

## Effects of the Booroola gene $FecB^B$ on somatic and germ cells of the fetal testis

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**Summary** — The present study was conducted to compare in homozygous  $FecB^B FecB^B$  (BB) Booroola and ++ male fetuses, the body and the testicular growths and the tissular and cellular compositions of the testis between 60 and 140 days of gestation. To eliminate differences in growth due to uterine environment, single embryos have been transferred in recipient Mérinos d'Arles ewes. At 60 and 100 days of gestation, the body masses of BB fetuses were significantly lower than those of ++ foetuses (11 and 13%;  $P = 0.05$ ); but their testis masses or their total contents of somatic (Leydig or Sertoli) cells did not differ significantly whatever the fetal age. At 100 and 140 days of gestation, testis and body masses were significantly correlated without difference between BB and ++ genotypes. In conclusion, the presence or absence of homozygous gene  $FecB^B$  does not induce significant differences in somatic or germ cell composition of the testis between 60 and 140 days of gestation.

**fetus / testis / gonocytes / Sertoli /  $FecB^B$**

**Résumé** — Effet du gène Booroola sur la composition en cellules germinales et somatiques du testicule fœtal. Le présent travail avait pour but de comparer chez des fœtus ovins mâles homozygotes, Booroola  $FecB^B FecB^B$  (BB) ou ++, les croissances corporelle et testiculaire et la composition cellulaire et tissulaire des testicules, en fonction de la présence du gène entre 60 et 140 j de gestation. Des gestations simples d'agneaux dans le même environnement utérin ont été obtenues par transfert embryonnaire d'un seul embryon, dans des brebis receveuses Mérinos d'Arles. À 60 et 100 j de gestation, les poids corporels des agneaux BB sont significativement plus légers que ceux des agneaux ++ (11 et 13 %;  $p = 0.05$ ); cependant les poids testiculaires ou les nombres de cellules de Sertoli ou de Leydig par testicule ne diffèrent pas significativement, quel que soit l'âge fœtal. À 100 et 140 j de gestation, les poids testiculaires et corporels sont très corrélés, cependant aucune différence liée au gène  $FecB^B$  n'a pu être mise en évidence. En conclusion, la présence du gène  $FecB^B$ , à l'état homozygote, n'entraîne aucune variation significative dans le contenu en cellules germinales ou somatiques du testicule foetal entre 60 et 140 j de gestation.

**foetus / testicule / gonocytes / Sertoli /  $FecB^B$**

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## INTRODUCTION

In the female, the Booroola FecB<sup>B</sup> (B) gene is known to affect first the onset of ovogenesis in the fetal ovary, the pattern of follicular development, the ovulation rate and the litter size (McNatty and Henderson, 1987; Driancourt, 1990; Smith et al, 1994; McNatty et al, 1995). The role of the Booroola B gene on the synthesis of oestrogen and on the FSH plasma levels is well documented (McNatty et al, 1987; Driancourt, 1990).

In the male, during the prepubertal period, a transient increase in LH and FSH plasma levels was observed in heterozygous carriers of the B gene by Seck et al (1988) but disputed by Purvis et al (1990). This genotype difference was mainly attributed to sire differences (Isaacs et al, 1995); however, in Booroola × Mérimos d'Arles cross, lambs were issued from three B+ sires; their progenies were half-brother B+ or ++ lambs, in this case, even a sire effect was observed, the genotype modified the gonadotropin plasma concentrations in prepubertal lambs (Seck et al, 1988). In adult ram, no obvious differences were recorded in hormonal secretions (Hochereau-de Reviere and Seck, 1990; Cazorla et al, 1997) or in somatic or germ cell compositions of the testis (Pissellet et al, 1990). In rams, the rise of FSH after castration is higher in carriers than in non-carriers of the Booroola B gene, resulting from a different sensitivity of the pituitary gland to negative testicular feedback (Price et al, 1990). In male fetuses, although temporal differences in body masses around 90 days of gestation were suspected, no long-term direct effect of the Booroola B gene on germ cells or on hormonal plasma or pituitary concentrations was recorded (Smith et al, 1996). This analysis did not analyse the somatic cells of the testis, and was performed on a mixed population of B+ and BB males obtained from litters of variable size (++ = 1.3 to 1.5 and BB/B+ = 2.3 to 2.9).

