Effects of ovarian follicle ablation on FSH, oestradiol and inhibin A concentrations and growth of other follicles in sheep

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The aim of this study was to examine the effect of removal of the largest follicle or all visible follicles during the first follicle wave on subsequent follicular growth, steroid, inhibin A and gonadotrophin secretion in sheep. On day 4.5 of a synchronized oestrous cycle, ewes ($n = 18$) were assigned to one of three groups which underwent either no treatment (control), ablation of the largest follicle (largest follicle aspirated and cauterized via laparotomy) or ablation of all follicles (all visible follicles ablated). Between day 0 and day 10 of the oestrous cycle, blood samples were collected every 8 h and ovaries were examined daily using transrectal ultrasonography. The lifespan of the second largest follicle (number of days > 3 mm in diameter) was longer (6.7 ± 0.9 days; $P < 0.05$) and the maximum diameter tended to be greater (4.8 ± 0.3 mm; $P = 0.07$) in ewes in which the largest follicle was ablated than in the control ewes (3.8 ± 0.4 days; 4.2 ± 0.3 mm). There was no difference in the day of emergence of the second follicular wave between groups (day 6.9 ± 0.4). However, the peak of the transient increase in FSH

concentrations after ablation was earlier (day 5.67 ± 0.15 ; $P < 0.05$) in ewes in which all follicles were ablated than in control ewes (day 6.72 ± 0.36); the timing in ewes that had only the largest follicle ablated was intermediate (day 6.11 ± 0.28). Serum inhibin A concentrations were about three-fold lower ($P < 0.05$) in both follicle ablation groups than in the control group. The numbers of follicles 2–3 mm in diameter during the first 3 days of the second follicular wave were greater in 'ablated ewes' (both groups had 2.6 ± 0.2 follicles day⁻¹) than in control ewes (1.7 ± 0.3 follicles day⁻¹). It is concluded that: (i) transient increases in FSH concentrations precede the emergence of follicle waves; (ii) ablation of all follicles on day 4.5 after oestrus advanced the timing of the next peak in FSH concentrations and the numbers of small follicles associated with the development of the second follicular wave; and (iii) ablation of the largest follicle resulted in an increase in the lifespan of the second largest follicle, indicating a regulatory role of large dominant follicles over smaller subordinate follicles.

Introduction

Waves of follicular growth and a dominant follicle are found in cattle (Fortune, 1994; Ginther *et al.*, 1996; Roche *et al.*, 1998) and, despite early controversy, it appears that there are also waves of follicular growth in sheep (Noel *et al.*, 1993; Ginther *et al.*, 1995; Leyva *et al.*, 1998; Evans *et al.*, 2000), although the occurrence of dominance remains to be established. In cattle, a wave of follicular growth involves the synchronous growth (> 4 –8 mm in diameter) of a cohort of follicles from the follicle reserve, followed by the continued development of one follicle (dominant follicle) and the atresia of all other follicles from the original cohort. Emergence of a new wave occurs after the dominant follicle from the previous wave undergoes atresia (Ginther *et al.*, 1996). A wave-like pattern of follicle growth similar to that in cattle has been described in sheep; however, the diameter of follicles in sheep is smaller than in

cattle: emergence occurs at a follicle diameter of 2 mm and the maximum diameter achieved by the ovulatory follicle is 6–7 mm (Ginther *et al.*, 1995; Evans *et al.*, 2000).

The existence of dominance in sheep is still unresolved. Some authors have reported that a weak form of dominance may exist on the basis of the observation that the largest follicle may delay or prevent the development of other follicles (Ravindra *et al.*, 1994; Ginther *et al.*, 1995; Rubianes *et al.*, 1997). Evans *et al.* (2000) argued for the occurrence of dominance in sheep on the basis of a hierarchy in terms of diameter and oestradiol concentration of the largest follicle, and an apparent relationship between the demise of the largest follicle and the emergence of the subsequent wave.

