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Gonadotrophin Levels and Ovarian Development in the Neonatal Ewe Lamb

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Abstract

Ovarian weight has been shown to increase markedly in the newborn ewe lamb, and factors which might contribute to this growth were examined. Follicle development was studied in the ovaries of 28 lambs aged 0, 2, 4, 6, 8 and 10 weeks. Plasma samples, pituitaries and one ovary were assayed for gonadotrophin or steroid hormone content.

No significant differences between age groups were found in pituitary LH concentration or mean plasma LH. Pituitary FSH concentration increased with age, except for a small decrease at 8 weeks, but no significant changes were seen in plasma FSH. Ovarian progesterone and oestradiol concentrations did not appear to be associated with follicular development.

Growing follicles were most numerous at 2 weeks whilst total vesicular follicles reached a peak at 4 weeks. Most vesicular follicles were less than 1·4 mm in diameter. Advanced atresia in vesicular follicles became apparent by 4 weeks although early signs were present in younger lambs.

Fluctuations in gonadotrophin levels do not appear to be responsible for variations in number and size of vesicular and growing follicles in the lamb ovary. Other possible explanations are discussed.

Introduction

Previous studies in this laboratory have indicated that there are striking morphological changes in the ovaries of ewe lambs during the first 8 weeks of the postnatal period (Kennedy *et al.* 1974). A significant increase in ovarian weight is accompanied by a marked proliferation and growth of vesicular follicles, and this development is unlike that seen in the adult. These events have now been studied in more detail in another group of ewe lambs, and some preliminary investigations were made to determine whether the ovaries secrete steroids. Concurrent changes in circulating gonadotrophin levels were recorded in an attempt to explain ovarian changes. An abstract of the data on hormone concentrations was presented by Tassell *et al.* (1976).

Materials and Methods

Single-born medium-wool Merino lambs were randomly allocated at birth in August–September to groups of four to six animals to be studied at birth, 2, 4, 6, 8 and 10 weeks of age. Prior to use, they were run with their dams under normal paddock conditions at Wellington, N.S.W. At appropriate ages, six blood samples were collected by venipuncture at 1-h intervals, and the animals were then slaughtered. Reproductive tracts and pituitary glands were removed, trimmed and weighed. One ovary and the uterus from each lamb were fixed in Bouin's solution for histological study. The other ovary, the plasma and the pituitary gland were frozen and stored at −12°C for hormone assay.

Levels of LH and FSH in plasma and pituitaries were determined by radioimmunoassay (LH: Goding *et al.* 1969; FSH: Salomonsen *et al.* 1973). Standards used in these assays were NIH.FSH.S6

and a purified LH preparation having biological potency twice that of NIH.LH.S1 (Papkoff *et al.* 1965). The antiserum used in the LH assay was an anti-ovine LH serum which was provided by Dr W. Hansel. Where gonadotrophin levels were undetectable, an arbitrary value equal to the lower limit of assay sensitivity was substituted for the purpose of calculating means. For LH this limit was 0·2 ng/ml, and for FSH 20 ng/ml.

Each frozen pituitary was homogenized in 10 ml phosphate buffer (0·1 M, pH 7·4). At the time of assay, final dilutions of the material at 1/2000, 1/10000 and 1/20000 were prepared, using the phosphate buffer. The frozen ovary was homogenized in the same type of buffer and assayed for progesterone using a radioimmunoassay (Hoppen *et al.* 1976) and for oestradiol by competitive protein binding (Chamley *et al.* 1972).

The fixed ovaries were serially sectioned at 10 μm and every 20th section was mounted, stained with a modified trichome stain (Gomori 1950) or periodic acid–Schiff reagent, and examined using a projection microscope. The numbers of vesicular and large growing follicles were counted, the nucleus of the ovum being used as a marker. Small growing follicles were counted in the first five and last five mounted sections in each ovary, and in every second mounted section in between these.

Follicles were divided into the following size classes. The types given in parenthesis represent approximate equivalents in the scale of Pedersen and Peters (1968).

