

Folliculogenesis in the sheep as influenced by breed, season and oestrous cycle

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Summary. In the sheep the total duration of folliculogenesis, i.e. from the start of development of a primordial follicle to ovulation, is thought to be about 6 months. The initiation of follicular growth as follicles enter the growth phase is influenced by gonadotrophins and, in the sheep, by factors such as breed, season and nutrition. Preantral follicles are characterized by a slow growth rate and no atresia. The number of preantral follicles is influenced by gonadotrophins, age, nutrition, season and unilateral ovariectomy. Antral follicles grow rapidly and produce steroids in response to gonadotrophins. The number of antral follicles varies according to factors such as breed of sheep, season, cycle, unilateral ovariectomy and gonadotrophins. Ovulation rate can be influenced in the short term by factors such as PMSG stimulation, short-term nutrition and unilateral ovariectomy that probably act by changing the number of follicles undergoing atresia. Factors such as breed, age and season probably act by changing the number of follicles entering the growth phase.

Introduction

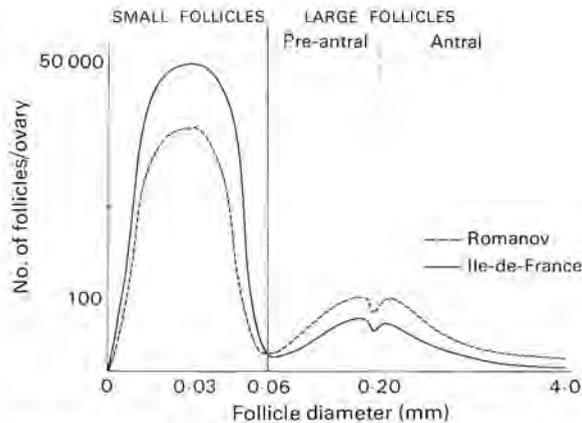
The adult ovary contains a definitive population of follicles, consisting of a large reserve of primordial follicles and a much smaller number of follicles at various stages of growth and development. The pool of primordial follicles is gradually depleted throughout the life of the animal as follicles enter the growth phase and develop towards ovulation or degenerate by the process of atresia.

The ovary of the adult ewe contains between 12 000 and 86 000 small follicles (i.e. with ≤ 2 layers of granulosa cells) and between 100 and 400 larger and developing follicles (Text-fig. 1; Cahill, Mariana & Mauléon, 1979). The mean time taken for a follicle to grow from 3 layers of granulosa cells (diameter 0.06 mm) to the preovulatory stage (6–8 mm) has been found to be approximately 6 months (Cahill & Mauléon, 1980). In rodents the transit time for the growth phase is 20–30 days (mouse: Peters & Levy, 1966; rat: Hage, Groen-Klevant & Welschen, 1978).

Initiation of follicular growth

In all species the precise point at which a follicle leaves the reserve pool and enters the growth phase is ill-defined. Primordial follicles are a heterogeneous group of follicles with a wide range of morphological and histochemical features. Mariana (1978) divided primordial follicles of the rat into two groups: the first group consisted of the smaller follicles in which no follicular

