

The Relation between Patterns of Ovarian Follicle Growth and Ovulation Rate in Sheep

K. E. Turnbull, P. E. Mattner, J. M. George and R. J. Scaramuzzi

Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148.

Abstract

The number and growth rate of follicles within classes based on granulosa volume were determined for ovaries taken from groups of 4-5-year-old, fine-wool Merino ewes drawn at different times of the year from a single strain flock maintained at Armidale, N.S.W. The breeding season of the flock normally extends from February to October and the mean ovulation rate rises from about 0.5 in February to about 1.8-1.9 during April-May.

Ewes sampled when they were anoestrous or had one (single-ovulatory) or two (twin-ovulatory) recent corpora lutea did not differ in respect to the mean total number of ovarian follicles, the mean number of follicles in individual classes, the time for follicles to complete their rapid growth stage, or the incidence of follicle atresia. However, the ovaries of twin-ovulatory ewes contained significantly more follicles in the two terminal classes within the rapid growth stage than did the ovaries of single-ovulatory or anoestrous ewes (2.2 v. 0.9 and 1.0). This difference was attributed to the differing numbers of follicles per day entering into the rapid growth stage (5.2, 4.5 and 3.7 respectively in twin-ovulatory, single-ovulatory and anoestrous ewes).

Introduction

Most of the studies dealing with the development of ovarian follicles in relation to ovulation rate in sheep have been concerned mainly with events immediately prior to or during the ovulatory period. However, relatively few developing follicles reach the stage of pre-ovulatory maturation; the majority undergo atresia and regress at an earlier stage.

Considerable attention has been given to patterns of growth and atresia of ovarian follicles in rodents (Lane and Davis 1939; Knigge and Leatham 1956; Greenwald 1961; Pedersen 1972), in sheep (Brand and de Jong 1973; Turnbull *et al.* 1977a) and in cattle (Rajakoski 1960; Marion *et al.* 1968). Also, Richards and Rees Midgley (1976) have reviewed the literature relating to the action of the protein hormones on follicular development. However, it is still not clear whether ovulation rate is determined primarily by events occurring shortly before ovulation or by follicle numbers, rate of follicle growth, or the incidence of atresia at much earlier stages of follicle development.

In the present study patterns of follicle growth preceding pre-ovulatory enlargement were investigated in a single strain flock of Merino ewes whose ovulation rate varied with stage of the breeding season.

Materials and Methods

Four-year-old ewes from a flock of fine wool Merino ewes located at Armidale, N.S.W., were used. The flock has been randomly bred with periodic infusion of rams from the same source. When mature (4-5 years old), the ewes exhibit cyclic oestrus from February to October and their

mean ovulation rate (number of ova shed per ewe) rises from approximately 1.0 in January–February to a maximum of about 1.8 in April–May and declines thereafter. From a mating in April–May of the year in which the present study was conducted the flock produced 165 lambs per 100 ewes.

Samples of 3–5 ewes were obtained from an unmated section of the flock in December, February, April, June, August and October. During the breeding season, only ewes in the mid-luteal stage of the oestrous cycle were used. The ovaries were removed at slaughter, fixed in Serra's fixative, serially sectioned at 15 μm and stained with haematoxylin and eosin. A microprojector was used to locate the section at which the diameter of individual follicles was maximal. Follicle diameter, the granulosa volume of follicles, the mitotic index of the granulosa, and the number of follicles >0.3 mm diameter in each ovary were assessed by the methods of Turnbull *et al.* (1977a). The mitotic index of the theca was not determined. Unlike the granulosa, the theca consists of several cell types interspersed with stroma and capillary beds. This feature, together with the difficulty of accurately defining the outer limits of this tissue (Turnbull *et al.* 1977a), renders estimates of the mitotic index of the theca less reliable than those of the granulosa.

Follicles were classified into 11 classes based on doubling of granulosa volume (Turnbull *et al.* 1977a) commencing with an arbitrary value of $2 \times 10^6 \mu\text{m}^3$ which coincides with a follicle diameter of approximately 0.15 mm. However, as follicles in class 4 are intermediate between rapidly growing follicles and those with a basal mitotic index, only follicles in classes 4 or greater were studied (see Table 2).

Counts of such follicles in ovaries included only follicles with normal mitotic divisions occurring in the granulosa cells and included the following two sub-types: (1) 'non-atretic' follicles in which no atretic bodies (Knigge and Leathem 1956) were present in the granulosa cells, and (2) 'early atretic' follicles in which a few atretic bodies were present among some granulosa cells.

The times (t) taken for follicles to double the volume of their granulosa (i.e. to pass from one class into the next) were estimated using the formula $(100 t \ln 2)/\text{M.I.}$ (Hoffman 1949), where t is the time required for granulosa cells to complete a mitotic division [0.43 h (Turnbull *et al.* 1977a)], and M.I. is the mitotic index.

