Aust. J. Biol. Sci., 1983, **36**, 351–5

Ovarian Follicles of New-born Merino Lambs from Genetic Lines which Differ in Fecundity

Robin J. Tassell,[A,B] *J. P. Kennedy,*[A] *B. M. Bindon*[C] *and L. R. Piper*[C]

[A] School of Wool and Pastoral Sciences, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.
[B] Present address: 'Willawong', Eastview Drive, Orangeville, N.S.W. 2570.
[C] Division of Animal Production, CSIRO, Armidale, N.S.W. 2350.

Abstract

Ovaries were obtained from 78 new-born lambs (12 singletons, 25 twins, 28 triplets, 10 quadruplets and 3 quintuplets) from flocks selected for (T902, T903 and Booroola) or against (O) multiple births. Sections of the ovaries were examined with a projection microscope and the numbers of all types of follicles were estimated. There were no differences between genetic lines in the number of primordial follicles, after adjustment for litter size and sire; however, there were significantly more of these follicles in single-born lambs than in lambs born in litters of two or more within genetic lines. The number of vesicular follicles was lower in Booroola than in O lambs.

Introduction

Ovulation rate differs in Merino flocks which have been selected for high and low incidence of multiple births (Bindon *et al.* 1971). These flocks include lines from a Peppin flock selected for (T) and against (O) multiple births and a high-fecundity, medium-wool, non-Peppin flock, the Booroola (B). Their genetic history and flock performances have been described by Turner (1978).

Trounson *et al.* (1974) reported that lambs from the O flock had more ovarian primordial follicles than lambs from the T flock and concluded that genetic selection for the incidence of multiple births has resulted in changes in the number of primordial follicles of the post-natal lamb. This study was designed to determine if this conclusion was substantiated from examination of ovaries from Booroola lambs and additional lambs from the T and O flocks.

Materials and Methods

Ovaries were collected from 78 lambs which were born dead or died soon after birth from exposure or desertion. Most ovaries were collected at 0800 h from lambs which died during the night. The animals included 10 from flock O, 26 from flock T (groups 902 and 903) and 42 from flock B. In groups 902 and 903, lambs in the T flock born to ewes which had been selected on their dam's lifetime litter-size records or their dam's lifetime ovulation records, respectively, are distinguished. Group 903 was, however, only created 2 years before the present lambs were born.

The tissues were fixed and processed according to standard histological procedures. Sections were cut at 6 μm and every 20th section was mounted, stained with a trichrome stain (Gomori 1950) and examined with a projection microscope. Ovaries were excluded if post-mortem degeneration interfered with counting techniques.

0004-9417/83/040351$02.00

Follicles were classified as described by Trounson *et al.* (1974) and Tassell *et al.* (1978). Primordial, small and large growing (non-vesicular), and partially and fully formed vesicular follicles were counted. All primordial follicles were counted in every 100th section and all other follicles were counted in every 40th section. Nuclear diameters were measured at magnification $\times 500$ and an estimate of follicle numbers per section was obtained, using the formula of Mandl and Zuckerman (1951). This estimate was multiplied by the number of sections in an ovary to derive an estimate for that ovary.

The data were analysed by least-squares procedures using both the actual and log transformed counts [$\log_{10}$ (count+1)]. Several different models were employed.

Model 1 (Table 1):

$$y = \mu + g_i + e_{ij},$$

where

y = follicle count for an individual animal,
g = effect of genetic line ($i = 1, ..., 4$),
e = random errors, assumed to be normally and independently distributed ($0, 0, \sigma_e^2$).

Model 2 (Table 2):

$$y = \mu + g_i + l_j + s_{ik} + e_{ijkl},$$

where y, g, and e are as defined in model 1, and

l = effect of litter size in which the lamb was born ($j = 1, ..., 5$),
s = effect of the sire of the lamb (within genetic line)—$k = 1, ..., 5$ for line 902, $k = 1, ..., 6$ for line 903 and $k = 1, ..., 7$ for line B.

Model 3 (twin-born lambs only):

$$y = \mu + g_i + x_j + s_{ik} + e_{ijkl},$$

where y, μ, g, s and e are as defined above and x is the effect of sex of litter mate (1 = male co-twin; 2 = female co-twin).

