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Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the oestrous cycle in breeds of sheep differing in prolificacy

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Transrectal ultrasonography of ovaries was performed each day in non-prolific Western white-faced ($n = 12$) and prolific Finn ewes ($n = 7$), during one oestrous cycle in the middle portion of the breeding season (October–December), to record the number and size of all follicles ≥ 3 mm in diameter. Blood samples collected once a day were analysed by radioimmunoassay for concentrations of LH, FSH and oestradiol. A cycle-detection computer program was used to identify transient increases in concentrations of FSH and oestradiol in individual ewes. Follicular and hormonal data were then analysed for associations between different stages of the lifespan of the largest follicles of follicular waves, and detected fluctuations in serum concentrations of FSH and oestradiol. A follicular wave was defined as a follicle or a group of follicles that began to grow from 3 to ≥ 5 mm in diameter within a 48 h period. An average of four follicular waves per ewe emerged during the interovulatory interval in both breeds of sheep studied. The last follicular wave of the oestrous cycle contained ovulatory follicles in all ewes, and the penultimate wave contained ovulatory follicles in 10% of white-faced ewes but in 57% of Finn ewes. Transient increases in serum concentrations of FSH were detected in all animals and concentrations reached peak values on days that approximated to follicle wave emergence. Follicular wave emergence was associated with the onset of transient increases in serum concentrations of oestradiol, and the end of the growth phase of the largest follicles (≥ 5 mm in diameter) was associated with peak serum concentrations of oestradiol. Serum FSH concentrations were higher in Finn than in Western white-faced ewes during the follicular phase of the cycle ($P < 0.05$). There were no significant differences in serum concentrations of LH between Western white-faced and Finn ewes ($P > 0.05$). Mean serum concentrations of oestradiol were higher in Finn compared with Western white-faced ewes ($P < 0.01$). It was concluded that follicular waves (follicles growing from 3 to ≥ 5 mm in diameter) occurred in both prolific and non-prolific genotypes of ewes and were closely associated with increased secretion of FSH and oestradiol. The increased ovulation rate in prolific Finn ewes appeared to be due primarily to an extended period of ovulatory follicle recruitment.

Introduction

In sheep, the ovulation rate is genetically predetermined and can vary between breeds from approximately one in mainly monovular Merino del Pais (Lopez-Sebastian *et al.*, 1997) and Australian Merino ewes (Campbell *et al.*, 1995), to approximately two in breeds with a high incidence of twinning, such as the Javanese thin-tail sheep (Sutama *et al.*, 1988), and to greater than three in prolific breeds, such as the Finnish Landrace and Romanov breeds (Campbell *et al.*, 1995). There are also specific genes, for example the *FecB* or Booroola gene, that result in ovulation rates greater than five (Cummins *et al.*, 1983). Higher ovulation rates are often accompanied by smaller ovulatory follicles (Driancourt, 1991; Driancourt *et al.*, 1996) and fewer granulosa cells per follicle with less oestradiol production (Avdi *et al.*, 1997). It has been suggested that increased ovulation rates could be due to a wider window of time for follicle recruitment or an increase in the numbers of follicles recruited (Scaramuzzi *et al.*, 1993). The physiological regulation of increased ovulation rate is unclear and, although the growth of antral follicles is largely regulated by FSH (Scaramuzzi *et al.*, 1993), the evidence that increased FSH secretion is responsible for increased ovulation rates is contradictory (Scaramuzzi *et al.*, 1993; Campbell *et al.*, 1995; Fry and Driancourt, 1996; Avdi *et al.*, 1997; Souza *et al.*, 1997). Souza *et al.* (1997), using

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ultrasonography, showed that ovarian follicular dynamics did not differ between sheep with or without the Booroola gene. However, ovulatory follicles and corpora lutea were smaller in ewes carrying the fecundity gene but serum gonadotrophin and ovarian hormone concentrations did not differ between the prolific and non-prolific ewes.

Early observations on the development of antral follicles in ewes led to the conclusion that follicles begin to grow early in the cycle and then remain in the ovaries to enlarge shortly before ovulation (Hutchinson and Robertson, 1966). Smeaton and Robertson (1971) reported that there were at least three distinct groups of ovarian follicles beginning to grow synchronously throughout the normal oestrous cycle in ewes. Brand and de Jong (1973) suggested a two-phase model of follicular development in cyclic ewes, with two distinct cohorts of antral follicles beginning to grow at about day 2 and 11 of the cycle. Studies using ink marking of follicles suggested waves of follicle production during the oestrous cycle (Driancourt *et al.*, 1991). Studies using endoscopy and ovarian ultrasonography have revealed that the growth of follicles to ≥ 5 mm in diameter occurs in an orderly fashion, at approximately 5 day intervals, throughout the oestrous cycle in ewes (Noel *et al.*, 1993; Ginther *et al.*, 1995). However, studies summarized by Lopez-Sebastian *et al.* (1997), including that of Schrick *et al.* (1993), did not find waves of follicle production.

