Plasma concentrations of FSH, LH, thyroid-stimulating hormone and growth hormone after exogenous stimulation with GnRH, TRH and GHRH in Booroola ewes that are homozygous carriers or non-carriers of the *Fec^B* gene

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The aim of the present study in Booroola ewes, either homozygous (BB) or non-carriers (++) of the *Fec^B* gene, was to test the specificity of the pituitary responses to exogenous hypothalamic releasing hormones by examining the plasma concentrations of FSH, LH, thyroid-stimulating hormone (TSH) and growth hormone (GH) after injecting the animals with different doses of GnRH, thyroid-releasing hormone (TRH) or growth-hormone-releasing hormone (GHRH) which were administered on separate occasions. The animals ($n = 8$ per dose) received 0, 3.1 or 12.5 μg of thyroid-releasing hormone and GnRH (i.v.), whereas they ($n = 9\text{--}13$ per dose) received 0, 6.0 or 16.0 μg GHRH (i.v.). For each experiment there were no differences between the genotypes in bodymass or age. Gene-specific differences in the mean pretreatment concentrations of plasma FSH (BB > ++; $P < 0.05$) but not of LH, TSH or GH were noted. After treatment with GnRH, TRH or GHRH, significant effects of dose were noted for all the hormones; however, a gene-specific effect was observed only for FSH in response to GnRH (BB > ++; $P < 0.01$) with no genotype $\times$ dose interaction (ANOVA). For LH, the effects of genotype and the genotype $\times$ dose interaction almost reached significance at the 5% level (genotype, $P = 0.055$; genotype $\times$ dose, $P = 0.067$). For TSH and GH the respective genotype $\times$ dose interactions were not significant. These results support the hypothesis that the *Fec^B* gene in Booroola ewes influences the release of pituitary hormones in response to hypothalamic releasing hormones only in the case of GnRH, which results mainly in different plasma concentrations of FSH.

Introduction

Booroola ewes possess a major gene, *Fec^B*, that influences their ovulation rate (i.e. the number of ovulations per oestrous cycle; for review, see Montgomery *et al.*, 1992). Homozygotes (BB), heterozygotes (B +) and non-carriers (++) of the *Fec^B* gene are segregated on the basis of ovulation rate recordings of ≥ 5 , 3 or 4, and 1 or 2, respectively (Davis *et al.*, 1982).

It is known that the immunoreactive (i) and bioactive (b) plasma concentrations of FSH (and, on some occasions, i-LH concentrations) are higher in BB ewes treated with GnRH than in ++ ewes that have intact ovaries that are ovariectomized or that are ovariectomized with hypothalamic–pituitary disconnection (McNatty *et al.*, 1987, 1989a, 1991, 1993; Phillips *et al.*, 1993). It is also known that polymorphisms of the *FSH β* or *LH β* gene are not linked to the *Fec^B* gene (Montgomery *et al.*, 1990, 1993) and that no gene-specific differences have been found for the GnRH-receptor-binding characteristics, FSH isoforms or plasma clearance rates, or steroid or inhibin feedback effects (Robertson *et al.*, 1984; Fry *et al.*, 1987; McNatty *et al.*, 1989b, 1992; Fleming *et al.*, 1990).

It is unclear whether the gene-specific difference in FSH (and possibly LH) concentrations resulting from a GnRH challenge are unique, as the pituitary responses in Booroola ewes to growth-hormone-releasing hormone (GHRH) and thyroid-releasing hormone (TRH) have not been reported. TSH shares a common α subunit with both FSH and LH (Ward *et al.*, 1991) and it is possible that this hormone may respond differently to TRH. There is evidence that when litter sizes are equal those with the *Fec^B* gene are lighter at birth (Montgomery *et al.*, 1992), raising the possibility that the pituitary response of growth hormone (GH) to GHRH may not be the same between the genotypes. The aim of the present study was to test the specificity of the pituitary responses in BB and ++ ewes by examining their plasma concentrations of FSH, LH, TSH and GH after injecting the animals with different doses of GnRH, TRH and GHRH.