The aim of the present work was to analyse the body and testis masses, the somatic and germ cell contents of the testis between 60 and 140 days of gestation. This work was performed on homozygous fetuses, after embryo transfer of single FecB<sup>B</sup>FecB<sup>B</sup> and ++ embryos in Merinos d'Arles ewes to eliminate the differences due to litter size or to the genotype of the dam.

## MATERIAL AND METHODS

The Booroola FecB<sup>B</sup> gene (referred to as B in the following) was progressively introgressed in a Mérimos d'Arles flock (ENSAM, Domaine du Merle, France) to obtain pure homozygous BB ( $n = 6$ ) and ++ ( $n = 4$ ) rams and ewes. Adult 2–4-year-old females were used; they were known to be either BB ( $n = 19$ ) or ++ ( $n = 13$ ), using the maximum likelihood analysis of their ovulation rates (Le Roy et al, 1989). They were induced to superovulate with a sequential pFSH (JF Beckers, Fac Vétérinaire, Liège, Belgium) treatment of six intramuscular injections with decreasing doses (Cognié et al, 1986). The gonadotropin treatment started 2 days before the end of an intravaginal progestagen treatment (Intervet: 40 mg/sponge for 14 days). The total dose of injected pFSH was 16 mg (4, 4, 2, 2, 2, 2) and 20 mg (5, 5, 3, 3, 2, 2) in BB and ++ genotypes, respectively.

To facilitate embryo transfer, treated BB ( $n = 12$ ) and ++ ( $n = 8$ ) females were divided into two groups with a 2-day interval between the end of treatment of each group. Raddle marked ewes were recorded daily. Embryo recoveries were performed on days six or seven post-oestrus by surgical flushing of the two uterine horns with PBS medium, under general anaesthesia. The ewes were fasted for 24 h and received an im injection of sodium thiopental (10 mg/kg of body weight) followed by halothan/oxygen inhalation through an endotracheal intubing. Corpora lutea were counted. All the embryos were examined under binocular microscope and their stage of development and quality were determined before transfer. Only good quality embryos at morula or blastocyst stages were transferred. Embryo transfer was performed in synchronised recipient ewes through laparoscopy. The recipient ewes

received an im injection of xylazine (0.11 mg/kg of body weight). One embryo was transferred, ipsilateral to the corpus luteum, per recipient female (34 ++ introgressed Mérinos d'Arles and three pure Mérinos d'Arles) to avoid side-effects of the number of fetuses and to ensure the same genetic gestation environment. The fetuses were then allotted into three groups for age at killing. The recipient females were either slaughtered (20) and the fetuses taken rapidly after death or the fetuses were obtained by caesarian (17) under general anesthesia, as described previously.

Fetuses were weighed immediately and males were identified by their anogenital length. Male genital tracts were carefully dissected and testes were weighed immediately to avoid dessication; at 60 days of gestation, the mesonephros, which was still present, was separated from the male genital tract and weighed separately from the testis. One testis per ovine fetus lamb was fixed at  $60 \pm 1$  ( $n = 16$ ),  $100 \pm 1$  ( $n = 10$ ) and  $140 \pm 1$  ( $n = 11$ ) days of age. They were fixed in Bouin Hollande's solution and processed as described previously (Hochereau-de Reviers et al, 1995). Paraffin sections (10  $\mu$ m thick) were stained by Feulgen reaction for nuclei and counterstained with Alcian blue. In 60-day-old fetuses, the sections were either equatorial or sagittal and this was taken into account for analysis. In older fetuses, the sections were parasagittal. In addition, to validate the mass measurement, testis volume was calculated from its mean diameter on the largest histological section for each testis.