The data were analysed by unpaired t -test.

Results

Follicle Populations

Comparing numbers of follicles in individual classes, the ewe groups slaughtered at various times of the year differed significantly only in regard to follicle class 4. The mean number of class 4 follicles was 26 (s.e. 4.4) in December (ewes in anoestrus) and declined throughout the subsequent breeding season to reach a minimum value of 8.0 ± 0.2 in August (see Fig. 1).

The data were then pooled on the basis of the ovulation rate (number of corpora lutea) of the ewes at the time of slaughter. The mean numbers of non-atretic and early atretic follicles per follicle class for anoestrous ewes, ewes with one corpus luteum (single-ovulatory) and ewes with two corpora lutea (twin-ovulatory) at the time of slaughter are shown in Table 1.

Using paired t -test, there were no significant differences between the three groups in respect to either the total number of non-atretic and early atretic follicles or the mean number of such follicles in individual classes 5–11 inclusive. However, there were significantly fewer class 4 follicles in the ovaries of twin-ovulatory ewes than in the ovaries of anoestrous ewes ($P < 0.05$). The number of class 4 follicles in the ovaries of the single-ovulatory ewes was intermediate and did not differ significantly from the corresponding numbers in the other two groups of ewes. Further, combining classes 10 and 11, the mean number of non-atretic follicles within these classes was significantly greater ($P < 0.02$) in twin-ovulatory ewes than in single-ovulatory or anoestrous ewes (2.2 *v.* 0.9 and 1.0 respectively) (see Table 1).

As seen in Table 1, signs of early atresia did not appear in follicles until they entered class 8 (1.0–1.5 mm diameter). The number of early atretic follicles within follicle classes 8–11 in anoestrous, single- and twin-ovulatory ewes was similar (7.3 ± 1.1 , 7.3 ± 1.5 and 8.3 ± 2.5 follicles respectively), the greatest incidence occurring in class 9. In addition, the time of year at which the ewes were sampled had no effect on the pattern of incidence of early atresia.

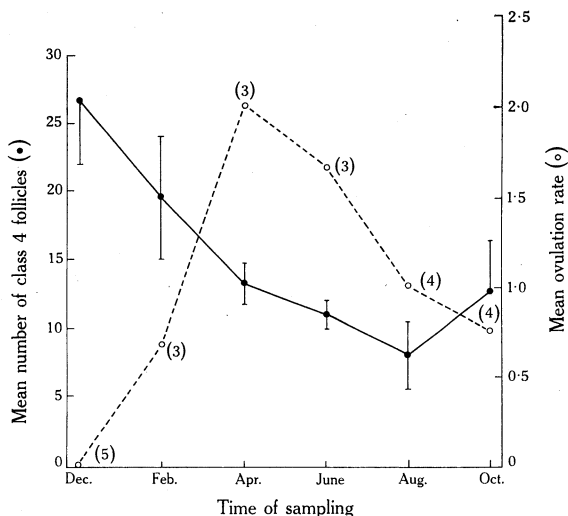


Fig. 1. Mean number of class 4 follicles and mean ovulation rate in the groups of Merino ewes examined at different times of the year. Standard errors are shown by vertical bars, and the numbers of ewes per sample are shown in parentheses.

Rate of Follicle Growth

The relationship between the mitotic index of the granulosa and log follicle diameter for anoestrous, single- and twin-ovulatory ewes is illustrated in Fig. 2. In each group follicular development was characterized by a rapid increase in growth rate of the granulosa tissue through classes 5–7 inclusive, maximal rates of growth being achieved in follicles within class 8 (c. 1.0–1.5 mm diameter). In larger follicles there was a progressive reduction in growth rate. As seen from Fig. 2, both the rapid increase in the mitotic index of the granulosa (phase 1 of rapid growth) and the subsequent decrease in the mitotic index of the granulosa (phase 2 of rapid growth) occurred at smaller follicle diameters in the twin-ovulatory ewes than in the single-ovulatory or the anoestrous ewes.

The times required for follicles to pass through the individual classes 4–11, as estimated from the times taken for doubling of the granulosa volume within each follicle class, are shown in Table 2. There was no significant difference between the three groups of ewes in regard to the overall time required for follicles to develop through classes 4–11. However, follicles from the anoestrous ewes or from the anoestrous and single-ovulatory ewes combined required a significantly longer period to grow through classes 4–7, likewise through classes 5–7 ($P < 0.05$), and a commensurately shorter time to pass through classes 8–11 than the follicles from the twin-ovulatory ewes.