Materials and Methods

Animals

All animal experimentation was done at the Wallaceville Animal Research Centre in accordance with the 1987 Animals

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Protection (Codes of Ethical Conduct) Regulations of New Zealand after approval was granted by the Animal Ethics Committee.

Booroola $\times$ Merino ($n = 42$ BB; $n = 42$ ++) and Booroola $\times$ Romney ($n = 12$ BB; $n = 12$ ++) ewes were studied. The overall mean $\pm$ SEM ages and bodymasses were 6.2 ± 0.3 years (++) and 6.1 ± 0.2 years (BB), and 44.8 ± 1.5 kg (++) and 43.3 ± 1.3 kg (BB), respectively; there were no genotypic differences in age or bodymass either within breeds or overall.

For the studies with GnRH and TRH, the same subset of 24 animals per genotype was used ($n = 16$ Booroola $\times$ Merino and $n = 8$ Booroola $\times$ Romney per genotype). The animals were tested first for responses of TSH to TRH and then two days later for responses of FSH and LH to GnRH; these experiments were performed in mid-anoestrus. For the studies with GHRH, a subset of 32 animals per genotype was used ($n = 27$ Booroola $\times$ Merino and $n = 5$ Booroola $\times$ Romney), of which only four were tested subsequently for their responses to TRH and GnRH. These animals were tested during either the breeding season (stage of oestrous cycle unknown; $n = 21$) or anoestrus ($n = 11$). The doses of GHRH and TRH were chosen after preliminary studies on Romney ewes. For TRH and GHRH (five ewes per dose) the doses tested were 0, 1.56, 3.13, 6.25, 12.5 and 25.0 μ g per ewe. The mean maximum TSH response (i.e. about 200%) was observed over the range of 6.25 to 25.0 μ g. Thus, the intermediate and highest doses chosen were 3.13 and 12.5 μ g. For GHRH, the mean maximum response (about 500%) was observed between 12.5 and 25.0 μ g and, accordingly, the intermediate and highest doses chosen were 6.0 and 16.0 μ g. The doses of GnRH administered (i.e. 3.13 and 12.5 μ g) were based on results from a previous study (McNatty *et al.*, 1987). For each experiment with TRH, GHRH or GnRH there were no differences between the genotypes in bodymass.

One day before experimentation, the animals, which were used to being handled, were brought indoors from pasture and a jugular venous catheter was fitted in each animal. The animals were fed *ad libitum* with meadow hay, lucerne pellets and had free access to water. They were accommodated in two well-ventilated rooms where they were free to walk, sit or stand. Throughout the experiments, the animals were sampled via the catheters with minimal restraint.

On the day of the experiment, the blood of the animals was sampled via the jugular venous catheter at intervals of 30 min for 2 h (first sample at time 0). Immediately after the fifth blood sample had been taken, 5 ml of GnRH (luteinizing-hormone-releasing hormone at 0, 3.13 or 12.5 μ g per 5 ml; Peninsular Laboratories Inc., Belmont, CA., Lot 022594) or TRH (thyrotrophin-releasing factor at 0, 3.13 or 12.5 μ g per 5 ml; Boehringer-Mannheim, Mt Wellington) in sterile saline plus 0.1% ovine serum albumin was injected as a bolus via the catheter. Subsequently, blood was collected every 15 min for the next 6 h. For the experiment with GHRH [growth-hormone-releasing factor (1-44, Sigma Chemical Co., St Louis, MO, Lot 55F-07061)], blood samples were collected at intervals of 10 min for 30 min (first sample at time 0) and after the fifth blood sample 5 ml GHRH (at 0, 6 or 16 μ g per 5 ml) dissolved in sterile saline plus 0.1% ovine serum albumin was injected via the catheter. Subsequently, blood was collected every 10 min for the next 90 min.

All blood samples, which were collected into heparinized tubes, were centrifuged at room temperature for 5–10 min at 400 *g* within 20 min of collection and the plasma stored at -20°C until assayed.