Small growing follicles: one to two layers of rounded or cuboidal granulosa cells.
Large growing follicles: more than two layers of granulosa cells but no separation between cells (type 5a, 5b).
Vesicular follicles:
Type A: antrum formation beginning (type 6).
Type B: larger spaces but antrum less than half formed (type 6–7).
Type C: antrum more than half formed but not mature (type 7).
Type D: antrum fully formed (type 7).

These divisions were chosen because there was relatively little overlap between classes. The diameter of all recorded follicles $\geqslant 1$ mm was measured microscopically, using the mean of two measurements taken at right angles. In addition the diameter of the largest follicle, regardless of the presence or absence of an oocyte nucleus, was measured macroscopically, using calipers.

Nuclear diameter was measured in three follicles of each type per ovary and a mean nuclear diameter was calculated for that type in each age group. Follicle numbers were corrected for nuclear size and section interval, according to the formula of Mandl and Zuckerman (1951), to give estimates of follicle populations and these were subjected to analysis of variance after square root transformation of (counts $+1$) to allow for zero values.

The abundance of follicles at an advanced stage of atresia (severe shrinkage and distortion, marked connective tissue invasion) was recorded on a semiquantitative scale.

Samples were taken from the uterus at the point where the bifurcation of the uterine horns becomes visible externally. Three sections 7 μm thick and 210 μm apart were stained with haematoxylin and eosin, and the height of the caruncular epithelium was measured at magnification $\times$ 500 (six measurements per section).

Results

Mean weights of pituitaries, ovaries and reproductive tracts are shown in Table 1. Pituitary weights increased steadily throughout the experimental period. Ovarian weights rose rapidly between 2 and 8 weeks of age, whilst reproductive tract weights followed a similar pattern but were maximal 2 weeks earlier.

Pituitary LH content increased until 6 weeks of age, fell at 8 weeks, then increased again at 10 weeks (Table 2*a*). Similar trends were seen in pituitary LH concentration. Plasma LH levels remained low and were relatively stable throughout the experiment. Small peaks were seen among values for individual animals from 2 weeks of age onwards.

Pituitary FSH, both concentration and content, increased steadily throughout the experimental period except for a small decrease in concentration at 8 weeks (Table 2*a*). Plasma FSH increased between 2 and 8 weeks. For individual animals

Table 1. Body weights and organ weights of ewe lambs

Values given are means ± s.e. Values in parentheses are the number of lambs per group for which body weight was available. Means with different superscripts differ significantly ($P < 0.05$)

	Age (weeks)					
	0	2	4	6	8	10
Pituitary (mg)	99^{d} ±8	145^{c} ±12	156^{bc} ±9	160^{bc} ±13	194^{ab} ±18	219^{a} ±20
Reproductive tract (g)[A]	$2{\cdot}243^{c}$ ±0·129	$2{\cdot}409^{c}$ ±0·289	$3{\cdot}374^{bc}$ ±0·333	$4{\cdot}773^{a}$ ±0·666	$4{\cdot}268^{ab}$ ±0·439	$4{\cdot}120^{ab}$ ±0·697
Paired ovaries (mg)	121^{c} ±40	104^{c} ±20	633^{b} ±196	848^{ab} ±222	1341^{a} ±155	1069^{ab} ±233
Live weight (kg)	—[B]	4·9 (1)	10·43 ±0·67 (3)	10·02 ±0·79 (5)	12·98 ±0·59 (4)	13·63 ±1·18 (4)
No. in group	4	5	6	5	4	4

[A] Ovaries and vagina removed. [B] Not available; similar lambs weigh 2·5–3·0 kg at birth.

plasma FSH levels were relatively constant and in those animals which showed pulsatile LH release, there was no concomitant peak of FSH.