Two methods were used to estimate the number of gonocytes per testis.

### Method I

The sex cords were considered as winding and contorted cylinders from which the length could be calculated from their volume and their mean cross-sectional area. The relative proportions of sex cords and intertubular tissue were determined with an ocular integrator (25 regularly spaced points), at a magnification of  $\times 800$ , on 20 fields per testis (500 points/testis), on the largest section of the fetal testis. Further, mean sex cord diameter was determined from 20 randomised round sex cord cross-sections per testis, taken in different loci of the testis with an ocular micrometer (at a magnification of  $\times 160$ ). The total vol-

umes of seminiferous tubules and of intertubular tissue were then calculated by multiplying their relative volumes in the parenchyma by the total mass of the testis. The total length of the seminiferous tubules was then calculated by division of their total volume by the mean seminiferous cross-sectional area (Attal and Courot, 1963). The Sertoli and gonocyte nuclei were counted on ten (100 and 140 days of gestation) or 20 (60 days of gestation) randomised cross-sections of sex cords per testis (at a magnification of  $\times 800$ ). The mean individual diameter of gonocytes, Sertoli cell nuclei and Leydig cells were determined with the ocular micrometer at a magnification of  $\times 1\,000$  on 20 cells per each cell type, taken in randomised loci. The mean corrected number of cells per unit length was calculated after correction for nuclear size and section thickness (10  $\mu$ m) according to Abercrombie (1946). The total numbers of Sertoli cells and gonocytes per testis (method I) were then obtained by multiplying their number per unit length by the total length of seminiferous tubules per testis.

### Method II

The second method estimated the total volume of gonocytes in the testicular parenchyma. The relative volume of gonocytes in the testicular parenchyma was determined with ocular integrator on 20 randomised fields per testis (at a magnification of  $\times 800$ ). The total volumes of gonocytes per testis were then calculated by multiplying the relative volume by the testis mass. The mean individual cellular volume of gonocytes was calculated from its mean cellular diameter, for each testis and the total number of gonocytes per testis were obtained by division of their total volume by their mean individual volume.

Fetal Leydig cells exhibited a dense cytoplasm with a relatively centrally located ovoid heterochromatic nucleus. They were first identified in semi-thin sections by Mauléon and Bézard (unpublished data) where they presented characteristics of steroidogenic cells. In 60-day-old fetuses, they formed islets of cells in between sex cords. Later on, as the blood vessels developed, Leydig cells surrounded the capillaries. The relative proportion of the Leydig cells in the intertubular tissue was determined with the ocular integrator (at a magnification of  $\times 800$ ) on 20

fields per testis. The total volume of Leydig cells was obtained by multiplying their relative volume in the intertubular tissue and the total volume of intertubular tissue. Their total numbers were obtained by division of their total volume per testis by their mean individual volume, calculated from their mean cross-sectional area, assuming they were round. All the values were corrected for shrinkage (shrinkage coefficient for Bouin Hollande's fixative solution = 0.64).

### Statistical analysis

The results were compared using Student's *t*-test or ANOVA with or without body weight or age as the covariate (CSS). Pearson pairwise correlations between method I and II evaluations of gonocytes per testis and between testis weights and their volumes calculated from testis section were calculated.

## RESULTS

### Embryo transfer data

The embryo transfer data are shown in table I. The ovulation rate obtained in BB ewes after a pFSH total dose of 16 mg/ewe was significantly lower (-36%;  $P = 0.05$ ) than that obtained with 20 mg/ewe in ++ ewes. After the transfer of one embryo per recipient ewe, no difference in viability of embryo according to genotype was observed and the same pregnancy rate after embryo transfer was recorded in each genotype (61 and 68.5%). One pregnant ewe died in each group before embryo recovery. The sex ratio was identical for each genotype (male/female = 0.47).