The mechanism involved in the selection of the dominant follicle is unclear. In cattle, it appears that the future dominant follicle is capable of switching its dependency from FSH secretion to LH and, therefore, can survive in an environment of decreasing FSH concentrations (Ginther *et al.*, 1996; Webb *et al.*, 1999). However, there is

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also evidence that the dominant follicle inhibits the growth of other follicles. In cattle, removal of the dominant follicle on day 3 of the oestrous cycle resulted in delayed regression of the largest subordinate follicle, early emergence of the second wave of follicular growth and an increase in the proportion of animals with three rather than two waves per cycle (Ko *et al.*, 1991; Adams *et al.*, 1993). The emergence of a new wave has been associated with a transient increase in FSH concentrations in cattle (Adams *et al.*, 1992; Evans *et al.*, 1997) and sheep (Souza *et al.*, 1998; Bister *et al.*, 1999; Gibbons *et al.*, 1999; Bartlewski *et al.*, 2000). Advancing the timing of the FSH increase and emergence of a new wave of follicles by ablation of the dominant follicle and postponement of the FSH increase and emergence of a new wave of follicles by i.v. treatment with follicular fluid demonstrates that the dominant follicle actively suppresses the growth of other follicles in cattle (Adams *et al.*, 1992). In sheep, the influence of the largest follicle on the increase in FSH concentrations that triggers the emergence of the subsequent wave has yet to be established.

The aim of the present study was to examine the influence of removal of the largest follicle or all visible follicles on subsequent follicular growth, oestradiol, FSH and inhibin A concentrations, and, hence, to determine whether dominance occurs in sheep, as in cattle. Follicles were removed by ablating (via laparotomy and cauterization) either the largest follicle or all visible follicles on day 4.5 of the oestrous cycle.

Materials and Methods

Animals and treatments

All procedures were in accordance with the Cruelty to Animals Act, 1876 (European Community Directive 86/609/EC) licensed by the Department of Health and Children, Ireland. During December, nine Suffolk-cross and nine Texel-cross maiden ewe lambs (40 ± 0.35 kg) were maintained at pasture ($530 \pm 18^\circ\text{N}$) where they had access to water and hay and 0.4 kg concentrates (19% (w/w) crude protein) per day. The oestrous cycles of the ewes were presynchronized using a 14 day intravaginal progestagen sponge (60 mg medroxyprogesterone acetate; Veramix, Upjohn Cranley). Follicular growth patterns were studied daily (at 08:00 h) from the day of sponge removal until day 10 of the subsequent oestrous cycle by transrectal ultrasonography using a real time B mode scanner (Dynamic Imaging Concept MLV) with a 7.5 MHz linear array transducer (Evans *et al.*, 2000). Oestrus was detected using a raddled vasectomized ram.

The day of follicle emergence was traced retrospectively as the day on which follicles were first recorded with a diameter of 2–3 mm. The lifespan of a follicle was defined as the number of days on which an identified follicle was detected with a diameter of ≥ 3 mm.

On day 4.5 of the oestrous cycle (between 15:00 h and 18:00 h) the ewes were assigned randomly to one of three

treatments: (i) control; (ii) ablation of the largest follicle; or (iii) ablation of all visible follicles. Ewes in all groups were sedated (400 μl i.m.; Rompun®; Bayer Plc, Bury St Edmunds) and follicles were ablated under local anaesthesia (20 ml s.c.; lignocaine plus adrenaline; Norbrook Laboratories Ireland Ltd, Monaghan) via mid-ventral laparotomy (control ewes were sedated only). Follicles were measured using callipers; follicular fluid was aspirated using a 25G needle and the collapsed follicle was cauterized completely (Ko *et al.*, 1991).