The least-squares means ($\pm$s.e.) presented in Tables 1 and 2 are from analyses of the untransformed data but the tests of significance were those obtained from the analyses of the log transformed counts. The line effects in models 2 and 3 were tested against the pooled sire (within-line) variance, but in all other tests, the error mean square was used as the denominator of the variance ratio. When the line or litter-size effects were significant, the individual means were compared according to Duncan's multiple range test.

Table 1. Least-squares mean numbers of primordial follicles for each genetic line

Means with different superscripts differ significantly ($P < 0{\cdot}05$)

Line	n	Mean No. of primordial follicles $\pm$s.e.
O	10	115072 ± 14675^{b}
T902	13	50746 ± 12870^{a}
T903	13	74124 ± 12870^{ab}
Booroola	42	89234 ± 7160^{b}

Results

Initially, the mean numbers of primordial follicles in the different genetic lines were calculated without correction for the influences of size of litter or sire (Table 1). There were significantly fewer primordial follicles in T902 than in O or Booroola lambs. However, after correction of numbers of primordial follicles for litter size and sire, both of which were significant effects, there were no significant differences between genetic lines (Table 2). These follicles were most numerous in singletons and least numerous in quintuplets.

Table 2. Least-squares means ±s.e. for each ovarian follicle class in each genetic line and litter size grouping

Means with different superscripts differ significantly ($P < 0.05$). ** $P < 0.01$; * $P < 0.05$; n.s., not significant

Line or litter size	n	$10^{-2} \times$ Mean No. of Primordial Follicles	Mean No. of Growing Follicles		Mean No. of Vesicular Follicles	
			Small	Large	Partial	Full
Line						
O	10	802±172	2110±400	643±130	174±56[a]	27±18[a]
T902	13	480±152	2140±360	215±115	103±49[b]	23±16[b]
T903	13	758±145	1630±340	239±109	177±47[a]	33±15[a]
Booroola	42	940±88	2350±210	583±67	41±29[c]	10±9[b]
Litter size						
1	12	1277±140[a]†	2560±330	651±106	266±45	66±15
2	25	663±91[b]	1940±210	498±69	90±30	8±10
3	28	738±109[b]	2530±260	465±83	80±35	14±12
4	10	925±173[ab]	2150±410	363±131	88±56	15±18
5	3	121±289[c]	1110±680	123±218	85±94	14±31
Tests of significance						
Line		n.s.	n.s.	n.s.	**	*
Litter size at birth		**	n.s.	n.s.	n.s.	n.s.
Sires		*	n.s.	n.s.	*	*

Line and litter size did not significantly influence the numbers of small or large growing follicles but line had a highly significant effect on the number of vesicular follicles (Table 2). There were very few follicles with an antrum in the Booroola lambs. Litter size at birth did not significantly affect numbers of this class of follicle although singles tended to have more than multiple-born lambs. Sires also had a significant effect on numbers in both vesicular follicle categories.

Sex of litter mate (model 3) did not significantly influence follicle numbers.

Discussion

The principal difference in numbers of primordial follicles was between single- and multiple-born lambs. In order to make realistic comparisons between genotypes, adjustment must therefore be made for differences in the distribution of birth types. In the earlier study of Trounson *et al.* (1974), in which data were not corrected for litter size at birth, the numbers of primordial follicles were significantly different in lambs from T and O flocks. After adjustment for litter size and sire in the present experiment the T and O were not significantly different. Likewise Land (1970) reported that pure-bred Blackface and Welsh lambs had significantly more oocytes at birth than the more fecund Finnish Landrace crosses with these breeds. However, he also did not correct for litter size at birth.

Differences in numbers of primordial follicles could be due to number of oogonial divisions (divisions per unit time or length of period in fetal life during which divisions occur), or to differences in rate of loss after formation. No striking differences in numbers of degenerating primordial follicles were noted but it is difficult to detect atretic changes in such small follicles with the light microscope.