### Body, mesonephros and testis masses

At 60 and 100 days of pregnancy, a significant difference in body mass was recorded, the BB being lighter than the ++ male fetuses (60 days = -11%;  $P = 0.04$ ; 100 days = -13%;  $P = 0.04$ ); at 140 days of gestation, such a difference was no longer recorded (table II).

At 60 days of gestation, the mesonephros was still present and was separated from embryonic male genital tract (deferent ducts and future cauda of epididymis) and weighed; but it did not differ significantly, according to the genotype (BB =  $18.9 \pm 2.4$ ; ++ =  $19.3 \pm 1.6$  mg). At a later age of pregnancy, the mesonephros was completely involuted except the part giving rise to the efferent ducts and the head of epididymis. However, no dissection was possible.

Testis masses did not differ significantly between carriers and non-carriers, whatever the age. The testis increased in weight 8-fold between 60 ( $14.8 \pm 2.7$  mg) and 100 days ( $123.7 \pm 7.1$  mg) and by 4-fold between 100 and 140 days of gestation ( $468 \pm 26$  mg), whatever the genotype. The testis mass was highly correlated ( $r = 0.90$ ;  $P < 0.01$ ) to the mean volume calculated from the mean cross-sectional diameter of the testis. The variation coefficient of calculated testis volume (30–40%) was higher than that of testis weight (17–20%).

**Table I.** Comparison of ovulation rate, pregnancy rate after embryo transfer and sex ratio in BB and ++ ewes (mean  $\pm$  sd).

Genotype	Ewes			Fetuses			
	Donors	Ovulation rate	Recipient pregnant (% pregnant)	Male	Female		
BB	19	$5.8 \pm 1.7$	73	50	68.5	23	26
++	13	$9.0 \pm 7.8$	61	37	61	17	19

**Composition of the testis.****Sex cords**

Table II also shows that the relative proportion of the sex cords in the testicular parenchyma did not vary between 60 and 140 days of age and that the intertubular tissue represented more than half of the testis parenchyma (sex cords = 46%; intertubular tissue = 54%). The mean diameter of sex cords (40  $\mu$ m) did not vary between 60 days and 140 days of gestation, whatever the genotype. The sex cords grew in length by a factor of 9.2 between 60 and 100 days and by a factor of 4.2 between 100 and 140 days

of gestation (60 days = 3.25 m; 100 days = 30 m; 140 days = 125 m). Sex cords did not differ in length with genotype, whatever the age.

Similarly, the total number of Sertoli cells per testis increased by a factor of 10 between 60 and 100 days and by a factor of 5.6 between 100 and 140 days of gestation (60 days =  $3.7 \times 10^6$ ; 100 days =  $37.4 \times 10^6$ ; 140 days =  $209 \times 10^6$ ); their nuclear diameter decreased slightly between 60 and 100 days of gestation (-10%) but did not change later on.

Concomitantly, the germ cells, ie, the total numbers of gonocytes per testis

**Table II.** Comparison of body and testis weights and of testicular composition in Booroola B B and ++ lambs between 60 and 140 days of gestation (m  $\pm$  sem).