Blood collection and hormone analysis

Blood samples (7 ml) were collected three times a day (at 07:00, 15:00 and 23:00 h) from day 0 to day 10 of the oestrous cycle by jugular venepuncture. The samples were stored at 4°C for 24 h and centrifuged at 1500 *g* for 20 min at 4°C . The serum was decanted and stored at -20°C until assayed for oestradiol, FSH and inhibin A concentrations. Follicular fluid samples collected from follicles that were ablated were centrifuged at 1000 *g* for 1 min, decanted and stored at -20°C until assayed for oestradiol and inhibin A concentrations.

Radioimmunoassays

Oestradiol concentrations in serum samples collected three times a day were assayed by validated radioimmunoassay (Prendiville *et al.*, 1995). The sensitivity of the assay was 0.03 pg per tube. The intra- and interassay coefficients of variation for reference samples with average concentrations of 1.78, 2.76 and 5.37 pg oestradiol ml^{-1} included in each assay were 5.8 and 7.9%, 6.0 and 7.0%, and 6.6 and 9.9%, respectively.

FSH concentrations were measured in all serum samples using a validated radioimmunoassay (Crowe *et al.*, 1997), which included an anti-ovine antibody (NIDDK anti-oFSH) but with the modification of ovine standards (USDA-oFSH-SIAFP-RP-2). The sensitivity of the assay was 0.06 ng ml^{-1} (defined as the least amount of unlabelled FSH tested that could displace iodinated FSH from the first antibody; $P < 0.05$, *t* test). Intra-assay coefficients of variation were 10, 10 and 3% for samples containing 0.23, 0.87 and 2.87 ng ml^{-1} , respectively. Interassay ($n = 2$) coefficients of variation for the same samples were 16, 9 and 12%, respectively.

Inhibin A concentrations were measured in follicular fluid samples (diluted 1:25 000) and in serum samples collected in the 12 h (two samples) before surgery and in the 48 h (six samples) after surgery using a modified version (Bleach *et al.*, 2001) of a two-site ELISA (Knight *et al.*, 1998). Sensitivity of the assay was 15 pg ml^{-1} and intra- and interassay coefficients of variation were 10.3 and 13.4%, respectively.

Statistical analysis

Data were analysed using ANOVA or paired *t* test and multiple comparisons were made by the method of least significant difference. The changes in FSH, oestradiol and

Table 1. Characteristics of follicular growth in waves 1 and 2 in control ewes (no treatment), ewes in which the largest follicle was ablated and ewes in which all visible follicles were ablated on day 4.5 after oestrus

	Control	Largest follicle ablated	All follicles ablated
Number of ovulations in previous oestrous cycle	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
First follicular wave			
Day of emergence of wave 1	2.2 ± 0.2	2.0 ± 0.2	2.2 ± 0.2
Diameter determined by ultrasonography at 8–10 h before ablation (mm)			
Largest follicle	4.8 ± 0.3	4.9 ± 0.3	5.0 ± 0.3
Second follicle	3.7 ± 0.2*	4.0 ± 0.3*	3.0 ± 0.3*
Diameter determined at surgery (mm)			
Largest follicle	–	6.2 ± 0.5	5.8 ± 0.6
Second follicle	–	4.0 ± 0.4*	4.2 ± 0.5*
Follicular fluid oestradiol (ng ml ⁻¹)			
Largest follicle	–	20.0 ± 2.0	11.0 ± 1.8
Second follicle	–	–	4.1 ± 2.2*
Follicular fluid inhibin A (µg ml ⁻¹)			
Largest follicle	–	4.8 ± 0.5	5.0 ± 0.4
Second follicle	–	–	4.1 ± 0.8
Second follicle of wave 1			
Maximum diameter at ultrasonography (mm) [†]	4.2 ± 0.3	4.8 ± 0.3	–
Lifespan (days)	3.8 ± 0.4 ^b	6.3 ± 0.9 ^a	–
Second follicular wave			
Day of emergence of wave 2	7.2 ± 0.3	6.8 ± 0.3	6.8 ± 0.2
Numbers of follicles (per day) during the first 3 days of wave 2			
2 and 3 mm in diameter	1.7 ± 0.3 ^b	2.6 ± 0.2 ^a	2.6 ± 0.2 ^a
4 and 5 mm in diameter	1.5 ± 0.1	1.2 ± 0.2	0.8 ± 0.3
≥ 6 mm in diameter	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

Values are mean ± SEM ($n = 6$).

