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Primordial Follicle Numbers in Ovaries and Levels of LH and FSH in Pituitaries and Plasma of Lambs Selected for and against Multiple Births

A. O. Trounson,[A] *W. A. Chamley*,[B] *J. P. Kennedy*[C] *and Robin Tassell*[C]

[A] Department of Animal Husbandry, University of Sydney Farms, Camden, N.S.W. 2570.
[B] Department of Physiology, University of Melbourne, S. S. Cameron Laboratory, Werribee, Vic. 3030.
[C] School of Wool and Pastoral Sciences, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

Abstract

The numbers of primordial follicles in ovaries of ewe lambs, from two groups of Peppin Merinos that had been selected for (T group) and against (O group) multiple births, were estimated at birth, 7 days and 5 months of age. Peripheral plasma and anterior pituitary levels of LH and FSH were determined by radioimmunoassay in the lambs at 5 months of age. These lambs were either entire or unilaterally ovariectomized (hemispayed) at 7 days of age.

There was no difference in primordial follicle numbers between lambs at birth and 7 days of age, but when paired ovaries of hemispayed lambs at 7 days and 5 months of age were compared there was a significant increase in follicle numbers with age (34 903 *v.* 48 047). O-group lambs had more primordial follicles than T-group lambs at birth and 7 days of age (65 501 *v.* 37 797) and there was a similar but not significant difference at 5 months of age (53 934 *v.* 44 057).

Peripheral plasma LH was higher in T-group than in O-group lambs and plasma LH levels were correlated to pituitary LH content ($r = 0{\cdot}75$). Vesicular follicles larger than 2 mm in diameter were present only in ovaries of lambs with plasma LH levels above 3·0 ng/ml. Pituitary FSH levels were considerably higher in hemispayed lambs than in entire lambs and there was a trend to higher levels in T-group lambs when compared with O-group lambs.

It is concluded that genetic selection for the incidence of multiple births has resulted in changes in the number of ovarian primordial follicles of the post-natal lamb and it is suggested that these changes may be related to the levels of pituitary gonadotrophins.

Introduction

Direct genetic selection for multiple births in sheep flocks derived from a common source has resulted in groups of animals with distinctly different lambing rates. The response has been described by Turner *et al.* (1962) and Turner (1966, 1968, 1969) in Peppin Merinos in which the difference in lambing rate appears to be due primarily to a correlated response in natural ovulation rate. Mean differences between groups selected for and against multiple births vary from 0·4 to 0·9 corpus luteum for ewes of various ages (Packham and Triffitt 1966; Bindon *et al.* 1971; Trounson and Moore 1972).

The effect of selection on mechanisms controlling ovulation rate has not yet been resolved, but Bindon (1973*a*, 1973*b*) has reported genetic differences in levels of LH in peripheral plasma of 30-day-old lambs from the Merino selection groups. Differences in the response to exogenous gonadotrophin have also been reported (Bindon *et al.* 1971; Trounson and Moore 1972) but there is no information on pituitary gonadotrophin levels or ovarian oocyte populations. The purpose of the present experiment was to determine the ovarian primordial follicle numbers of ewe lambs from the Merino selection groups at birth, 7 days and 5 months of age. Further, anterior pituitary and peripheral plasma levels of LH and FSH in lambs

at 5 months of age were examined in relation to the selection group of the lamb and to the effects of unilateral ovariectomy at 7 days of age.

Materials and Methods

Animals

The lambs used in this study were progeny of the Peppin Merino selection groups described by Turner *et al.* (1962). Selection had either been for (T group) or against (O group) the incidence of multiple births. The lambs were the result of an ova transfer study (Trounson and Moore 1972), where fertilized ova from the O group and T group were transferred to recipient ewes of both groups. All lambs born to recipient ewes were identified at birth and were run under field conditions at the University of New South Wales Field Station, Hay, N.S.W.

