional dose of 250 i.u. (low), 500 i.u. (medium) or 1000 i.u. (high) PMSG. At 56 h after pessary removal all ewes received 1000 i.u. hCG (Chorulon: Intervet) to stimulate ovulation and 3–4 days later the ovulation rate was determined by laparoscopy.

Design. A 2×3 factorial experiment was designed to compare the ovulation rate between Booroola F+ and ++ ewes receiving a low ($N = 9$, $N = 8$), medium ($N = 7$, $N = 12$) or high ($N = 11$, $N = 12$) dose of PMSG.

Statistical analysis. Treatments were compared by analysis of variance on log-transformed data.

Results

LH and FSH were undetectable in the plasma of the hypophysectomized ewes after the GnRH challenge, confirming that no viable pituitary tissue remained.

Dose-response curves for Booroola F+ and ++ ewes were derived by plotting the log of the mean ovulation rate for each treatment group against the total dose of gonadotrophin administered (Fig. 1). The induced ovulation rates were significantly higher ($P < 0.01$) in the Booroola F+ ewes than in ++ ewes (Fig. 1). There was a significant ($P < 0.01$) dose-dependent effect of PMSG on induced ovulation rate which appeared to reach a peak (V_{max}) at the medium gonadotrophin dose for both ewe groups (Fig. 1). There were no significant interactions between genotype and dose of gonadotrophin. This is reflected in the similar values of the K sensitivity (the dose of gonadotrophin required to achieve a 50% maximal ovulatory response) for Booroola F+ and ++ ewes (Fig. 1). The proportion of hypophysectomized ewes ovulating did not differ between

Fig. 1. The ovulatory response of hypophysectomized Booroola F+ and ++ ewes to a low, medium or high dose of gonadotrophin.

groups and only 53% of all the ewes ovulated after gonadotrophin treatment. The number of Booroola F+ and ++ ewes ovulating in each treatment group were respectively 4 and 3 for a low dose, 6 and 4 for a medium dose and 7 and 6 for a high dose.

Discussion

These results show that, for a given level of exogenous gonadotrophin, hypophysectomized Booroola F+ ewes have double the ovulation rate of ++ ewes. This demonstrates that the high ovulation rate of the Booroola ewe is not due to pituitary factors and is probably due to ovarian factors.

Previous studies have shown that Booroola ewes possessing at least one copy of the F gene (FF or F+) had a greater ovulatory response to PMSG treatment than did Booroola ++ ewes (Kelly *et al.*, 1983; Bindon *et al.*, 1985), which indicated that the mechanism of action of the fecundity gene was at the ovarian level. However, these studies were not definitive as differences in the level or type (Chappel, 1981; Wide, 1985) of endogenous gonadotrophin may have influenced subsequent ovulation rates. Removal of the pituitary gland from the Booroola ewes eliminated the influence of endogenous gonadotrophin variability on ovulation rate. We demonstrated that, with three levels of PMSG treatment, hypophysectomized Booroola F+ ewes exhibit twice the ovulatory response of hypophysectomized ++ ewes (Fig. 1), which confirms the earlier postulate.

Ovulation occurs from Booroola follicles that are smaller than normal (Baird *et al.*, 1982; Driancourt *et al.*, 1985; McNatty *et al.*, 1985), contain half the number of granulosa cells and have a lower oestrogen content (Baird *et al.*, 1982), indicating that the gonadotrophin requirement per follicle to reach ovulation may be less. This fits with other facets of the growth pattern of Booroola follicles such as the extended period of follicular recruitment, the decreased amount of atresia amongst these recruited follicles and the ability of early maturing follicles to retain their viability until the time of ovulation (Driancourt *et al.*, 1985).

The induced ovulation rate appeared to plateau with the medium dose gonadotrophin treatment (total 1500 i.u. PMSG, 1000 i.u. hCG) for both types of ewe. No difference was evident in the dose required to achieve a 50% increase in ovulation rate (*K* sensitivity), but there was a difference in the maximum response ($V_{\max}$) to gonadotrophin treatment (Fig. 1). The ovarian difference between the types of ewe may therefore not be in their sensitivity to gonadotrophins (Henderson *et al.*, 1985) but may result from other changes.

Work with acutely hypophysectomized ewes has demonstrated that the administration of steroid-free ovine follicular fluid will inhibit PMSG-induced folliculogenesis (Cahill *et al.*, 1984). This indicates the possible existence of a local mechanism by which the larger follicles in monotocous species may suppress the growth of smaller follicles. Ink labelling of individual large follicles *in vivo*, and following their progression through the late luteal and early follicular phase, indicated that the follicle growth inhibitor (FGI) present in follicular fluid suppresses the 'recruitment' of follicles for growth rather than their 'selection' for ovulation (M. A. Driancourt, R. C. Fry, L. P. Cahill & J. K. Findlay, unpublished results). One unique facet of folliculogenesis in the Booroola ewe is the extended period of recruitment. Recruitment in control Merino ewes occurs between Days 13 and 15 of the oestrous cycle (Day 0 = oestrus) while in the Booroola Merino recruitment continues to Day 17 (Driancourt *et al.*, 1985). This presents the possibility that the Booroola may be deficient in 'follicular growth inhibitor' or exhibit a decreased follicular sensitivity to it, allowing more follicles to be recruited and thereby increasing the ovulation rate. In support of this local action of follicular fluid upon ovulation rate, Cummins *et al.* (1983) demonstrated that the suppression of FSH in ovariectomized F+ and ++ ewes to follicular fluid (72 000 units of inhibin activity) was similar.

In conclusion, we propose that the prolificacy of the Booroola ewe is at least partly due to changes at the ovarian level, with further investigations required to elicit the mechanism by which these changes are implemented.

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