As no atresia occurs in follicles prior to class 8, estimates of the number of follicles entering phase 1 of rapid growth could be obtained by dividing the mean number of follicles in classes 5–7 by the time required for follicles to grow through these

Table 1. Number (mean and s.e.) of normal and early atretic follicles per follicle class in anoestrous (group 0), single-ovulatory (group 1) and twin-ovulatory ewes (group 2)

Ovulation group	Follicle type	Follicle class and diameter (mm)										
		4	5	6	7	8	9	10	11	Grand mean		
		(0.33-0.42)	(0.43-0.54)	(0.55-0.69)	(0.70-1.02)	(1.03-1.51)	(1.52-2.24)	(2.25-3.32)	(3.33-5.0)			
0	Non-atretic	20.8 ± 3.6*	13.6 ± 1.7	6.6 ± 1.1	5.6 ± 1.2	5.7 ± 1.0	4.4 ± 1.2	0.6 ± 0.2	0.4 ± 0.2	64.9 ± 7.4		
	Early atretic					2.8 ± 1.1	3.7 ± 0.7	0.3 ± 0.2	0.6 ± 0.2			
1	Non-atretic	14.3 ± 3.0	12.3 ± 1.1	8.7 ± 1.0	5.9 ± 1.1	7.4 ± 1.3	3.7 ± 1.4	0.6 ± 0.3	0.3 ± 0.2	60.4 ± 6.4		
	Early atretic					1.1 ± 0.3	5.7 ± 1.8	0.1 ± 0.1	0.3 ± 0.2			
2	Non-atretic	10.3 ± 2.1*	12.3 ± 1.7	7.7 ± 1.9	5.5 ± 1.1	5.3 ± 0.2	6.2 ± 1.6	1.7 ± 0.6	0.5 ± 0.3	57.8 ± 7.0		
	Early atretic					3.0 ± 0.9	5.0 ± 2.1	0.3 ± 0.3	0.0			

* Means differ significantly at the 5% level.

Table 2. Time required for follicles to pass through individual follicle classes in anoestrous (group 0) single-ovulatory (group 1) and twin-ovulatory ewes (group 2)
M.I. = mitotic index of granulosa (mean ± s.e.). D.T. = time (in hours) for doubling of granulosa volume (mean ± s.e.)

Follicle class	10 ⁻⁶ × Granulosa volume (μm ³)	Mean follicle diameter (μm)	Ewe group			
			0		1	
			M.I.	D.T.	M.I.	D.T.
4	16-32	376	0.22±0.02	135.5±9.5	0.24±0.03	124.2±16.1
5	32-64	482	0.39±0.03	76.4±5.4	0.43±0.05	69.3±9.0
6	64-128	675	0.70±0.04	42.6±2.3	0.72±0.06	41.4±4.7
7	128-256	953	0.96±0.03	31.0±1.1	1.00±0.07	29.8±2.2
Sub-total 4-7				285.5±15.5		264.7±20.3
8	256-512	1376	1.03±0.04	28.9±1.2	1.04±0.08	28.7±3.8
9	512-1024	1986	0.75±0.04	39.7±1.7	0.70±0.07	42.6±5.9
10	1024-2048	3169	0.60±0.13	49.7±2.0	0.51±0.09	58.4±8.0
11	2048-4096	4353	0.50±0.10	59.6±10.1	0.44±0.08	67.7±16.4
Sub-total 8-11 ^A				177.9		197.4
Grand total 4-11 ^A				463.4		462.1

^A Standard error not calculated since both class 10 and class 11 follicles were not present in all ewes.

classes (Turnbull *et al.* 1977a). Such estimates indicated that approximately 3.7 ± 0.3 , 4.5 ± 0.3 and 5.2 ± 0.6 follicles entered the rapid growth phase per day in anoestrous, single- and twin-ovulatory ewes respectively. The differences between the relevant numbers in twin-ovulatory ewes and those in either anoestrous ewes or anoestrus and single-ovulatory ewes combined closely approached statistical significance at the 5% level ($t_{13} = 2.142$ and $t_{20} = 1.992$ respectively).

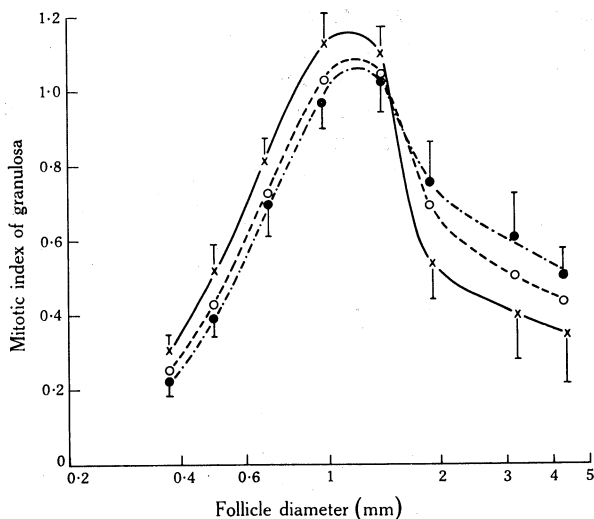


Fig. 2. Change in the mitotic index of the granulosa with growth of follicle in anoestrous (●) single-ovulatory (○) and twin-ovulatory ewes (×). Standard errors are shown by vertical bars.