Experimental Procedures

Ovaries obtained from 12 lambs within 24 h of birth were immediately fixed for histology. Fourteen ewe lambs from the O and T selection groups were unilaterally ovariectomized (hemispayed) 7 days after birth by removing the left ovary which was fixed for histology. Nine of the hemispayed lambs survived to 5 months of age and were slaughtered along with nine entire lambs. The remaining ovary of the hemispayed lambs and both ovaries of the entire lambs were recovered, weighed and fixed for histology. A jugular vein blood sample was taken by venipuncture just prior to slaughter and the plasma was frozen in aliquots for hormone assays. The anterior pituitary glands of the slaughtered lambs were dissected free of adjoining tissue and frozen for gonadotrophin assays.

Histological Examination

Ovaries were fixed in Bouin's fixative for 24 h, embedded in paraffin, coded and sectioned at 8 μm. Sections were stained with trichrome (Gomori 1950) and haematoxylin.

Primordial follicles were counted at 320-μm intervals in four sections at each end of the ovary and at 640-μm intervals between these. Oocytes were included when the nucleus was visible, the outline of the oocyte could be determined, and not more than a single complete or incomplete layer of cells surrounded the oocyte. When more than half of this layer had become distinctly rounded the follicle was classified as a growing follicle and omitted. Such follicles almost invariably had a double layer of cells at some point. Atretic follicles and oocytes that lacked visible follicle cells were included if the above conditions were met. Nuclear diameter was measured in five oocytes in every second section counted. The total number of primordial follicles per ovary was calculated using the formula derived by Mandl and Zuckerman (1951*a*). The amount of follicular growth and development in the ovaries was noted and the diameter of the largest vesicular follicles in each ovary measured.

Hormone Assay Procedures

Peripheral plasma was assayed for progesterone by the competitive protein binding assay described by Thorburn *et al.* (1969) and total oestrogens by the radioimmunoassay described by Abraham (1969). The peripheral plasma levels and anterior pituitary content of LH and FSH were determined by the radioimmunoassays reported by Goding *et al.* (1969) and Salamonsen *et al.* (1973). Pituitaries were homogenized in 0·01M phosphate buffer (pH 7·4) and diluted 3×10^3 times prior to assay.

Statistical Procedures

The raw data are presented in tabular form with respective standard errors (S.E.). Primordial follicle numbers and pituitary FSH values were transformed to logarithms and plasma and pituitary LH values were transformed to square roots: $(x+0{\cdot}5)^{\frac{1}{2}}$. The raw or transformed data were submitted to *t*-tests or analyses of variance.

Results

Primordial Follicles at Birth and 7 Days of Age

The total numbers of primordial follicles in ovaries of lambs at birth and 7 days of age did not differ. In T-group lambs, where numbers were large enough for com-

parisons, at birth there was a mean of 38 662 primordial follicles (five lambs) compared to 37 257 (eight lambs) at 7 days of age. The data from lambs of both ages have been pooled for further comparisons and are presented in Table 1. Litter size had no apparent effect on total primordial follicles and has not been included in the classification of the lamb. The selection group of the recipient dam had no effect on the number of primordial follicles of lambs of either group (Table 1). When lambs were compared solely on the basis of their own genetic group, O lambs had larger numbers of primordial follicles than T lambs (65 501 *v.* 37 797; $P<0{\cdot}05$). The mean diameter of fixed ovaries from O-group lambs ($4{\cdot}32\pm0{\cdot}63$ mm, $n=9$) was slightly larger than from T-group lambs ($3{\cdot}36\pm0{\cdot}27$ mm, $n=16$).

Table 1. Total ovarian primordial follicles in ovaries of O- and T-group lambs at birth and 7 days of age

Genetic group: Lamb	Recipient dam	No. of lambs	Mean no. of primordial follicles (±S.E.)
T	T	4	37009±7214
T	O	9	38148±5802
	Total:	13	37797±4223
O	O	3	53320±12922
O	T	4	74638±26558
	Total:	7	65501±15618

Primordial Follicles at 5 Months of Age

When paired ovaries of the nine hemispayed lambs were compared, the total number of primordial follicles was higher at 5 months than at 7 days of age (48 047 *v.* 34 903; $P<0{\cdot}001$). However, there was no detectable difference between 5-month-old entire and hemispayed lambs (Table 2). At 5 months of age the difference between T- and O-group lambs was not marked although there was a trend to larger numbers of primordial follicles in ovaries of O lambs (O, 53 934 *v.* T, 44 057; $t=1{\cdot}7$, n.s.).

