Pattern and manipulation of follicle development in sheep and goats

A.C.O. Evans

Department of Animal Science, The Centre for Integrative Biology, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland. E-mail alex.evans@ucd.ie

Summary

Improving our understanding of the pattern of follicle development and its manipulation by exogenous hormones in sheep and goats will improve our ability to manipulate their fertility. Repeated ultrasonographic observations of the growth of individual follicles have shown that follicles develop in a wave-like pattern during oestrous cycles, with two or three follicular waves per cycle being most common in sheep and three or four follicular waves per cycle being most common in goats. The largest follicles that are present at luteolysis continue development to ovulation and these usually derive from the last follicular waves but in some cases also develop from the penultimate follicular wave. Follicle development is often stimulated by using eCG to enhance the recruitment of small follicles, increase ovulation rates and increase the synchrony of oestrus after oestrous synchronisation treatments. Superovulation treatments also use gonadotrophin stimulation, the success of which seems to be related to the number of small follicles at the start of treatment. Long-term (12 - 14 days) progestagen treatments used for oestrus synchronisation can promote prolonged growth of ovulatory follicles in sheep but has not been studied in goats. The ovulation of aged follicles in cattle has a detrimental effect on fertility, but this relationship is less clear and seems to be less critical in sheep.

Introduction

A large pool of resting primordial follicles is laid down during fetal development in sheep, with the first follicles being formed about 70 days of gestation (54). This pool is non-renewable and during the lifetime of the animal, follicles develop to primary, secondary and tertiary (4) stages before ovulating. Very few follicles progress to ovulation and most die by atresia at any stage of development. While it is highly variable, it has been estimated that there are about 100,000 to 200,000 follicles in lambs at birth (48) and that at any one time there are about 50 antral follicles in the ovaries of an adult sheep (14,21). It has been suggested that it takes about 6 months for a follicle to develop from the resting stage to the preovulatory stage and approximately 34 to 43 days from initial antrum formation to the preovulatory phase (15,87).

Manipulation of reproduction often relies on the manipulation of follicle development and this desire has driven investigations to better understand the pattern of ovarian follicle development and in particular the relationships among follicles that develop in the ovaries.

Follicle development during the oestrous cycle

A detailed study of the ovaries of cattle collected at slaughter led Rajakoski (1960) to coin the term “follicle wave”. He observed that the number of follicles ≥5 mm in diameter was not evenly distributed throughout the estrous cycle and that these follicles were apparently organised
into two periods of growth, observed temporarily as waves of follicle growth in the ovaries. It was not until follicle development could be monitored on a daily basis in the same animals, using ultrasonography, that the proposition of follicle waves in cattle was unequivocally demonstrated (64,76,82). A follicle wave is considered to be the initial synchronous growth of a cohort of follicles (emergence), followed by one or more that continues growing (the dominant follicle) while others regress (subordinate follicles).

**Sheep**

Studies since Rajakoski (1960) on the pattern of follicle growth in sheep have discussed the presence or absence of follicle waves. Early experiments were inconclusive and involved India ink studies, slaughter studies or oestradiol secretion studies. They suggested that there were either two (13) or three (55,83) phases of follicle growth and atresia or that there was no reliable pattern of follicle growth during an oestrous cycle (40,47,87). It has also been estimated that three or four follicles develop to the antral phase per day in sheep (87).

With the development and use of repeated ultrasonography or laparoscopy, evidence for (30,61,84) and against (66,67,79) an organized pattern of follicle waves has been presented. However, many of these studies did not closely examine changes in the number of follicles among days of the cycle, as per the original definition of waves by Rajakoski (1960). In the light of these studies we examined the day-to-day changes in the numbers and pattern of follicle growth in sexually mature ewes (23). This showed that there are fluctuations in the number of follicles in different size classes consistent with the definition of follicle waves (65), and that there were predominantly two or three follicle waves during an oestrous cycle. The numbers of small, medium and large follicles significantly fluctuated among days of the cycle and also peaked on successive days, demonstrating that there was a progressive growth of follicles from one size class to another over a number of days in a wave-like fashion (23). Further support for the concept that follicle waves occur in sheep, in a similar way to cattle, is that follicle waves in sheep are preceded by a transient increase in FSH concentrations that stimulate follicle wave emergence (7,10,24,28,85) (Figure 1).