Discussion

The ovaries of the ewes that were anoestrous or single-ovulatory at the time of slaughter did not differ significantly in respect to either the mean number of follicles in individual or combined follicle classes, the time for follicles to complete their rapid growth stage, or the relative incidence of follicle atresia. This suggests that neither the hormonal events around ovulation nor the presence of a corpus luteum was important in determining the population of follicles supported by the ovaries, at least when the ewes were single-ovulatory. However, the ovaries of the twin-ovulatory ewes contained significantly more non-atretic class 10 and 11 follicles (2.2 v. 0.9 and 1.0) and fewer class 4 follicles (10.3 v. 14.3 and 20.8) than the ovaries of the single-ovulatory and anoestrous ewes.

In ewes development of follicles into class 10 and 11 (diameter ≥ 2.4 mm) is not restricted to a period around the time of ovulation (Hutchinson and Robertson 1966; Smeaton and Robertson 1971; Turnbull *et al.* 1977a). Throughout the luteal stage of the oestrous cycle, and evidently through the anoestrous period, there is continuing and concurrent emergence and loss of representatives of these classes as a result of follicles growing and regressing asynchronously (Turnbull *et al.* 1977a). A similar pattern of follicle growth and decline also occurs in cows (Priedkalns *et al.* 1968). The resultant or prevailing population of class 10 and 11 follicles supported at a particular time or stage of the breeding season is likely to be a major factor determining the current ovulation rate. This is in accord with the findings that only follicles within these classes have reached the critical size (about 2.5 mm diameter) at which

ovine ovarian follicles can respond to human chorionic gonadotrophin by resumption of meiosis of the oocyte nucleus (Dzuik 1965) or commence pre-ovulatory dilation and ovulate within 2–3 days if an appropriate stimulus occurs (Smeaton and Robertson 1971).

Although the number of class 4 follicles declined throughout the course of the breeding season and was lower in twin-ovulatory than in single-ovulatory ewes, there were no corresponding changes in the population of class 5 follicles. Accordingly, it appeared that the above changes occurred as a result of the differing rates of uptake of class 4 follicles into phase 1 of rapid growth (5.2, 4.5 and 3.7 follicles per day respectively in twin-ovulatory, single-ovulatory and anoestrous ewes). Despite these different rates of uptake an associated increase in the number of class 5–7 follicles present in the breeding season did not occur because the time for follicles to develop through these classes (5.1, 5.9 and 6.3 days respectively in twin-ovulatory, single-ovulatory and anoestrous ewes) was correspondingly reduced as the rate of uptake increased.

Approximately 5.2, 4.5 and 3.7 follicles per day must also have entered phase 2 of rapid growth in twin-ovulatory, single-ovulatory and anoestrous ewes respectively, for no follicles are lost through atresia prior to the onset of phase 2 of rapid growth (class 8). This circumstance, together with the much longer period required in twin-ovulatory ewes for follicles to develop through classes 9–11 (see Table 2), would be expected to induce a slight increase in the number of follicles present in these classes in twin-ovulatory ewes relative to the number in other ewes (Turnbull *et al.* 1977b). However, through classes 8–11 in all ewes the number of follicles was progressively reduced by the advent of atresia. Although the relative incidence of follicle atresia was similar for the three groups of ewes, it appears to have risen slightly with increase in current ovulation rate. Thus the ratio of the number of non-atretic class 8–11 follicles to the daily uptake of class 4 follicles into the rapid growth stage was similar for each group (approximately 2.7 : 1). It seems, therefore, that the potential for single or twin ovulation was determined in large part by the current population of class 10 and 11 follicles which, in turn, was strongly influenced by factors controlling the development of class 4 follicles.

Accordingly, the present findings indicate that, in ewes, the number of ova shed at ovulation is influenced by effects on follicle development occurring about 19 days (the time for development through classes 4–11, see Table 2) prior to the pre-ovulatory dilation of follicles—in all, about 20–21 days before ovulation. This is not in conflict with the general finding that ewes will release more ova than normal if they are treated with PMS gonadotrophin a few days prior to ovulation. An immediate action of such treatment is to eliminate follicle atresia (Greenwald 1962; Peters *et al.* 1975; Turnbull *et al.* 1977a) with the result that follicles which would otherwise have regressed remain as part of the population of follicles capable of responding to an ovulatory stimulus.

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