Table 2. Total primordial follicles and ovary weights of O- and T-group lambs at 5 months of age

Values are means±S.E.

Genetic group of lamb	State	No. of lambs	Wet wt of ovary (mg)	No. of primordial follicles
T	Entire	5	248±19	45927±6564
T	Hemispayed	5	319±44	42187±5846
Total		10	284±26	44057±4190
O	Entire	4	213±48	52675±11306
O	Hemispayed	4	364±76	55194±8875
Total		8	289±51	53934±6671

Fresh ovarian weights were similar for T and O lambs but compensatory hypertrophy was observed in the ovary remaining after unilateral ovariectomy (Table 2). In entire lambs at 5 months of age the mean of fresh ovarian weights was 232±23 mg compared to 339±40 mg in hemispayed lambs ($P<0{\cdot}05$). Compensatory hyper-

trophy of the remaining ovary appeared to be more pronounced in O-group than in T-group lambs (70·9 *v.* 28·6%), although the difference was not significant.

Plasma and Pituitary LH and FSH Levels at 5 Months of Age

Peripheral plasma LH levels (Table 3) were higher in T-group than in O-group lambs (4·4 *v.* 2·4 ng/ml; $P<0·05$) and there was a trend to higher mean LH levels in hemispayed lambs. There appeared to be no direct relationship between plasma LH levels and total primordial follicles in individual lambs, but lambs with plasma LH levels higher than 3·0 ng/ml had one or more vesicular follicles larger than 2 mm in diameter and those with LH levels below 3·0 ng/ml had no large vesicular follicles present in the ovary studied.

Table 3. Plasma and pituitary LH levels in O and T lambs 5 months of age

Values are means±S.E.

Genetic group	State	No. of lambs	Plasma LH levels (ng/ml)	Pituitary fixed wt (mg)	Pituitary LH: Content (μg)	Pituitary LH: Concentration (μg/g)
T	Entire	5	3·9±0·8	301±52	24·9±8·5	96·9±27·6
T	Hemispayed	5	4·8±0·4	355±35	34·7±3·2	100·4±7·4
Total		10	4·4±0·3	328±31	29·8±2·7	98·7±13·5
O	Entire	4	1·7±1·5	275±34	20·2±9·6	80·8±25·3
O	Hemispayed	4	3·1±1·9	272±14	24·8±8·4	91·5±18·2
Total		8	2·4±0·7	274±17	22·5±3·3	86·2±14·6

Pituitary LH content and concentration tended to follow the same pattern as peripheral plasma LH (Table 3) although the means were not significantly different. Pituitary LH content was closely related to plasma LH levels ($r=0·75$; $P<0·01$) but there was no direct relationship between pituitary LH and primordial follicle numbers in individual lambs. The fixed weight of the pituitary was similar for entire and hemispayed lambs and there was little difference between genetic groups (Table 3).

Table 4. Plasma and pituitary FSH levels in O and T lambs 5 months of age

Values are means±S.E.

Genetic group	State	No. of lambs	Plasma FSH level (ng/ml)	Pituitary FSH: Content (μg)	Pituitary FSH: Concentration (μg/g)
T	Entire	5	83·8±18·5	891·5±60·6	3332·3±562·5
T	Hemispayed	5	120·5±15·9	4347·6±2839·0	13486·6±8841·7
Total		10	102·2±13·0	2614·6±1457·3	8409·5±4506·3
O	Entire	4	113·7±20·4	592·2±80·0	2250·8±410·7
O	Hemispayed	4	120·4±18·7	2330·9±1440·4	8692·3±5436·1
Total		8	117·1±12·9	1461·6±744·2	5471·6±2801·9

The levels of FSH in peripheral plasma were not related to genetic group of lamb nor to unilateral ovariectomy (Table 4) and there were no direct relationships between plasma or pituitary FSH and numbers of primordial follicles or the presence of larger vesicular follicles. Mean pituitary FSH content and concentration, shown in

Table 4, were considerably higher in hemispayed lambs than in entire lambs ($P<0{\cdot}05$). In some hemispayed lambs the levels were massive compared to levels in the entire lambs. There was a trend for higher pituitary FSH levels in T-group lambs (Table 4) but the differences were small compared to the effect of unilateral ovariectomy.