Hormone assays

FSH, LH and TSH. Most of the reagents for these radioimmunoassays were supplied by the National Hormone and Pituitary Program (NIADDK, NIAMDD), Bethesda, MD. The assays for FSH and LH were identical to those described by McNatty *et al.* (1993). The ovine (o) FSH for iodination was NIAMDD-oFSH-I-1, the oFSH preparation was NIAMDD-oFSH-RP-1 (biopotency, $75 \times$ NIH-FSH-S1), and the oFSH antiserum was NIAMDD-anti-oFSH-1 (AFP-C5288113). The oLH for iodination was NIDDK-oLH-I-3 (AFP-9598B), the LH antiserum was raised at Wallaceville Animal Research Centre and is described by McNatty *et al.* (1987), the oLH reference preparation was NIAMDD-oLH-23 (biopotency, $2.3 \times$ NIH-LH-S1). The TSH for iodination was bovine TSH-I-1, the oTSH reference preparation was NIAMDD-oTSH-12 (biopotency, $27 \times$ the international bovine TSH standard), and the oTSH antiserum was anti-oTSH-1, which was used at a final dilution of 1:300 000. The crossreactivities of oFSH (NIAMDD-oFSH-S17) and oLH (NIAMDD-oLH-23) in the TSH assay were $< 0.2\%$. For FSH, LH and TSH, the volume of plasma assayed was 0.2 ml. The internal standards and standard curve samples were prepared in FSH, LH and TSH-free hypophysectomized ewe plasma. For FSH, LH and TSH, plasma samples demonstrated parallelism to the standard curves. The overall intra-assay and interassay coefficients of variation for the internal standards in all three assay systems were $< 10\%$ and the overall detection limits for FSH, LH and TSH were ≤ 0.2 ng ml^{-1} .

GH. Ovine GH was measured using a homologous radioimmunoassay. The oGH antiserum was raised in a New Zealand white rabbit against NIH-GH-S11; oGH was then incubated in 8 mol urea l^{-1} (Hart *et al.*, 1975) for 1 h and iodinated with ^{125}I by the iodogen method (Salacinski *et al.*, 1981). The assay was performed by preincubating the unknown (0.1 ml) or standard in hypophysectomized ewe plasma with the oGH antiserum (final dilution, 1:7500) in 20 mmol barbitone l^{-1} , 10 mmol EDTA l^{-1} , 150 mmol NaCl l^{-1} , 13 iu heparin ml^{-1} and 0.02% (w/v) sodium azide, 0.5% (w/v) normal rabbit serum and 0.2% (w/v) BSA (pH 8.6) in a volume of 0.3 ml. After preincubation at 4°C for 3 days, 0.1 ml of [^{125}I]-labelled oGH (500 pg) was added to each tube. After 17 h each tube received 1.7 ml of sheep anti-rabbit γ -globulin serum (final dilution, 1:50) in 10 mmol sodium phosphate l^{-1} , 10 mmol EDTA l^{-1} , 150 mmol NaCl l^{-1} , 0.02% sodium azide, 3% (w/v) polyethylene glycol 8000 and 0.1% (w/v) BSA buffer at pH 7.5. After incubation for 24 h, the tubes were centrifuged at 3000 *g* for 15 min and the ^{125}I activity of the precipitate determined. Plasma samples demonstrated parallelism with the standard curve. The antibody displayed a low crossreactivity with ovine prolactin of $< 0.04\%$. The limit of detection was 0.3 ng ml^{-1} and the intra-assay and interassay coefficients of variation were 5.8 and 7.1%, respectively.

Table 1. Changes in plasma hormonal concentrations in Booroola ewes to the hypothalamic releasing hormones GnRH, TRH and GHRH with response to dose and Booroola genotype