Fetal age	60 days		100 days		140 days	
	BB	++	BB	++	BB	++
No of foetus	7	8	5	5	5	6
Body mass (g)	51.4 $\pm$ 2.0a	57.5 $\pm$ 1.5b	694 $\pm$ 22a	804 $\pm$ 38b	3240 $\pm$ 144	3108 $\pm$ 185
Testis mass (mg)	16.2 $\pm$ 2.9	15.4 $\pm$ 1.0	109 $\pm$ 10	136 $\pm$ 19	481 $\pm$ 27	457 $\pm$ 44
Sex cord						
diameter ( $\mu$ m)	41 $\pm$ 1	41 $\pm$ 1	42 $\pm$ 5	39 $\pm$ 5	37 $\pm$ 1	39 $\pm$ 2
total length /testis (m)*	3.3 $\pm$ 0.3	3.2 $\pm$ 0.2	27.0 $\pm$ 2.6	32.8 $\pm$ 1.7	130.1 $\pm$ 14.4	118.7 $\pm$ 14.4
Sertoli cells						
No/testis ( $10^6$ )	3.8 $\pm$ 0.5	3.6 $\pm$ 0.3	38.8 $\pm$ 5.7	35.9 $\pm$ 3.7	216.9 $\pm$ 21.0	201.3 $\pm$ 25.9
nucl diam ( $\mu$ m)	5.8 $\pm$ 1.1	6.0 $\pm$ 1.2	5.3 $\pm$ 0.7	5.5 $\pm$ 1.8	5.3 $\pm$ 0.5	5.4 $\pm$ 1.0
Gonocytes						
nuc diam ( $\mu$ m)*	7.3 $\pm$ 0.1	7.4 $\pm$ 0.1	8.9 $\pm$ 0.1	8.0 $\pm$ 0.2	8.6 $\pm$ 0.2	8.4 $\pm$ 0.2
cell diam ( $\mu$ m)*	10.5 $\pm$ 0.1	10.8 $\pm$ 0.1	15.0 $\pm$ 0.1	14.4 $\pm$ 0.2	13.4 $\pm$ 0.3	13.6 $\pm$ 0.4
No/testis ( $10^6$ )						
Meth I	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	2.5 $\pm$ 0.2	3.2 $\pm$ 0.4	7.4 $\pm$ 1.3	8.1 $\pm$ 1.5
Meth II	0.8 $\pm$ 0.1	0.7 $\pm$ 0.1	3.1 $\pm$ 0.2	5.1 $\pm$ 0.9	13.8 $\pm$ 0.5	12.4 $\pm$ 0.3
Leydig cells						
No/testis ( $10^6$ )	2.20 $\pm$ 0.30	2.32 $\pm$ 0.38	34.1 $\pm$ 3.8	45.7 $\pm$ 12.9	87.1 $\pm$ 16.1	142.1 $\pm$ 45.5
cell diam ( $\mu$ m)*	10.0 $\pm$ 2.9	9.4 $\pm$ 3.2	7.8 $\pm$ 1.7	7.6 $\pm$ 2.2	6.9 $\pm$ 1.9	7.2 $\pm$ 1.6

\* m, meter;  $\mu$ m, micrometer.

increased during the fetal life, whatever the method employed (I or II). At 60 days of gestation, the total number of gonocytes was slightly but not significantly higher in BB than in ++ fetal testis, whatever the method employed. The total numbers of gonocytes per testis (method I) did not differ with the orientation of the section (equatorial:  $0.7 \pm 0.16$ ; parasagittal:  $0.7 \pm 0.13$ ). The cellular and nuclear mean diameters of gonocytes increased by 38 and 15%, respectively, between 60 and 100 days of fetal life, whatever the genotype and did not vary later on. Prophase and mitoses of gonocytes were recorded often at 60 and 100 days of gestation. The prophase nuclei of gonocytes exhibited larger nuclei ( $9.5 \pm 0.5 \mu\text{m}$ ) than the whole population of gonocyte interphase nuclei. The total numbers of gonocytes per testis increased by a factor of 4 during the period 60–100 days and a factor of 2.8 during the period 100–140 days of gestation (60 days =  $0.75 \times 10^6$ ; 100 days =  $2.8 \times 10^6$ ; 140 days =  $7.75 \times 10^6$ ). The relative volume of gonocytes in the testicular parenchyma decreased significantly ( $-50\%$ ;  $P < 0.01$ ) with fetal age. The two methods of estimation of total numbers of gonocytes per testis gave values which were highly correlated to each other in each individual ( $r = 0.841$ ;  $P < 0.001$ ) and of the same order of magnitude but higher (50%) with method II. The number of gonocytes expressed per milligram of testis decreased significantly with increasing fetal age, whichever the method used, but did not differ with the presence of the B gene.