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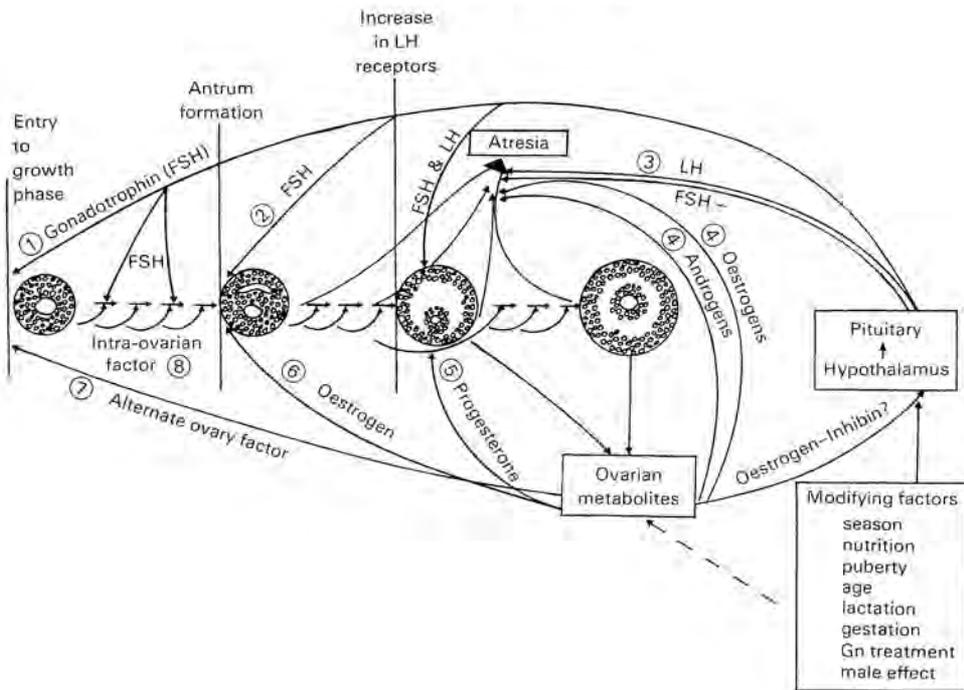
Text-fig. 1. Schematic representation of the total follicular population in ewes of high (---) and low (—) ovulation rates. Follicle diameters refer to measurements from fixed ovaries. (From Cahill, Mariana & Mauléon, 1979.)

cells incorporated tritiated thymidine, while the second comprised larger follicles with at least one follicular cell undergoing division. Mauléon (1975) suggests that the first group contains follicles formed very late during oogenesis and which probably remain in this state throughout the life of the animal. Lintern-Moore & Moore (1979) also found two distinct groups of primordial follicles in the mouse ovary, with the larger follicles exhibiting significantly greater synthesis of RNA within the oocyte.

There is some controversy as to whether gonadotrophins influence the rate at which primordial follicles enter the growth phase. Small preantral follicles with 4–5 layers of granulosa cells have been observed following hypophysectomy in the rat (Smith, 1930) and hamster (Moore & Greenwald, 1974), suggesting that gonadotrophins may not be obligatory for initiation of follicular growth. Nevertheless, after hypophysectomy of the sheep there was a large decrease in the number of small preantral follicles (Dufour, Cahill & Mauléon, 1979), and a similar effect in the hamster was overcome by exogenous gonadotrophin (Chiras & Greenwald, 1978), demonstrating that, although gonadotrophins are not obligatory for the initiation of follicle growth, they are facilitatory (Text-fig. 2). However, further work is required to determine which specific gonadotrophins initiate follicular growth and their mode of action.

Initiation of follicular growth is influenced by the level of nutrition in the rat (Lintern-Moore & Everitt, 1978). If we extrapolate this finding to the sheep, a nutritional effect would not be manifest in terms of a change in ovulation rate until 6 months later, this being the time interval between the initiation of follicular growth and ovulation. Evidence for this hypothesis was reported by Fletcher (1974) who applied different nutritional treatments to ewes. They were then placed on the same pasture and the live-weights equilibrated. The mean ovulation rate of the treated ewes reflected the original nutritional treatments 6 months earlier with the ovulation rate being 30% lower in ewes previously subjected to a restricted diet. Lamming (1966) suggested that severe undernutrition of females produces a “pseudohypophysectomy” which could explain the decreased rate at which follicles enter the growth phase.

More follicles appear to enter the growth phase in breeds with a higher ovulation rate because more preantral follicles are observed in the ovaries of Romanov ewes than in those of Ile-de-France ewes (Text-fig. 1). Similarly, more follicles appear to enter the growth phase during the anoestrous season (Cahill & Mauléon, 1980). The mechanism driving more follicles to enter the growth phase, according to breed or seasonal influences, is probably the end result of a number of interacting factors that are not fully understood.