Based on the above, it is possible to conclude that follicles do develop in cohorts (or waves) during the oestrous cycle in sheep. Despite this, it is possible to reconcile the views of others that follicle development in ewes is continuous (47) or random (53) or the view that follicles grow and regress asynchronously at any time of the luteal phase of the oestrous cycle (87). I suggest that the explanation is largely due to the numbers of follicles per cohort and the number of cohorts developing in each oestrous cycle. In our studies (23), ewes with two follicle waves showed an organized pattern of follicle development of follicles from one size class to another; however, this was less clear in ewes with three follicle waves. This was possibly due to the increased number and speed of change-over of follicles, and may explain why in other studies (53,79) a follicle wave pattern was not detected since follicle waves were apparently numerous and frequent. Ginther et al (1995) noted that follicles reaching maximum diameters of 3 or 4 mm did not exhibit an organized pattern of follicle growth and regression. However, follicles grew from 3 to ≥5 mm approximately every 5 days during the cycle, indicating a level of organization of growth regression of large follicles. When investigators limit their considerations to only large follicles (reaching ≥5 mm in diameter), a wave-like pattern is usually described (30,50,85). Hence, follicle development occurs in a wave-like pattern in sheep, but the ease with which this is identified is dependent on the number of follicles and number of waves per oestrous cycle. During oestrous cycles, the dominant and largest subordinate follicles reach maximum diameters of 5 to 7 mm and 3 to 5 mm, respectively (6,23,30). In most cases the ovulatory follicles develop from the cohort of follicles from the last follicular wave. However, the ovulatory follicles can also derive from the penultimate follicular wave (6,28) giving rise to a situation where follicles ovulate from both the last and the second last follicular waves of the cycle.
Figure 1. Schematic presentation of the pattern of follicle development and associated concentrations of FSH and oestradiol during the oestrous cycle in sheep with three follicular waves (from 22). In the second panel only the largest follicles are shown for clarity and the dotted lines indicate the possible growth profiles of ovulatory follicles where the ovulation rate exceeds one (star indicates ovulation).
**Goats**

The dynamics of follicle wave development in goats have received less attention than in cattle or sheep and breed differences likely contribute to the differences reported. Prior to the use of ultrasonography to repeatedly monitor ovarian follicle development, laparoscopic observations in cyclic goats have documented the growth of ovarian follicles but were not able to describe an organised pattern (16). Studies since then using ultrasonography have shown that the pattern of follicle development during oestrous cycles in goats does follow a wave-like pattern (reviewed in 74) and that there are between 2 and 6 waves of follicle development during oestrous cycles with 3 or 4 waves being the most prevalent (19,29,31,56,58,80) (Figure 2). On any one day of the oestrous cycle there are 5 to 10 follicles ≥3 mm in diameter in the ovaries and follicles ovulate at between 6 and 9 mm in diameter (16,19,29,31). As in sheep, when double ovulations occur they are usually of follicles derived from the same wave but in a few cases they derive from two consecutive follicle waves (29).

**Figure 2.** Follicular waves in representative goats. Cycling goats # 28 and # 21 developed three and four follicular waves, respectively, during an interovulatory cycle. In pregnant goat # 26 six follicular waves emerged during the early pregnancy. Only follicles that grow over 3 mm are drawn (from 74).
Manipulation of follicle development and fertility

Exogenous substances have been given to sheep for many years to manipulate follicle development and ultimately fertility (35,39,42,51,71). In recent years the more precise effects of these agents on follicle development have been characterised.

Gonadotrophins

Follicle development is often stimulated by using eCG, enhancing the recruitment of small follicles (62), increasing ovulation rates (3,11,49,62) and increasing the synchrony of oestrus after oestrous synchronisation treatments (11,17,44). Superovulation treatments also use gonadotrophin stimulation (12,52,69), the success of which seems to be related to the number of small follicles at the start of superovulation treatment (32,33) and may (33,52,75) or may not (20,32) be affected by the presence of a large follicle at the time of treatment.

Progesterone

Progesterone and progestagens are widely used to induce oestrus during the non-breeding season (36-38,88) and to synchronise oestrus during the breeding season (35). These methods result in a synchronous decrease in circulating progestagen concentrations at device withdrawal, but the timing of ovulation is variable and dependent on the stage of follicle development at the time of progestagen withdrawal (73). The characteristics of the ovulatory follicle are also dependent on the ability of the progestagen treatments to control gonadotrophin secretion. It has been shown in cattle (46,77,86) and sheep (27,50) that, in the absence of a corpus luteum, LH pulse frequency increases as the progestagen release from synchronisation devices decreases over time (46,70,86). In cattle, an association between intermediate progesterone concentrations, increased LH pulse frequency and the development of large prolonged dominant follicles has been described (8,60,78,81,86). In cattle, it is clear that ovulation of these follicles results in a decrease in fertility (5,18,59,86). This may or may not be partially due to an effect of the uterine environment on the embryo (9,91), but it is likely also to involve the deleterious early activation of the oocytes (60,68).