### *Intertubular tissue*

The Leydig cell mean cross-sectional diameter decreased with fetal age by  $-17\%$  between 60 and 100 days and by  $-10\%$  between 100 and 140 days of gestation. In the intertubular tissue, the Leydig cells increased by a factor of 18, during the 60–100 days and by a factor 3 during the period 100–140 days of gestation (60 days =

$2.3 \times 10^6$ ; 100 days =  $40 \times 10^6$ ; 140 days =  $115 \times 10^6$ ). No genotype differences in Leydig cell number or size were observed, whatever the fetal age (table II).

## DISCUSSION

A relatively low ovulatory response of BB ewes after injection of 16 mg pFSH/ewe was obtained in this experiment. The enhanced ovarian sensitivity to gonadotropins in ewes carrying the F gene (Webb et al. 1995) was the reason for using a lower FSH dose than in non-carrier ++ ewes. It is of interest to report that the same FF ewes retreated with a similar protocol 1 year later but with a higher dose, 20 mg pFSH/ewe, presented a higher ovulation rate (mean  $\pm$  sd:  $15.0 \pm 8.5$ ) than with 16 mg/ewe; and their mean ovulation rate was doubled as compared to ++ ewes. A mean number of seven transferable embryos per treated donor ewe was obtained (Cognié and Baril, 1995, pers comm).

To avoid variations due to the uterine occupancy and capacity, we have transferred one fetus per recipient ewe which was a synchronised Mérinos d'Arles or ++ introgressed Mérinos d'Arles. The mean body weight of BB was lighter than that of ++ fetuses at 60 and 100 days of gestation and the differences observed in body weight were not related to the presence of multiple gestation or differences in uterine environment. By contrast, in the female 60-day-old fetuses, collected in the same experiment, no genotype difference in body weight was recorded (Courot and Driancourt, pers comm).

At 60 days of gestation, there was no relationship between testis and body weights while later on, at 100 days of gestation, a positive and significant relationship between testis and body weights has been recorded and corroborated the results of Smith et al (1996) observed at 90 days of gestation. The

testis weights observed in Mérinos d'Arles BB or ++ fetuses are slightly lower than those observed previously in Ile de France or crossbred Ile de France lambs (Hochereau-de Reviers et al, 1995). At the end of gestation, the smaller testis weight observed in Mérinos d'Arles (BB or ++) as compared to previous data obtained in Ile de France or Ile de France crossbred lambs, could explain the delayed rise in testis growth after birth, observed in Mérinos d'Arles [16–20 weeks, Hochereau-de Reviers and Seck (1991)] as compared with that observed in Ile de France (7 weeks) or Romanov [5 weeks; Lafortune et al (1984)]. Testis weights and testis volume calculated from mean testicular cross-sectional diameter measured on the largest histological sections were compared; they were highly correlated ( $r > 0.9$ ); the calculated volume was lower (40–60%) than testis weight owing to shrinkage. The testis mass was preferred as it was precise, not dependent on the histological treatment and its coefficient of variation was always the smallest.

Two methods to estimate the total numbers of germ cells per testis were compared here and both used the testis mass. The first method, largely developed in our group is based on the tubular nature of the seminiferous epithelium in mammals and birds. Further, we previously demonstrated that the diameter of the sex cords did not vary in sheep testis during fetal life (Hochereau-de Reviers et al, 1995). In ungulates, the sex cords originated and ended in the central rete testis by the tubuli recti; sex cords were contorted and winding cylinders. Their diameter did not change with the position, near the tunica albuginea or near the rete testis; the proportion of testicular parenchyma occupied by sex cords did not vary with the equatorial or parasagittal orientation of the histological section.