*Indicates a lower value ($P < 0.05$) for the second largest compared with the largest follicle within a treatment (column).

^{ab}Values within a row with no common superscript are significantly different ($P < 0.05$).

[†]Difference between treatments tends towards significance ($P = 0.07$).

inhibin A concentrations before and at about 24 h after surgery were calculated using the mean of samples taken over the 4–12 h interval before surgery (samples from days 4.00 and 4.33 of the oestrous cycle) and the 20–28 h interval after surgery (samples from days 5.33 and 5.67 of the oestrous cycle). These times were chosen as optimal to reflect changes in hormone concentrations caused by follicle ablation.

Results

All of the ewes ovulated a single follicle at the end of the synchronized oestrous cycle as determined by ultrasonography and by counting corpora lutea in those ewes that underwent laparotomy. The dynamics of growth of the two largest follicles per wave are shown (Fig. 1). The day of

emergence of the first follicular wave was similar in all groups (combined mean 2.1 ± 0.1 days after oestrus; Table 1). On day 4 in all groups, the largest follicle had a significantly greater ($P < 0.05$) diameter than did the second largest follicle, as measured initially by ultrasonography and confirmed at surgery in ewes that had follicles ablated (Table 1). The concentration of oestradiol in follicular fluid was greater ($P < 0.05$) in the largest follicle than in the second largest follicle in ewes in which all follicles were ablated, but there was no difference in the follicular fluid concentration of inhibin A between these follicles (Table 1). The lifespan of the second largest follicle (number of days that the diameter was ≥ 3 mm) was on average 2.5 days longer ($P < 0.05$) and tended ($P = 0.07$) to have a greater maximum diameter in ewes in which the largest follicle was ablated than in control ewes (Table 1).

Fig. 1. Diameters of the largest and second largest follicles in the first and second follicular waves measured using transrectal ultrasonography. (a) Control ewes (sedation only). (b) Ewes in which the largest follicle was ablated on day 4.5 of the oestrous cycle. (c) Ewes in which all visible follicles were ablated on day 4.5 of the oestrous cycle. (■) Largest follicle, first wave; (●) largest follicle, second wave; (□) second largest follicle, first wave; (○) second largest follicle, second wave. Values are mean $\pm$ SEM ($n = 6$ per group). Data were aligned to the mean day of follicle wave emergence. Vertical dotted line indicates the time of follicle ablation.

The second follicular wave emerged at a similar time in the three groups (combined mean 6.9 ± 0.2 days after oestrus; Table 1). There were a higher ($P < 0.05$) number of small follicles (2–3 mm in diameter) during the first 3 days of the second follicular wave in ewes that had the largest or all follicles ablated than in the control ewes (Table 1). There was a tendency ($P = 0.07$) for a difference among groups for the number of medium-sized follicles (4–5 mm in diameter) during the first 3 days of the second wave, with the lowest number in ewes that had all follicles ablated (0.8 ± 0.3) and the highest number in control ewes (1.5 ± 0.1 ; Table 1). There were no differences among groups in the numbers of

large follicles (> 6 mm in diameter) present during the first 3 days of the second follicular wave (Table 1).