Plasma Progesterone and Oestrogen at 5 Months of Age

The levels of progesterone and oestrogen detected in peripheral plasma of all the lambs were below the values obtained for adult ovariectomized ewe plasma and were considered negligible.

Discussion

Mauléon (1967) has stated that oogonal mitosis in the sheep ceases almost 90 days after fertilization. Mauléon (1969) also stated that a decrease in numbers of primordial follicles was difficult to demonstrate in ewe lambs in the few months after birth. However, in the present experiment there was an increase in numbers of primordial follicles between 7 days and 5 months of age when paired ovaries of hemispayed lambs were compared. The increase did not appear to be due to unilateral ovariectomy as similar numbers of primordial follicles were found in entire and hemispayed lambs at 5 months of age. There is substantial evidence in several species that there are no changes in oocyte numbers after unilateral ovariectomy, although an increase in the number of vesicular follicles may be found (Arai 1920; Mandl and Zuckerman 1951*b*; Jones and Krohn 1960; Peters and Braathen 1973). The reason for the increase in primordial follicles in the present experiment is obscure, since Kennedy *et al.* (1974) have reported considerable growth and atresia of follicles shortly after birth but Worthington (personal communication) has also found increases in oocyte numbers between birth and 4 months of age in Merino lambs. Further information is required for pre- and postnatal lambs to clarify these observations.

The difference between O and T lambs in the number of primordial follicles is similar to the breed difference in total oocytes reported by Land (1970) in comparisons between pure-bred Blackface and Welsh ewes and their Finnish Landrace crosses. Genetic selection has resulted in changes in oocyte numbers in T and O Merino lambs, but the difference at 5 months was not as obvious as that in newborn lambs. It would appear unlikely from the present results that the selection group of the recipient dam had any effect on the determination of primordial follicle numbers of the lamb at birth, which supports the results of Land (1970), where differences in oocyte numbers and growing vesicular follicles could be demonstrated even when lambs had the same breed of dam.

Foster *et al.* (1972) bilaterally ovariectomized lambs at 1–4 days of age and did not detect differences in serum or pituitary LH between intact and ovariectomized lambs over the next 14 days. However, bilateral ovariectomy at 5 days of age resulted in higher serum LH and lower pituitary LH compared to intact controls at 44 days of age (Leifer *et al.* 1972). The trend in the present experiment was towards higher pituitary and plasma LH levels in hemispayed lambs and is similar to the response observed in mature ewes (Roche *et al.* 1970; Reeves *et al.* 1972; O'Reilly and Dzuik 1973). The variable but often large increases in pituitary FSH levels

found in the present experiment strongly implicate this hormone in the ovarian–pituitary feedback system. Failure to detect oestrogen in the peripheral plasma may not preclude differences between entire and hemispayed lambs in ovarian oestrogen secretion which could have long-term effects on pituitary gonadotrophin levels.

The observation that T lambs had higher plasma LH levels than did O lambs and the correlation of pituitary and plasma LH levels supports the hypothesis that selection for multiple births may result in changes in the synthesis and release of pituitary gonadotrophins. The presence of large vesicular follicles in ovaries of lambs with plasma LH levels higher than 3·0 ng/ml and higher plasma LH levels in T lambs suggests that this hormone may contribute to the observed differences in primordial follicle numbers and possibly to differences in ovulation rate after puberty. In more detailed studies on plasma LH levels in lambs of the T and O group, Bindon (1973*a*, 1973*b*) observed both fluctuations during the day and considerable overlap between lambs of each group, but LH levels tended to be higher in T lambs and even higher again in a flock of more highly selected animals. Evidence for LH involvement in ovulation rate and the incidence of multiple births has also been reported by Thimonier *et al.* (1973) for lambs of different breeds and Land *et al.* (1972) for adult ewes of different breeds at mid-cycle. The involvement of FSH in multiple ovulation in the ewe has not been established and the only evidence in the present study was a tendency for pituitary levels to be higher in T-group lambs.

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