The second method is based on the proportion of gonocytes in the testicular parenchyma, estimated from a 25-point ocu-

lar integrator: for each testis, 500 points are counted. The technique employed as discussed by Solari (1973, 1977) is similar in nature to that described and developed later on by Gundersen (1986). Both methods I and II gave highly correlated results, of the same order of magnitude, the second giving higher number (30–50% more) than the first one. Method I implied four succeeding calculations and added four sources of errors while method II implied only three. Method II should be more precise, provided that the relative proportion of gonocytes in the testicular parenchyma did not often take null values. The mean total numbers of gonocytes at 140 days of gestation were 8 to  $12 \times 10^6$  and were not very different from those recorded ( $450 \cdot \text{testis} \cdot 10^{-4}$ ) at 135 days of gestation by Smith et al (1996). During the fetal life, the increment of the sex cords is mainly due to an increase in length. The germ cells present in the fetal ovine testis are essentially gonocytes, characterised by a prominent round nucleus. Mitotic figures are often recorded but no meiotic prophase has been observed, except in one fetal ovine ovotestis, observed in a 60-day-old ++ male fetus (excluded from these data), in which zygotene spermatocytes were the most advanced meiotic cells recorded. The pre-meiotic nuclei described by Smith et al (1996) were in fact prophase of gonocytes, characterised by round nuclei, larger than those of interphase gonocytes. In the female ovary, such figures were present and corresponded to ovogonial prophases. By contrast the nuclei of type A spermatogonia, as described by Ortavant (1959) were pale and devoided of heterochromatin, ovoid with a mean diameter of  $9.2 \pm 0.1 \mu\text{m}$  and with a single central nucleolus; progressively with spermatogenic differentiation, their nuclei became spherical, heterochromatic with small nucleoli and their mean nuclear diameter decreased to  $6.8 \pm 0.05 \mu\text{m}$  (Ortavant, 1959). To promote the differentiation of gonocytes in spermatogonia and spermatocytes and to see whether its kinetic differ

with BB or ++ genotypes, we grafted 60-day-old fetal testes in the scrotum of Nude immunotolerant mice, one testis per Nude mice. The testes survived but did not grow; however, appearance of spermatogonia with pale ovoid nuclei and central nucleolus, was observed in grafted testes but with no genotype variation (Hochereau-de Reviers and Perreau, 1997). Thus, as in prepubertal or adult rams carrying the B gene no difference in kinetic of differentiation of gonocytes or in efficiency of the process was observed. Presence of the gene appeared to affect only transiently the body growth in the male.

In addition to the data of Smith et al (1996) on testis weight and gonocyte numbers, we analysed somatic cells of the testis. In the adult females, one major difference was the number of granulosa cells per ovulating follicle (McNatty et al, 1990); we never obtained genotype differences in Sertoli or Leydig cell total numbers per testis between 60 and 140 days of gestation or in adult rams (Pisselet et al, 1990). In Mérinos d'Arles male lambs at the end of gestation, the individual cellular cross-sectional area of Leydig cells was smaller than those recorded for Ile de France or Romanov lambs and could be related to the low level of testosterone observed after birth at 2 weeks of age in these crossbred lambs (Seck et al, 1988).

Collectively, these results found, for single embryos transferred in the same genetic uterine environment, only lighter body weights until 100 days of gestation in BB as compared to ++ fetal lambs. Later on the differences vanished. These variations in body weights were not related to significant variations of the testis parameters. No important differences in the expression of FecB<sup>B</sup> in the male gonad was observed, whatever the age, by contrast to the early fetal expression observed in the female (Smith et al, 1994; McNatty et al, 1995). Even the gene is autosomal and located on ovine chromo-

some 6, homologous to human chromosome 4 (Montgomery et al, 1993; Lanneluc et al, 1994). We could suspect that the expression of the FecB<sup>B</sup> is repressed by the maleness, except during short periods of the life: until 100 days of fetal life, during prepubertal testis growth between 2 and 10 weeks of age after birth and during seasonal spring testis growth.

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