Ovarian levels of oestradiol-17β fluctuated widely between animals within groups and bore no obvious relationship to age or follicle development. Mean progesterone

Table 2. (*a*) Pituitary and plasma gonadotrophin levels in ewe lambs and (*b*) progesterone and oestradiol levels in ovarian tissue

Values given are means ± s.e. Means with different superscripts differ significantly ($P < 0.05$)

	Age (weeks)					
	0	2	4	6	8	10
	(*a*) Pituitary and plasma gonadotrophin levels					
Pituitary LH concn (μg/g)	$102{\cdot}49^{a}$ ±53·67	$135{\cdot}92^{a}$ ±38·27	$173{\cdot}93^{a}$ ±46·04	$304{\cdot}96^{a}$ ±86·19	$104{\cdot}82^{a}$ ±10·82	$220{\cdot}98^{a}$ ±79·88
Pituitary LH content (μg/gland)	$9{\cdot}02^{b}$ ±3·82	$18{\cdot}72^{ab}$ ±4·19	$27{\cdot}41^{ab}$ ±7·98	$45{\cdot}43^{a}$ ±10·89	$19{\cdot}86^{ab}$ ±1·22	$45{\cdot}67^{a}$ ±15·88
Plasma LH (ng/ml)	$1{\cdot}39^{a}$ ±0·11	$1{\cdot}36^{a}$ ±0·42	$1{\cdot}44^{a}$ ±0·32	$1{\cdot}86^{a}$ ±0·56	$1{\cdot}58^{a}$ ±0·90	$1{\cdot}41^{a}$ ±0·85
Pituitary FSH concn (μg/g)	$160{\cdot}5^{b}$ ±25·8	$178{\cdot}61^{ab}$ ±15·9	$283{\cdot}9^{ab}$ ±69·7	$326{\cdot}5^{a}$ ±44·9	$285{\cdot}2^{ab}$ ±30·2	$346{\cdot}6^{a}$ ±58·8
Pituitary FSH content (μg/gland)	$15{\cdot}53^{d}$ ±2·08	$25{\cdot}58^{cd}$ ±2·42	$42{\cdot}38^{bc}$ ±8·38	$50{\cdot}05^{b}$ ±3·50	$54{\cdot}57^{ab}$ ±5·73	$73{\cdot}53^{a}$ ±9·91
Plasma FSH (ng/ml)	$34{\cdot}99^{ab}$ ±6·17	$25{\cdot}85^{b}$ ±2·03	$48{\cdot}97^{b}$ ±7·95	$51{\cdot}85^{a}$ ±10·75	$58{\cdot}85^{a}$ ±8·47	$49{\cdot}16^{ab}$ ±8·50
	(*b*) Progesterone and oestradiol levels in ovarian tissue					
Progesterone concn (ng/g)	$168{\cdot}75^{ab}$ ±23·38	$253{\cdot}20^{a}$ ±44·23	$50{\cdot}17^{c}$ ±15·19	$57{\cdot}8^{bc}$ ±10·88	$157{\cdot}25^{abc}$ ±74·83	$140{\cdot}75^{abc}$ ±22·10
Total progesterone (ng/ovary)	$7{\cdot}98^{b}$ ±1·69	$11{\cdot}58^{b}$ ±1·66	$10{\cdot}92^{b}$ ±1·41	$21{\cdot}04^{b}$ ±1·43	$96{\cdot}98^{a}$ ±37·89	$66{\cdot}6^{a}$ ±5·29
Oestradiol concn (ng/g)	$7{\cdot}06^{a}$ ±4·54[A]	$0{\cdot}42^{b}$ ±0·11[B]	$0{\cdot}09^{b}$ ±0·02	$0{\cdot}14^{b}$ ±0·06	$0{\cdot}16^{b}$ ±0·11	$0{\cdot}04^{b}$ ±0·01
Total oestradiol (ng/ovary)	$0{\cdot}441^{a}$ ±0·315[A]	$0{\cdot}017^{a}$ ±0·004[B]	$0{\cdot}036^{a}$ ±0·02	$0{\cdot}079^{a}$ ±0·053	$0{\cdot}109^{a}$ ±0·073	$0{\cdot}019^{a}$ ±0·005

[A] Based on three ovaries. [B] Based on four ovaries. All animals in other groups were represented.

concentrations fell sharply between 2 and 4 weeks of age, then increased slightly in older lambs (Table 2*b*).