Code	Hormone involved	Action	Reference
1	Gonadotrophin (probably FSH) FSH	Initiation of follicle growth Facilitation of growth of preantral follicles	Ryle (1971); Lintern-Moore & Moore (1979) Chiras & Greenwald (1978)
2	FSH	Antrum formation	Evans <i>et al.</i> (1932)
3	LH	Enhancement of atresia of antral follicles	Armstrong & Dorrington (1977)
	FSH	Inhibition of atresia of antral follicles	Richards & Midgley (1976)
4	Androgens	Enhancement of atresia of antral follicles	Payne & Runsen (1958)
	Oestrogens	Inhibition of atresia of antral follicles	Harman <i>et al.</i> (1975)
5	Progesterone	Inhibition of the growth rate of large antral follicles	Jesel (1971)
6	Oestrogens	Synergistic effect with FSH on follicles at antrum formation	Richards & Midgley (1976)
7	Unknown	Enhancement of follicles to enter growth phase following hemicastration	Dufour <i>et al.</i> (1979)
8	Unknown	Intra ovarian regulation resulting in correlations between number and growth rates of follicles	Cahill & Mauléon (1980)

Text-fig. 2. Schematic representation of the documented regulating mechanisms controlling pre-antral and antral follicles.

Preantral follicles

After leaving the reserve pool a follicle enters the preantral phase and grows by an increase in the size of the oocyte and multiplication of granulosa cells. In the sheep ovary, preantral follicles are more numerous (approximately 2:1) than antral follicles and atresia is seldom seen. Preantral follicles also have a relatively slow growth rate. In the sheep the mean time for a follicle to pass through this phase was estimated to be 4.3 months (Cahill & Mauléon, 1980). In large domestic animals, therefore, preantral follicles may act as a short-term reserve or buffer between the non-growing or dormant primordial follicles and the actively growing antral follicles.

Various studies have suggested that preantral follicles are under the control of gonadotrophins (Text-fig. 2), especially FSH, e.g. the in-vitro study of the mouse ovary (Ryle, 1971), the in-vivo studies of the rat (de Reviens & Mauléon, 1973), the study of gonadotrophin receptors in the rat (Presl, Pospisil, Figarov & Krabec, 1974), and the in-vivo study of the sheep (Dufour *et al.*, 1979).

The number of preantral follicles can be influenced by numerous factors (age, season, nutrition, gonadotrophin treatment, unilateral ovariectomy) but, generally, to demonstrate an effect these factors need to operate over a relatively long time span. Unilateral ovariectomy and hypophysectomy in the sheep result in a significant increase and decrease respectively in the number of preantral follicles. These effects were not evident at 4 days but were significantly different at 70 days after treatment (Table 1). It is interesting to speculate on the mechanism leading to the increased number of preantral follicles following unilateral ovariectomy. Findlay & Cumming (1977) have shown a rise in peripheral FSH concentration 5–12 h after unilateral ovariectomy but no long-term changes have been documented. This long-term effect of unilateral ovariectomy might therefore be mediated by an intra-ovarian mechanism rather than via the hypothalamic-pituitary axis.

Table 1. The number of preantral follicles per ovary at 4 and 70 days after hypophysectomy and unilateral ovariectomy in Ile-de-France ewes (adapted from Dufour *et al.*, 1979)

Treatment	No. of preantral follicles per ovary	
	4 days	70 days
Control	166 ± 50 ^a	266 ± 32 ^b
Hypophysectomy	146 ± 46 ^a	89 ± 30 ^c

Values with different superscript letters are significantly different (*t* test, *P* < 0.05).

Antral follicles

At a follicle diameter (fixed ovaries) of 0.1–0.4 mm (rat 0.1 mm, sheep 0.2 mm, cow 0.4 mm; from Mariana & Machado, 1976), intercellular spaces (diffuse antrum) appear and with further growth a fluid-filled cavity is formed (discrete antrum).