It is not clear why litter size should affect numbers of primordial follicles. Fetuses in large litters clearly compete for limited nutritional resources during pregnancy. Whether this competition would be strong enough to affect the formation of new oogonia and/or oocytes at the stage when oogenesis is occurring is not known. It might depend on the nutritional status of the ewe which has been reported to affect embryos even before day 35 of pregnancy (Parr and Williams 1982). On the other hand, low nutritional status may in fact reduce the rate of atresia as in young rats (Lintern-Moore and Everitt 1978).

The other feature of the results which is noteworthy is the lack of vesicular follicles in the Booroola lambs. This did not appear to be strongly related to litter size and can be regarded as a real genetic difference. The results suggest reduced stimulus for development in Booroola lambs, or a later onset of development, or a greater loss of developing follicles. There was no histological evidence for the last possibility. Bindon and Turner (1974) have shown significant differences in concentrations of LH in plasma at 30 days of age due to birth type (litter size), as well as differences due to line, in the same flocks as were used in this experiment. Their animals from the B flock had higher LH than lambs from the O flock and FSH also was higher at 30 days of age (Findlay and Bindon 1975). It is possible that these differences in gonadotrophin levels are a reflection of the lack of ovarian development in Booroola lambs at birth, leading to a reduced steroid feedback at 30 days of age. This could be tested by studying changes in plasma gonadotrophin concentrations following castration of lambs from these genotypes in the first weeks of life.

Sires were a significant effect on numbers of primordial and vesicular follicles. The genotypes of the Booroola rams represented in this study with respect to the putative Booroola gene are not known. Even though the data are drawn from selection experiments each with relatively few sires, it is not unreasonable to find significant differences between progeny of different sires. No attempt has been made to attach any genetic significance to these sire effects in the data.

These findings contribute further to our knowledge of early ovarian development in the Booroola. They are of little value, however, in identification of potentially fecund ewes since the ovaries must be removed to obtain the measurements.

Acknowledgment

The assistance of Brigitte McCracken in the histological studies is greatly appreciated.

References

Bindon, B. M., Ch'ang, T. S., and Turner, H. N. (1971). Ovarian response to gonadotrophin by Merino ewes selected for fecundity. *Aust. J. Agric. Res.* **22**, 809–20.

Bindon, B. M., and Turner, H. N. (1974). Plasma LH of the prepubertal lamb: a possible early indicator of fecundity. *J. Reprod. Fertil.* **39**, 85–8.

Findlay, J. K., and Bindon, B. M. (1975). Plasma FSH in Merino lambs selected for fecundity. Proc. 7th Ann. Conf. Aust. Soc. Reproductive Biology, Sydney. p. 33.

Gomori, G. (1950). A rapid one-step trichrome stain. *Am. J. Clin. Pathol.* **20**, 662–4.

Land, R. (1970). Number of oocytes at birth in the ovaries of pure, and Finnish Landrace cross, Blackface and Welsh sheep. *J. Reprod. Fertil.* **21**, 517–21.

Lintern-Moore, S., and Everitt, A. V. (1978). The effect of restricted food intake on the size and composition of the ovarian follicle population in the Wistar rat. *Biol. Reprod.* **19**, 688–91.

Mandl, A. M., and Zuckerman, S. (1951). The relation of age to number of oocytes. *J. Endocrinol.* **7**, 190.

Parr, R. A., and Williams, A. H. (1982). Nutrition of the ewe and embryo during early pregnancy. *Aust. J. Biol. Sci.* **35**, 271–6.

Tassell, R., Chamley, W. A., and Kennedy, J. P. (1978). Gonadotrophin levels and ovarian development in the neonatal ewe lamb. *Aust. J. Biol. Sci.* **31**, 267–73.

Trounson, A. O., Chamley, W. A., Kennedy, J. P., and Tassell, R. (1974). Primordial follicle numbers in ovaries and levels of LH and FSH in pituitaries and plasma of lambs selected for and against multiple births. *Aust. J. Biol. Sci.* **27**, 293–9.

Turner, H. N. (1978). Selection for reproduction rate in Australian Merino sheep: direct responses. *Aust. J. Agric. Res.* **29**, 327–50.

Manuscript received 24 January 1983, accepted 28 June 1983