Hormone	Genotype	Pretreatment hormone concentrations (mean $\pm$ SEM ng ml ⁻¹)	Percentage hormonal response with respect to dose of releasing hormone ^a		
			Control	Low	High
FSH	BB	2.0 $\pm$ 0.2*	108 (86, 135)	160 (129, 201)	241 (193, 300)**
	++	1.6 $\pm$ 0.1	87 (70, 100)	130 (104, 163)	139 (111, 174)
LH	BB	0.3 $\pm$ 0.01	108 (70, 168)	1246 (808, 1921)	1361 (883, 2098)
	++	0.3 $\pm$ 0.01	96 (62, 147)	1621 (1051, 2498)	3332 (2161, 5135)
TSH	BB	0.5 $\pm$ 0.05	95 (80, 112)	118 (101, 140)	133 (113, 158)
	++	0.5 $\pm$ 0.04	97 (82, 115)	119 (83, 141)	106 (89, 123)
GH	BB	3.4 $\pm$ 0.4	103 (61, 175)	339 (219, 526)	442 (268, 729)
	++	2.8 $\pm$ 0.4	88 (51, 149)	277 (180, 434)	415 (251, 684)

Hormonal response values are expressed as geometric means (and 95% confidence limits) expressed as percentages of the pretreatment values.

There were 8 animals per genotype per dose for the FSH, LH and TSH measurements and 9–13 animals per genotype per dose for GH. ^aValues are geometric means (and 95% confidence limits) * $P < 0.05$, ** $P < 0.01$ compared with the ++ genotype. FSH and LH were measured in ewes treated with GnRH, TSH after treatment of ewes with TRH and GH after treatment with GHRH.

Presentation of data and statistical analyses

As there was a gene-specific difference in the pretreatment means for FSH, the post-treatment responses of all hormones for each ewe were expressed as a percentage of the mean of the pretreatment concentration. There were no obvious effects of breed (i.e. Booroola $\times$ Romney compared with Booroola $\times$ Merino). Consequently, the results for both breeds were pooled. The hormonal responses to the releasing hormones were presented as mean ($\pm$ SEM) percentage responses with respect to time and genotype. Preliminary data analyses revealed no significant interactions for any hormone between genotype and time. To test for possible effects of genotype a mean percentage response over time (i.e. 15–180 min for FSH, LH and TSH, and 10–60 min for GH) was obtained for each ewe. Thereafter, these values were log-transformed to normalize the variances and then tested by two-way analysis of variance, with genotype and dose being the two variables. These results with respect to the dose of releasing hormone and genotype are expressed as geometric means (and 95% confidence limits).

Results

The response of GH to GHRH in each genotype did not differ between the breeding or non-breeding seasons, and the results over these times were therefore pooled. The pretreatment mean $\pm$ SEM concentrations of FSH, LH, TSH and GH are shown in Table 1. During the pretreatment period, a genotypic difference was noted in the response of FSH but not of the other hormones. The mean percentage ($\pm$ SEM) hormonal responses to the releasing factors GnRH, TRH and GHRH are shown in Figs 1 and 2. For all hormones (i.e. LH, FSH, TSH and GH) the peak concentrations were reached within the first 2 h. For LH, but not the other hormones, the peak response to GnRH at the highest dose (12.5 μ g) occurred appreciably later than that observed at the intermediary dose (3.13 μ g) of GnRH (Fig. 1; 90 min compared with 30 min). Irrespective of dose of GnRH or TRH, the response of FSH, LH and TSH had declined by 3 h. The response of GH to GHRH was over after 1 h.

The geometric mean (and 95% confidence limits) changes in FSH, LH and TSH concentrations over the first 3 h or in GH concentration over the first hour following injection of the hypothalamic releasing hormone are shown in Table 1. Two-way analysis of variance of the responses of GH and TSH with respect to dose and genotype showed significant main effects of dose ($P < 0.01$ for both hormones) but no effects of genotype and no interactions. Likewise, there was a significant main effect of dose on the response of LH ($P < 0.01$) and the effects of genotype and the genotype $\times$ dose interaction almost reached significance at the 5% level (genotype, $P = 0.055$; genotype $\times$ dose, $P = 0.067$). For the response of FSH there were significant main effects of genotype ($P < 0.01$) and dose ($P < 0.001$) but no genotype $\times$ dose interaction. With respect to the response of FSH, there was a genotypic effect with 12.5 μ g but not 3.1 μ g of GnRH (Table 1). The aforementioned difference with respect to genotype for FSH but not LH, TSH or GH was also noted when the areas under the dose–response curve or maximum hormone responses were tested.