The pattern of circulating hormone concentrations is shown (Fig. 2). Oestradiol concentrations peaked before surgery at a similar time and concentration in all three groups (combined means: 3.7 ± 0.2 days and 1.91 ± 0.17 pg ml $^{-1}$; Table 2). Similarly, FSH concentrations reached nadir concentrations at a similar time and concentration in all three groups (combined means: day 3.8 ± 0.2 and 1.0 ± 0.13 ng ml $^{-1}$; Table 2). Concentrations of oestradiol and inhibin A decreased and FSH concentrations increased from 8 h before to 24 h after surgery in all groups of ewes ($P < 0.05$; Table 2). Concentrations of each of these hormones were similar among groups before surgery but FSH concentrations were higher ($P < 0.05$) and inhibin A concentrations were lower ($P < 0.05$) at 24 h after surgery in the two groups of ewes in which follicles were ablated compared with control ewes (Table 2). The day of the oestrous cycle on which the circulating oestradiol concentrations reached a nadir was not significantly different among the groups, but was earliest in ewes in which all follicles were ablated and latest in the control ewes (Table 2). FSH reached peak concentrations sooner ($P < 0.05$) after surgery in ewes in which all follicles were ablated than in control ewes, and was intermediate in ewes in which only the dominant follicle was ablated (Table 2). In addition, there was a tendency ($P = 0.06$) for a difference among groups for the peak concentration of FSH, being lowest in control ewes and highest in ewes in which the largest follicle was ablated (Table 2).

When comparing the timing of peak and nadir hormone concentrations between oestradiol and FSH (within a treatment group), the day of the oestrous cycle on which oestradiol concentrations reached a peak and FSH concentrations reached a nadir was not different within any group (combined means: day 3.7 ± 0.2 of the oestrous cycle; Table 2). The days on which FSH concentrations reached a peak and oestradiol concentrations reached a nadir were also not different between control ewes and ewes that had the largest follicle ablated, but was later in ewes in which all follicles were ablated (see Table 2).

Discussion

The results of the present study indicate that removal of the largest follicle results in continued survival of the second largest follicle, implying that the largest follicle has a role in regulation of the lifespan of other follicles. In addition, removal of the largest, or all follicles, resulted in a rapid decrease in inhibin A concentrations and an increase in FSH concentrations, leading to an increase in the numbers of small follicles in the subsequent follicular wave.

The emergence of follicular waves was associated with transient increases in FSH concentrations in agreement with previous studies (Souza *et al.*, 1998; Bister *et al.*, 1999; Gibbons *et al.*, 1999; Bartlewski *et al.*, 2000). It is accepted that FSH secretion is controlled by both oestradiol and

inhibin negative feedback (Mann *et al.*, 1992; Knight *et al.*, 1998); however, the relative roles of each are not clear. In the present study, circulating concentrations of oestradiol peaked in all groups (at about day 3.7) before surgery (on day 4.5). Oestradiol concentrations were decreasing at about the time of surgery in all groups but the results indicated that the more follicles that were ablated, the earlier oestradiol reached nadir concentrations. Inhibin A concentrations decreased from before surgery to about 85% at 24 h after surgery in control ewes but decreased to 38% in ewes that had the largest follicle ablated and to 29% in ewes that had all follicles ablated. This finding is in agreement with another study in which all follicles were ablated and immunoreactive inhibin concentrations in the ovarian vein decreased to 65% from before to 10 min after ablation (Findlay *et al.*, 1990). The fluctuations in FSH concentrations, although advanced in the two groups of ewes in which follicles were ablated, reached similar concentrations in all groups. The temporal relationship between the decrease in inhibin A concentrations and the increase in FSH concentrations observed in ewes in which follicles were ablated indicates that inhibin A may be an important regulator of FSH secretion in sheep. However, peak FSH concentrations were not different among groups and small changes in inhibin A concentrations in control ewes resulted in a similar pattern of fluctuation in FSH concentrations to that of ewes in which a large decrease in inhibin A concentration was observed (ablated ewes). If inhibins were the main regulators of FSH concentrations, a greater increase after follicle ablation (and inhibin removal) would have been expected. Hence, changes in oestradiol concentrations may be significant regulators of FSH concentrations in ewes, even when these changes are small and not detectable. An alternative suggestion is that the pituitary may be maximally responsive to small changes in inhibin or steroid (possibly including androgens) concentrations. Once a small threshold change occurs (as in inhibin A concentrations in the control ewes), a maximum response is elicited that cannot be increased by a larger change in negative feedback (as in the large changes in inhibin A concentrations in the ewes in which follicles were ablated). In cattle, it has been suggested that inhibins regulate the background FSH secretion and that cyclic changes in oestradiol drive daily cyclic changes in FSH (Glencross *et al.*, 1994); however, a role for inhibin A in controlling cyclic changes in FSH concentrations has been proposed (Bleach *et al.*, 2001). Further experiments are needed to determine accurately the relative roles of oestradiol and inhibins in the regulation of FSH concentrations during the luteal phase of ewes. It should also be noted that inhibins have a local regulatory function in the ovary (Findlay *et al.*, 2000; Campbell and Baird, 2001; Knight and Glister, 2001).