Numbers of large growing follicles at 2 weeks were greater than at 6, 8 and 10 weeks (Table 3). Although small growing follicle numbers appeared to peak at 2 weeks and then decline, the differences were not significant. Total vesicular follicles

Table 3. Numbers of growing and vesicular follicles per ovary in ewe lambs

Values given are means ± s.e. Means with different superscripts differ significantly ($P < 0.05$)

Follicles	Age (weeks) 0	2	4	6	8	10
Growing:						
Small	2441^{a}	3284^{a}	2903^{a}	1908^{a}	1734^{a}	2825^{a}
	±422	±727	±748	±423	±335	±921
Large	248^{ab}	319^{a}	192^{ab}	105^{bc}	53^{c}	81^{c}
	±77	±106	±29	±25	±23	±58
Vesicular:						
Type A	$114{\cdot}0^{abc}$	$143{\cdot}1^{abc}$	$239{\cdot}7^{ab}$	$269{\cdot}6^{a}$	$87{\cdot}4^{bc}$	$64{\cdot}8^{c}$
	±44·0	±38·8	±79·2	±64·0	±16·3	±36·4
Type B	$88{\cdot}3^{a}$	$48{\cdot}8^{a}$	$121{\cdot}0^{a}$	$60{\cdot}8^{a}$	$55{\cdot}7^{a}$	$35{\cdot}0^{a}$
	±20·8	±25·3	±56·2	±17·4	±26·4	± 5·4
Type C	$58{\cdot}1^{ab}$	$5{\cdot}6^{d}$	$58{\cdot}9^{a}$	$18{\cdot}2^{bcd}$	$45{\cdot}6^{abc}$	$10{\cdot}5^{cd}$
	±21·7	±3·6	±12·9	±5·9	±22·0	±4·0
Type D < 1 mm	$9{\cdot}5^{bc}$	$6{\cdot}2^{c}$	$86{\cdot}9^{a}$	$38{\cdot}5^{ab}$	$89{\cdot}3^{a}$	$47{\cdot}9^{a}$
	±5·6	±3·8	±22·2	±12·2	±24·6	±13·3
Type D ⩾ 1 mm	$1{\cdot}1^{b}$	$0{\cdot}9^{b}$	$43{\cdot}3^{a}$	$38{\cdot}4^{a}$	$60{\cdot}9^{a}$	$38{\cdot}8^{a}$
			±16·9	±13·1	±15·9	±9·0
Total	$270{\cdot}9^{ab}$	$204{\cdot}7^{b}$	$549{\cdot}7^{a}$	$425{\cdot}5^{ab}$	$339{\cdot}0^{ab}$	$196{\cdot}9^{b}$
	±55·4	±66·7	±139·0	±89·4	±71·5	±59·4

reached a peak at 4 weeks, and numbers decreased throughout the remainder of the experiment (Table 3). The numbers of larger follicles (type D) showed two peaks at 4 and 8 weeks. The size of the largest follicle increased between 2 and 6 weeks of age (Table 4) but most vesicular follicles were less than 1·4 mm, and usually they were

Table 4. Diameters of follicles in ewe lambs

Values given are means ± s.e. Values in parentheses represent the number of ovaries per group containing measurable follicles. Ovaries without measurable follicles are omitted. Means with different superscripts differ significantly ($P < 0.05$)

	Age (weeks) 0	2	4	6	8	10
Diameter of largest follicle[A] (mm)	$4{\cdot}0^{a}$ (1/4)	$1{\cdot}05^{c}$ ±0·25 (4/5)	$1{\cdot}95^{bc}$ ±0·43 (6/6)	$2{\cdot}28^{ab}$ ±0·22 (5/5)	$2{\cdot}08^{bc}$ ±0·08 (4/4)	$2{\cdot}70^{ab}$ ±0·42 (4/4)
Mean diameter of follicles[B] ⩾ 1 mm (mm)	$1{\cdot}18^{ab}$ (1/4)	$1{\cdot}05^{b}$ (1/5)	$1{\cdot}16^{b}$ ±0·02 (6/6)	$1{\cdot}33^{a}$ ±0·04 (5/5)	$1{\cdot}29^{ab}$ ±0·06 (4/4)	$1{\cdot}31^{a}$ ±0·07 (4/4)

[A] Macroscopic measurements. [B] Measured with a projection microscope.

less than 1·0 mm in diameter. The peak in the total number of vesicular follicles preceded the peak in ovarian weight, although numbers of follicles $\geqslant 1 \cdot 0$ mm were more closely related to ovarian weight.