Discussion

The present results suggest that the main effect of the *Fec^B* gene is its influence on the action of GnRH to affect the plasma concentration of FSH. Our present results on the response of LH to a GnRH challenge in BB and ++ animals is consistent with previous findings that the differences between the genotypes approach significance at the 5% level (McNatty *et al.*, 1991), and that this difference may be confounded by an interaction with the dose of GnRH (Price *et al.*, 1991). The results reported here show that the gene-specific difference in the pituitary response to GnRH was not observed with respect to the GH response to GHRH or the TSH response to TRH. It therefore seems reasonable to suggest that the difference in the pituitary response to GnRH between BB and ++ ewes is unique to this hypothalamic releasing hormone. These results confirm and extend previous findings that the pituitary gland in BB ewes responds differently to exogenous GnRH compared with

Fig. 1. Change in mean ($\pm$ SEM) plasma concentrations of (a) FSH and (b) LH expressed as the percentage response with respect to time from GnRH or saline injection. Eight ++ ($\circ$) and eight BB ($\bullet$) ewes were tested with the different doses of GnRH and with saline.

Fig. 2. Change in mean ($\pm$ SEM) plasma concentrations of (a) thyroid-stimulating hormone (TSH) and (b) growth hormone (GH) expressed as the percentage response with respect to time from injection of thyroid-releasing hormone (TRH) or growth-hormone-releasing hormone (GHRH), respectively, or saline injection. There were eight ++ (○) and eight BB (●) ewes for each of the TRH treatments, including the saline controls, whereas there were 10 ++ and 10 BB saline controls, 13 ++ and 13 BB animals given 6 µg GHRH, and 9 ++ and 9 BB animals given 16 µg of GHRH.

that in ++ ewes with respect to the plasma concentrations of FSH (McNatty *et al.*, 1987, 1991, 1993).

The interpretation of the present results might be constrained by the numbers of animals used or because the doses of releasing hormone may not be sufficiently extensive to rule out completely any minor differences between the genotypes in their responsiveness to releasing hormone. In an earlier study on anoestrous ewes, we reported that the response of FSH in BB ewes to 5 or 25 µg of GnRH (two ewes per genotype per dose) was less than twice the normal concentration of FSH, whereas that in ++ ewes was greater (i.e. 2.5–4-fold). Although these animals were studied in February while the present animals were studied in December, the most likely explanation for this discrepancy is that there were too few animals in the former study for a meaningful interpretation of the genotypic effect.

The consistent finding of a greater response of LH to GnRH in the BB compared with the ++ genotype, where the level of significance is between 0.05 and 0.10, suggests that this differential LH response may be of biological importance. The lack of any similar trend in the concentration of TSH suggests that if the response of LH has any significance it is likely to relate to LH β -subunit synthesis. In preliminary studies of pituitary glands in Booroola ewes it is evident that although many cells contain both FSH and LH immunoreactivity, there are many more cells immunoreactive for LH than for FSH (D. A. Heath and K. P. McNatty, unpublished). It is possible that the cells co-synthesizing FSH and LH function differently from those synthesizing LH alone.

The mean LH responses to GnRH and the mean GH responses to GHRH were substantial (i.e. $\geq 500\%$) at both the intermediary and high doses of releasing hormone, and similar to those reported in other studies (McNatty *et al.*, 1987; Wrutniak *et al.*, 1987). In contrast, the mean FSH and TSH responses (i.e. 100–300%) to GnRH and TRH, respectively, were modest. The time-course and magnitude of the TSH response to TRH given i.v. was similar to that described by others for ewe lambs (Wrutniak *et al.*, 1987), as were those for FSH in response to GnRH given i.v. (McNatty *et al.*, 1987).