An inverse relationship between LH pulse frequency and progesterone concentrations is well documented in sheep (34). While progesterone is used in some synchronisation protocols in sheep, many products use more potent synthetic progestagens (72). The initial release of progestagen from intravaginal sponges is high, but decreases with time (37). When low progestagen concentrations in ewes were experimentally created this lead to larger follicles (90), older larger more persistent ovulatory follicles (25,27,50) and higher oestradiol concentrations (27,45).

The effect of ovulation of aged follicles on fertility in sheep is not clear. When progesterone in corn oil was injected subcutaneously into prostaglandin-treated ewes, follicles were larger and older at ovulation and conception rates were lower in ewes with low progesterone concentrations (<1 ng/ml) than control ewes (progesterone > 1 ng/ml; no prostaglandin treatment) (45). This conception decrease was thought to be due to a reduction in fertilisation rates or early embryo survival. Another study has reported that the ovulation of older, larger follicles also results in a decrease in fertility (89) and another has suggested that the reduction in fertility of ewes that received an 11-day progesterone treatment during anestrus was due to the ovulation of an aged follicle (88). In contrast, other studies have found no adverse effects of ovulation of aged follicles on embryo quality and fertility in ewes. Ewes that ovulated aged follicles had the same proportion of good quality embryos as those that ovulated younger follicles, and the number of ewes lambing (mean 84%) was also not different among groups (25). The age of follicles at ovulation in ewes therefore appears to be less critical than in cattle, and, within the confines of a 14-day progestagen synchronisation treatment, the ovulation of older follicles is unlikely to lead to a major reduction in fertility. However, it is possible that progestagen treatments for more than 14 days may be deleterious to fertility. While few studies have examined this in detail, oral
progestagen treatments for 16 to 20 days resulted in fertility rates of between 61 and 75% (26,43,51); compared to values of 86 to 94% for ewes fed for 14 days (1,41).

Since long-term progestagen treatments can result in the ovulation of aged follicles and that this may have a negative effect on oocytes development, short-term progestagen treatments for oestrus synchronisation have been examined (reviewed in 57). To ensure acceptable oestrus response after at 5 to 7 day progestagen oestrus synchronisation the treatment has to include a dose of prostaglandin F2α (PGF2α). When cycling goats were treated with PGF2α and then a progestagen device for 5 to 7 days (and 200 IU eCG at device withdrawal) pregnancy rates after cervical insemination with fresh semen were between 60 and 65 % (57). Using ultrasonography, the pattern of follicle growth has been characterised in response to the combined PGF2α and 5-day progestagen treatment when started at random stages of the cycle in goats. When a follicle that had emerged four or more days previously was present at the start of treatment that it regressed, a new follicle wave emerged and a growing large follicle was available for ovulations at the time of device withdrawal. When a new follicle wave emerged about the time of the start of treatment a growing large follicle was also present at the time of device withdrawal. Hence, irrespective of the status of follicle development at the start of treatment, a young healthy, growing follicle was able to ovulate at the end of treatment (57).

Prostaglandin

Prostaglandin F2α has no significant direct effect on follicle development but has been used to synchronise oestrus in sheep (2) and goats (63) by regulating development of the corpus luteum. Traditionally treatment consists of two PGF2α doses given at a 9- to 14-day interval based on the concept that the corpus luteum is refractory to PGF2α for the first few days of the oestrous cycle. This has recently been challenged and a regimen of two doses at a 7-day interval has been shown to be successful in goats (57). The interval to oestrus after PGF2α administration (and hence the synchrony in a group) depends on the status of the corpus luteum (how long it takes progesterone concentrations to reach basal values) and the status of follicle development at treatment. If a healthy growing follicle is present at the time of PGF2α treatment then oestrus and ovulation will occur sooner than if a new follicle needs to emerge and develop to ovulation.

Conclusions

Daily observations of the pattern of follicle growth have lead to the understanding that follicle development occurs in a wave-like pattern in sheep, with two to four waves per cycle being the most common. However, the ease with which follicle waves are described seems to depend on the frequency of the waves and the number of follicles per wave. In general, follicle waves are preceded by a transient increase in FSH concentrations, and there is a hierarchy among the follicles of a wave for diameter and follicular fluid oestradiol concentrations. The manipulation of follicle development during oestrous cycles is mainly concerned with manipulating ovulation rate using gonadotrophins and synchronizing oestrus-using progestagens. While long-term progestagen treatments (12 to 14 days) are effective at synchronizing oestrus for breeding the significance of fertility of ovulating aged follicles is not clear. This has lead to the recent investigation of the use of short-term progestagen treatments (7 days) to synchronize oestrus and to explore the possibility of using PGF2α only treatments.
References