At birth and 2 weeks of age the ovaries contained no growing or vesicular follicles in advanced stages of atresia but these began to appear in the 4-week-old group in which four ovaries showed occasional atretic scars and two had more numerous remnants. In the 8- and 10-week groups the number of follicles in advanced states of atresia was larger. All recorded follicles $\geqslant 1$ mm in diameter showed some signs of atresia, such as rupture and folding of the membrana propria and disorganization of the membrana granulosa layers.

The height of the uterine epithelium did not differ significantly between groups. The uteri of three lambs slaughtered at birth lacked uterine glands, whilst those from 2-week-old lambs showed early development of these. Some (2/4) 4-week-old and all older lambs showed full development of glands.

Discussion

Values for both concentration and content of LH in the pituitary increased up to 6 weeks of age which agrees with the report of Foster *et al.* (1972) that LH concentration in the pituitary was decreased at birth but increased over the first 18 days postnatally.

Mean plasma FSH and LH values did not vary significantly throughout the 10-week period. The low levels of LH were similar to those present in anoestrous and dioestrous adults (Foster *et al.* 1975*b*), and were comparable with levels in lambs of the same age studied by Leifer *et al.* (1972) and Foster *et al.* (1975*b*). Earlier, Foster *et al.* (1972) had described a marked increase in serum LH between birth and 18 days.

Small peaks of LH in plasma were first seen in individual lambs as early as 2 weeks of age. The sampling regime employed here would not have allowed the exact pulsatile pattern to be determined. Previously peaks have been reported to occur by 30 days of age (Bindon and Turner 1974) or as late as 9 weeks (Foster *et al.* 1975*a*). Our observation that pulsatile LH release can occur in the presence of a tonic secretion of FSH is in agreement with the pattern described by these authors. We agree with their suggestion that acute secretion of LH and FSH may be under separate control mechanisms in the neonatal ewe lamb. It is unlikely that the LH pattern is implicated in the ovarian changes we have described, since pulsatile LH activity was seen up to 10 weeks of age, i.e. after the time when the vesicular follicle population reached a peak.

The increase in ovarian weight between 2 and 8 weeks of age would be due, in part, to the increase in number and size of vesicular follicles and the associated accumulation of follicular fluid, since the greatest rate of increase in weight coincided with peaks in numbers of large (types C and D) vesicular follicles.

Many follicles seem to begin growth at about the time of birth and to continue growth until about 4 weeks of age when signs of atresia appear. The stimulus for the initiation of follicular growth is not known, and the hypothesis of Peters *et al.* (1975) that the beginning of such growth is regulated by intra-ovarian mechanisms does not explain events in the lamb because, although the initiation of follicle growth slows down at about 4 weeks of age, the pool of primordial follicles does not decline significantly between birth and 20 weeks of age (Trounson *et al.* 1974; Worthington

and Kennedy 1976). A possible explanation is that degenerating follicles contain a substance which reduces the initiation of follicular growth (Peters *et al.* 1973).

Treatment of 6-week-old lambs with PMSG and HCG can induce follicular growth and ovulation (Worthington and Kennedy, unpublished data) which indicates that atretic changes can be prevented or delayed as has been shown by Peters *et al.* (1975) in the mouse. The implication is that although gonadotrophins in the lamb are sufficient to stimulate growth of follicles up to antrum formation, they are insufficient to prevent atresia in follicles greater than approximately 1 mm in diameter.

In adult cycling ewes oestradiol levels are highest in the ovarian vein draining the ovary containing the largest non-atretic follicle (Bjersing *et al.* 1972). In the lamb it is difficult to determine which ovary contains the largest follicle and this may explain the variability in oestradiol concentration between lambs.

The weight of the reproductive tract increased markedly between 2 and 6 weeks of age but there was no evidence of stimulation of the uterine epithelium by steroids, apart from glandular development.

Circulating levels of gonadotrophins in ewe lambs appear to be adequate for follicular growth when compared to those of the adult (Salomonsen *et al.* 1973), but marked changes in follicular development do not seem to be associated with variation in plasma LH or FSH. Studies of the gonadotrophin receptor populations in the ovary might help to explain these observations.

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