In Booroola ewe lambs and ewes that carry the *Fec^B* gene, a consistent feature has been the higher concentrations of plasma FSH but not LH relative to the non-carriers (Montgomery *et al.*, 1989; Braw-Tal and Gootwine, 1993; McNatty *et al.*, 1991, 1993). Although further studies are needed, the present results suggest that the plasma concentrations of TSH and GH are not different between the genotypes. Collectively, these results indicate that the difference in plasma FSH but not LH, TSH and GH concentration between BB and ++ ewes is due, at least in part, to the presence of the *Fec^B* gene influencing the responsiveness of the pituitary gland to GnRH. This interpretation is consistent with our previous finding in GnRH-stimulated, ovariectomized Booroola BB and ++ ewes with hypothalamic-pituitary disconnection: in these animals, the mean response of FSH to each physiological GnRH pulse was significantly higher in BB than in ++ ewes (McNatty *et al.*, 1991).

The underlying reason for this difference in pituitary responsiveness to GnRH is not known. The *Fec^B* gene has been excluded from linkage to the *FSH β* region of chromosome 15 and appears to have no influence on the number or size of the *FSH β* gene transcripts in pituitary mRNA preparations

(Montgomery *et al.*, 1990, 1993); however, the temporal changes in *FSH- β* mRNA, including stability of the *FSH β* message, needs to be studied further. No influence of the *Fec^B* gene on pituitary GnRH-binding characteristics, on the concentration of GnRH in the brain, or on hypophyseal portal GnRH secretory characteristics have been noted (Gale *et al.*, 1988; Fleming *et al.*, 1990; McNatty *et al.*, 1993). In studies of Booroola fetuses, differences have been noted in the development of the mesonephros and germ cells in the newly forming gonad (Smith *et al.*, 1993). Parallel studies on the development of pituitary gonadotrophs have not been carried out, and the possibility remains that the pituitary gland in BB ewes simply contains more cells that can synthesize β -FSH or co-synthesize β -FSH and β -LH, but not α -gonadotrophin, when stimulated with GnRH.

In conclusion, our results support the hypothesis that the *Fec^B* gene in Booroola ewes influences the release of pituitary hormones in response to hypothalamic releasing hormones only in the case of GnRH, and affects mainly the plasma concentrations of FSH.

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References

- Braw-Tal R and Gootwine E (1989) Pituitary response to GnRH and ovariectomy in Booroola-Awassi and Awassi ewe lambs *Journal of Reproduction and Fertility* **87** 73–80
- Braw-Tal R and Gootwine E (1993) Plasma immunoreactive inhibin and FSH in prepubertal Assaf and Booroola-Assaf ewe lambs *Domestic Animal Endocrinology* **10** 87–94
- Davis GH, Montgomery GW, Allison AJ, Kelly RW and Bray AR (1982) Segregation of a major gene influencing fecundity in progeny of Booroola sheep in New Zealand *New Zealand Journal of Agricultural Research* **25** 525–529
- Fleming JS, Lun S, Smith P and McNatty KP (1990) Pituitary receptors for gonadotrophin-releasing hormone in Booroola Merino ewes which were non-carriers or homozygotes of the fecundity gene F *Journal of Neuroendocrinology* **2** 601–604
- Fry RC, Cahill LP, Cummins JT, Bindon BM, Piper LR and Clarke IJ (1987) The half-life of follicle-stimulating hormone in ovary-intact and ovariectomized Booroola and control Merino ewes *Journal of Reproduction and Fertility* **81** 611–615
- Gale JS, Smith P, Truman P and McNatty KP (1988) Gonadotrophin releasing hormone immunoreactivity in Booroola Merino ewes *Journal of Reproduction and Fertility* **82** 581–586
- Hart I, Flux DS, Andrews P and McNeilly AS (1975) Radioimmunoassay for ovine and caprine growth hormone *Hormone and Metabolic Research* **7** 35–40
- McNatty KP, Hudson N, Henderson KM, Gibb M, Morrison L, Ball K and 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 *Journal of Reproduction and Fertility* **80** 577–588
- McNatty KP, Fisher M, Collins F, Hudson NL, Heath DA, Ball K and Henderson KM (1989a) Differences in the plasma concentrations of FSH and LH in ovariectomized Booroola FF and ++ ewes *Journal of Reproduction and Fertility* **85** 705–713
- McNatty KP, Hudson NL, Collins F, Heath DA and Henderson KM (1989b) Effects of oestradiol-17 β , progesterone or bovine follicular fluid on the plasma concentrations of FSH and LH in ovariectomized Booroola ewes