The rate of division (mitotic index) of granulosa cells increases rapidly to a maximum soon after antrum formation and then decreases slowly to zero in preovulatory follicles (sheep: Turnbull, Braden & Mattner, 1977; cow: Scaramuzzi, Turnbull & Nancarrow, 1980). From these studies it has been estimated that approximately 3 follicles per animal per day enter the antral phase and most of these follicles undergo atresia. The mean time for a follicle to grow from antrum formation to the preovulatory phase in sheep is 34–43 days (Turnbull *et al.*, 1977; Cahill & Mauléon, 1980).

FSH has long been implicated in antrum formation (Evans, Simpson, Tolksdorf & Gensen, 1932) and a synergistic action of FSH and oestrogens which results in the rapid growth of follicles soon after antrum formation (Text-fig. 2) has been shown by Richards & Midgley (1976). FSH binds primarily to granulosa cells of follicles at all stages of growth and the number of FSH binding sites per cell does not appear to vary greatly (Nimrod, Erickson & Ryan, 1976) although the number of cells per follicle increases throughout development.

Luteinizing hormone (LH) binds primarily to thecal cells at all stages of follicular development and to granulosa cells of the largest antral follicles, with only limited binding to granulosa cells of small antral and preantral follicles (Ryle, 1971; Midgley, 1973). Furthermore FSH has been shown to increase the number of LH receptors on granulosa cells in follicles of the immature rat (Zeleznik, Midgley & Reichert, 1974). One of the important features of the late antral phase is a large increase in the number of LH receptors per follicle (Channing & Kammerman, 1974); Carson, Findlay, Burger & Trounson (1979) have shown that there is a 10-fold increase in the number of LH receptors on granulosa cells of sheep follicles of > 4 mm diameter compared to follicles of 1–4 mm. With the large increase in LH receptors the follicle becomes increasingly sensitive to LH and it is well documented that following the LH surge at oestrus, or after hCG administration, the largest antral follicles which do not ovulate become atretic (mouse: Engle, 1927; sheep: Turnbull *et al.*, 1977). It is therefore apparent that antral follicles are of two distinct types reflecting their LH receptor population. Those in one group are susceptible to LH and after an LH surge ovulate, luteinize or become atretic, whilst follicles in the other group do not respond to LH.

The influence of prolactin on ovarian function remains unresolved. Granulosa cells of small antral follicles in the pig bind prolactin and this binding capacity decreases as follicle size increases (Rolland, Hammond, Schellekens, Lequin & de Jong, 1976). A parallel decrease in the prolactin concentration of follicular fluid as the antral follicles increase in size is well documented (McNatty, Hunter, McNeilly & Sawers, 1975). Prolactin may therefore act directly at the ovarian level by interfering with steroidogenesis, follicular maturation or both.

Antral follicles, especially the largest antral follicles, are mainly responsible for the ovarian production of oestrogens and androgens (Baird & Scaramuzzi, 1976) which have an important role in the control of gonadotrophin levels by feed-back on the hypothalamic–pituitary axis (see Baird & McNeilly, 1981). Thus large antral follicles not only influence their own growth and development by controlling the levels of gonadotrophins but they also indirectly influence the growth and development of all other follicles in the growth phase via the gonadotrophins.

More antral follicles are present in sheep ovaries during the breeding season than in the anoestrous season (Kammerlade, Welch, Nalbandov & Norton, 1952); therefore the number of follicles which can be ovulated in response to PMSG is low during the non-breeding season (Gherardi & Lindsay, 1980). The increased number of antral follicles during the breeding season could be due to the increased peripheral concentrations of FSH (Findlay & Cumming, 1976).