With hindsight, the optimal time for surgery would have been earlier than day 4.5 as was performed in this study. Day 4.5 after oestrus was selected for surgery as it was the earliest time at which the identity of the largest follicle could be predicted accurately using ultrasonography (Evans

Fig. 2. (a) FSH, (b) oestradiol and (c) inhibin A concentrations in control ewes ($\blacktriangle$; sedation only), ewes in which the largest follicle was ablated ($\square$: largest follicle cauterized on day 4.5 after oestrus) and ewes in which all visible follicles were ablated ($\circ$: all visible follicles cauterized on day 4.5 after oestrus). FSH concentrations were aligned to the mean day of emergence of the first follicular wave and from two samples before surgery. Oestradiol and inhibin A concentrations were aligned (forwards and backwards) to the time of surgery. Values are mean $\pm$ SEM ($n = 6$). Vertical dotted line indicates the time of follicle ablation. Some error values were small and bars are contained within the plotted symbol.

et al., 2000). If oestradiol concentrations had still been increasing, and FSH concentrations had been decreasing at the time of surgery, the changes brought about by follicle ablation (such as the timing of nadir oestradiol concentrations and peak FSH concentrations) would have been expected to be more marked. Nevertheless, significant differences in oestradiol and FSH concentrations among groups are in agreement with the findings of other studies (Findlay *et al.*, 1990; Souza *et al.*, 1998) that follicle products regulate FSH concentrations and that the largest follicle has the most significant regulatory role.

Table 2. Characteristics of plasma oestradiol, FSH and inhibin A concentrations in control ewes (no treatment), ewes in which the largest follicle was ablated and ewes in which all visible follicles were ablated on day 4.5 after oestrus

	Control	Largest follicle ablated	All follicles ablated
Peak oestradiol			
Day of oestrous cycle	3.82 ± 0.37	3.38 ± 0.27	3.78 ± 0.11
Concentration (pg ml ⁻¹)	1.8 ± 0.2	1.8 ± 0.3	2.1 ± 0.3
Nadir FSH			
Day of oestrous cycle	4.11 ± 0.42	3.33 ± 0.34	3.83 ± 0.21
Concentration (ng ml ⁻¹)	0.90 ± 0.19	1.08 ± 0.22	1.14 ± 0.27
Surgery			
Day of oestrous cycle	4.5	4.5	4.5
Hormone concentration			
FSH (ng ml ⁻¹)			
Before ablation	1.17 ± 0.17*	1.80 ± 0.33*	1.45 ± 0.32*
After ablation	1.56 ± 0.15 ^b	2.70 ± 0.21 ^a	2.56 ± 0.33 ^a
Oestradiol (pg ml ⁻¹)			
Before ablation	1.1 ± 0.2*	1.1 ± 0.2*	1.2 ± 0.2*
After ablation	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.2
Inhibin A (pg ml ⁻¹)			
Before ablation	142 ± 15*	89 ± 4*	95 ± 22*
After ablation	122 ± 11 ^a	34 ± 10 ^b	28 ± 6 ^b
Nadir oestradiol			
Day of oestrous cycle [‡]	6.00 ± 0.30	5.71 ± 0.24	5.00 ± 0.19
Concentration (pg ml ⁻¹)	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
Peak FSH			
Day of oestrous cycle	6.72 ± 0.36 ^a	6.11 ± 0.28 ^{ab}	5.67 ± 0.15 ^b
Concentration (ng ml ⁻¹) [‡]	2.23 ± 0.36	3.46 ± 0.21	2.83 ± 0.42

Values are mean ± SEM (*n* = 6).