- which were homozygous carriers or non-carriers of a fecundity gene *Journal of Reproduction and Fertility* **87** 573–585
- McNatty KP, Hudson NL, Shaw L, Condell LA, Ball K, Seah S-L and Clarke IJ (1991) GnRH-induced gonadotrophin secretion in ovariectomized Booroola ewes with hypothalamic–pituitary disconnection *Journal of Reproduction and Fertility* **91** 583–592
- McNatty KP, Heath DA, Hudson NL, Ball K and Condell L (1992) Immuno-reactive inhibin concentrations in ovarian and peripheral venous plasma and follicular fluid of Booroola ewes which were homozygous carriers or non-carriers of the *Fec^B* gene *Journal of Reproduction and Fertility* **95** 489–502
- McNatty KP, Hudson NL, Lun S, Heath DA, Shaw L, Condell L, Phillips DJ and Clarke IJ (1993) Gonadotrophin-releasing hormone and the control of ovulation rate by the *Fec^B* gene in Booroola ewes *Journal of Reproduction and Fertility* **98** 97–104
- Montgomery GW, Scott IC, Littlejohn RP, Davis GH and Peterson AJ (1989) Concentrations of FSH are elevated in newborn ewe lambs carrying the Booroola *F* gene but not in lambs from a prolific Romney strain *Reproduction, Fertility and Development* **1** 299–308
- Montgomery GW, Sise JA, Greenwood PJ and Fleming JS (1990) An insertion/deletion polymorphism around the *FSH β* gene in sheep excluded from tight linkage to the Booroola *F* gene *Journal of Molecular Endocrinology* **5** 167–173
- Montgomery GW, McNatty KP and Davis GH (1992) Physiology and molecular genetics of mutations that increase ovulation in sheep *Endocrine Reviews* **13** 309–328
- Montgomery GW, Crawford AM, Penty JM, Dodds KG, Ede AJ, Henry HM, Pierson CA, Lord EA, Galloway SM, Schmack AE, Sise JA, Swarbrick PA, Hanrahan V, Buchanan FC and Hill DF (1993) The ovine Booroola fecundity gene (*Fec^B*) is linked to markers from a region of human chromosome 4q *Nature Genetics* **4** 410–414
- Phillips DJ, Hudson NL and McNatty KP (1993) Effects of ovariectomy and genotype on bioactive FSH in plasma and pituitary of Booroola ewes *Journal of Reproduction and Fertility* **98** 559–565
- Price CA, Hudson NL and McNatty KP (1991) Plasma, LH, FSH and testosterone in adult rams which were homozygous carriers and non-carriers of the Booroola fecundity gene *Journal of Reproduction and Fertility* **91** 267–275
- Robertson DM, Ellis S, Foulds LM, Findlay JK and Bindon BM (1984) Pituitary gonadotrophins in Booroola and control Merino sheep *Journal of Reproduction and Fertility* **71** 189–197
- Salacinski PRP, McLean C, Sykes JEC, Clement-Jones VV and Lowry PJ (1981) Iodination of proteins, glycoproteins and peptides using a solid-phase oxidizing agent 1,3,4,6-tetrachloro-3 α -diphenyl glycolic acid (iodogen) *Analytical Biochemistry* **117** 136–146
- Smith P, O W-S, Hudson NL, Shaw L, Heath DA, Condell L, Phillips DJ and McNatty KP (1993) Effects of the Booroola gene (*Fec^B*) on body weight, ovarian development and hormone concentrations during fetal life *Journal of Reproduction and Fertility* **98** 41–54
- Ward DN, Bousfield GR and Moore KH (1991) Gonadotropins. In *Reproduction in Domestic Animals* pp 25–80 Ed. PT Cupps. Academic Press, New York
- Wrutniak C, Cabello G, Charrier J, Dulong J-P, Blanchard M and Barenton B (1987) Effects of TRH and GRF administration on GH, TSH, T4 and T3 secretion in the lamb *Reproduction, Nutrition, Development* **27(2B)** 501–510