The number of antral follicles in the sheep ovary also varies according to the breed and breeds with a higher ovulation rate have more antral follicles (Cahill *et al.*, 1979). Large variations exist between individuals and this is believed to be the major cause of the large variation in ovulation rate induced by treatment with PMSG late in the oestrous cycle. This belief arises out of the close correlation between observed ovarian responses to PMSG and the calculated number of follicles likely to respond (Moor, Cahill & Stewart, 1981).

Relationship between the number of follicles and their growth rate

A major problem in the use of exogenous gonadotrophin for ovarian stimulation is that the number of follicles in a given class (e.g. the class of follicles destined to ovulate) and the growth rate of these follicles are not independent factors. When all non-atretic follicles in an ovary were

divided into 13 classes according to follicle size, the number of follicles in a class and the growth rate of that class were negatively correlated and the slopes of the regression equation for these two factors were significantly different between ewes (Cahill & Mauléon, 1980). If this relationship is causal then the administration of exogenous gonadotrophin may produce a response in follicular growth rate contrary to that desired as the ovary compensates for this change and attempts to revert to its former state. This relationship could explain the initial follicular growth in the neonate when the few follicles that have entered the growth phase grow at an accelerated rate (rat: Hage *et al.*, 1978). In addition, if this relationship is causal it could suggest the existence of an intra-ovarian mechanism regulating the number of follicles by changing the follicular growth rate.

Atresia and ovulation rate

Most follicles terminate their growth in the antral phase and become atretic. The few surviving follicles undergo maturation changes following the LH surge at oestrus (Thibault, 1977; Moor & Warnes, 1978). The follicles which undergo atresia do not have any apparent deficiency or abnormality of the oocyte because atretic follicles have been 'rescued' *in vitro* and the oocytes, after transplantation, have produced offspring (Moor & Trounson, 1977). The actual mechanisms of atresia and why a follicle undergoes atresia while neighbouring follicles continue to grow remain unknown.

Short-term treatments that result in changing the ovulation rate per ovary (e.g. PMSG stimulation, unilateral ovariectomy, short-term nutritional effects) probably act primarily by changing the number of follicles that undergo atresia in the final stages of follicular growth. In sheep, unilateral ovariectomy as late as 3 days before ovulation can result in the rupture of follicles that would normally have undergone atresia, ovulating to maintain the normal ovulation rate (Land, 1973). On the other hand, long-term treatments which influence ovulation rate (e.g. breed, season and age) are likely to act primarily by changing the number of non-atretic antral follicles. The difference in ovulation rate observed between breeds (Cahill *et al.*, 1979) was not achieved by a difference in the proportion or number of atretic follicles, but rather by a difference in the number of normal follicles (Table 2).

Table 2. The mean number (\pm s.e.m.) of normal and atretic follicles in the right ovaries of 8 Romanov and 12 Ile-de-France ewes

Breed	Ovulation rate	No. of normal follicles	No. of atretic follicles	% of atretic follicles	
				Per total no.	Per no. of antral follicles
Romanov	3.1*	233 \pm 24†	10.0 \pm 1.6	4.1	12.0
Ile-de-France	1.4*	150 \pm 20†	6.8 \pm 0.9	4.3	13.1

* $P < 0.01$.

† $P < 0.05$.

The use of hormonal agents during the last few days before oestrus to induce a controlled increase in ovulation rate in ewes (2 or 3 ovulations) has been generally unsuccessful. These treatments have attempted to overcome the innate atresia mechanisms but a highly variable response has been obtained. Immunization of ewes against androstenedione which presumably increases the secretion of endogenous gonadotrophin, has led to an increased proportion of twin ovulations (Van Look, Clarke, Davidson & Scaramuzzi, 1978) and could prove to be a most useful research tool.

Conclusions

Our knowledge of folliculogenesis in the sheep is far from complete. Perhaps the most intriguing problems at present are those relating to the selection of (1) primordial follicles to start development and enter the growth phase and (2) antral follicles to undergo atresia whilst the seemingly similar neighbouring follicles remain unaltered.

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