*Indicates a difference (*P* < 0.05) between before ablation and after ablation values, within a hormone

[‡]Hormone concentrations before and after ablation are the mean of two samples collected before (days 4.00 and 4.33) and 24 h after (days 5.33 and 5.67) ablation.

[‡]Difference among treatments tends towards significance (*P* ≤ 0.10).

^{ab}Within rows, values with no common superscript are significantly different (*P* < 0.05).

Our findings are similar to those in cattle in which removal of the dominant or largest follicle resulted in delayed regression and larger diameter of the second largest follicle (Ko *et al.*, 1991; Adams *et al.*, 1993). New wave emergence occurred at a similar time in treated ewes and control ewes in the present study, but in cattle, follicle cauterization leads to the advancement of emergence of a new follicle wave. The failure to hasten new wave emergence in the ewes in the present study could be related to the timing of follicle ablation. At surgery, circulating concentrations of oestradiol were decreasing and FSH concentrations were increasing, and the mechanism to initiate new wave emergence had possibly already been initiated in all groups. However, ablation did increase FSH concentrations and stimulate the growth of more small follicles than in control ewes. In agreement with work in cattle (Evans *et al.*, 1997), there was a temporal and negative relationship between the timing of peak and nadir oestradiol and FSH concentrations. In agreement with earlier studies, the follicular fluid samples indicated that the

dominant follicle was the main source of oestradiol (Bjersing *et al.*, 1972; Carson *et al.*, 1981).

The increased lifespan of the second largest follicle in the ewes in which the largest follicle was ablated compared with the control ewes indicates that a form of dominance exists in mono-ovulatory sheep. All ewes in the experiment had ovulated a single follicle at the end of the previous cycle (Evans *et al.*, 2000). The hierarchy of follicles in terms of diameter and oestradiol concentration in follicular fluid also supports the idea of dominance in sheep in agreement with other reports (Bjersing *et al.*, 1972; Carson *et al.*, 1981; Evans *et al.*, 2000). The presence of a dominant follicle in cattle is associated with suppression of the number of follicles (Ginther *et al.*, 1989). The increased number of small follicles (2–3 mm in diameter) observed in ewes in which the largest or all follicles were ablated provides further evidence for the occurrence of dominance in sheep. Hence, it appears that the largest or dominant follicle encourages the regression of other, subordinate follicles. How the dominant follicle influences the lifespan of the

subordinate follicles is unclear but the effect is either indirect via the gonadotrophins or via an as yet undescribed local direct mechanism. Other data suggest that clear dominance does not occur in sheep. New follicle waves have been shown to emerge in the presence of large follicles from a previous wave (Flynn *et al.*, 2000) and in some cases ovulated (Bartlewski *et al.*, 1999) and new follicle waves emerged in the presence of steroidogenically active follicles from a previous wave in anoestrous ewes (Evans *et al.*, 2001). Therefore, the occurrence of dominance may be reliant on a number of factors including breed of ewe, stage of reproductive cycle and time of year.

In conclusion, the emergence of new waves of follicular growth was associated with transient increases in FSH concentrations and these fluctuations in FSH concentrations appear to be regulated by follicle products (oestradiol and inhibin A). In addition, the largest follicle appears to regulate the growth of other follicles. The demise of the largest follicle increased the lifespan of the second largest follicle and resulted in an increase in the numbers of follicles in the subsequent follicular wave, indicating that ovarian follicle dominance occurs during the luteal phase of sheep.

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