In sheep, the growth of antral follicles is primarily dependent on FSH, and the terminal phase of follicular development, culminating in ovulation, is under the control of LH (Baird *et al.*, 1976; Scaramuzzi *et al.*, 1993; Campbell *et al.*, 1995). During the ovine oestrous cycle, serum concentrations of FSH increase and decrease at relatively regular intervals (Campbell *et al.*, 1991; Ginther *et al.*, 1995), and there is a temporal relationship between the emergence of follicular waves (follicles growing from 3 to ≥ 5 mm in diameter) and these transient increases in serum FSH concentration (Ginther *et al.*, 1995; Fry and Driancourt, 1996).

The aim of the present study was to use daily ultrasound imaging of ovaries to describe and compare the patterns of ovarian antral follicle turnover throughout the oestrous cycle in two breeds of sheep with different ovulation rates (non-prolific Western white-faced and prolific Finn sheep). Particular interest was taken in differences that might explain the mechanism used to increase ovulation rate in prolific sheep. In this respect, attempts were made to correlate alterations in circulating concentrations of FSH and oestradiol, and the development of the largest ovarian follicles.

Materials and Methods

Animals and experimental procedures

Twelve Western white-faced (aged approximately 5 years, average body weight 90 ± 7 kg) and seven Finn ewes (3–4 years of age, average body weight 57 ± 4 kg) were used in the present study during the middle portion of the breeding season (October–December). Western white-faced sheep are largely of Rambouillet × Columbia breeding, and the average number of lambs born per ewe is 1.5 ± 0.2 (Rawlings *et al.*, 1987). The Western white-faced ewes used in this study were nulliparous animals and the Finn sheep had lambed twice. The average number of offspring born per ewe for the Finn sheep was 2.4 ± 0.3. Animals were housed outdoors in sheltered dry lots and fed daily maintenance rations of alfalfa pellets. Water, hay and cobalt iodized salt bars were available *ad libitum*.

Oestrus was detected with three vasectomized crayon-harnessed rams and an electronic oestrous detector (Firma Draminski, Olsztyn, Poland). This instrument measures changes in vaginal mucous impedance near the cervix uteri and was validated for the present application in sheep by Szczepanski *et al.* (1994). A decrease in vaginal electrical resistance below 40 Ω occurs at the beginning of behavioural oestrus and persists for approximately 24 h (Szczepanski *et al.*, 1994).

Each animal underwent transrectal ovarian ultrasonography each day for one ovulatory cycle starting on the day on which the ewe was first marked by the rams or a vaginal impedance reading below 40 Ω was recorded. The day of ovulation were regarded as the day on which the large ovarian antral follicles that had been identified by ultrasonography was no longer detected. Ovarian ultrasonography used a B-mode, real-time scanner (Aloka SSD 500 Echo Camera, Overseas Monitor Corp. Ltd, Richmond, BC), equipped with a 7.5 MHz human prostate transducer (shaft length, 35 cm; shaft diameter, 1.6 cm), validated for use in sheep (Ravindra, 1993; Schrick *et al.*, 1993). Images were displayed at a magnification of × 1.5. The number, diameter and relative position of all ovarian follicles ≥ 3 mm in diameter were sketched on ovarian charts. All ovarian images were recorded on high-grade video tapes (Fuji S-VHS, ST-120 N), using a compatible cassette recorder (Panasonic, Super VHS, AG 1970), for retrospective analysis of ovarian data.

Blood samples (5 ml) were collected each day by jugular venepuncture, using vacutainers (Becton Dickson, Rutherford, NJ), and were allowed to coagulate for 18–24 h at room temperature. After centrifugation at 3000 r.p.m. for 15 min, serum was harvested and stored at –20°C for analysis at a later date.

Follicular data summary and analysis

Follicular data (follicles ≥ 3 mm in diameter) were combined for both ovaries. Data from two Western white-faced ewes were excluded from analyses as they had abnormally long cycles (both 23 days). A follicular wave was defined as one or more antral follicles that grew from 3 to ≥ 5 mm in diameter. The day the follicles were first detected at 3 mm was the day of wave emergence. Groups of follicles emerging within 48 h were regarded as a single follicular wave. For follicular waves in which follicles emerged within 24 h, the day of wave emergence was the day on which the first follicle of the group was detected at 3 mm and, for follicular waves in which follicles emerged within 48 h, the central day was considered the day of wave emergence (see Figs 1 and 2). The following characteristics of follicular

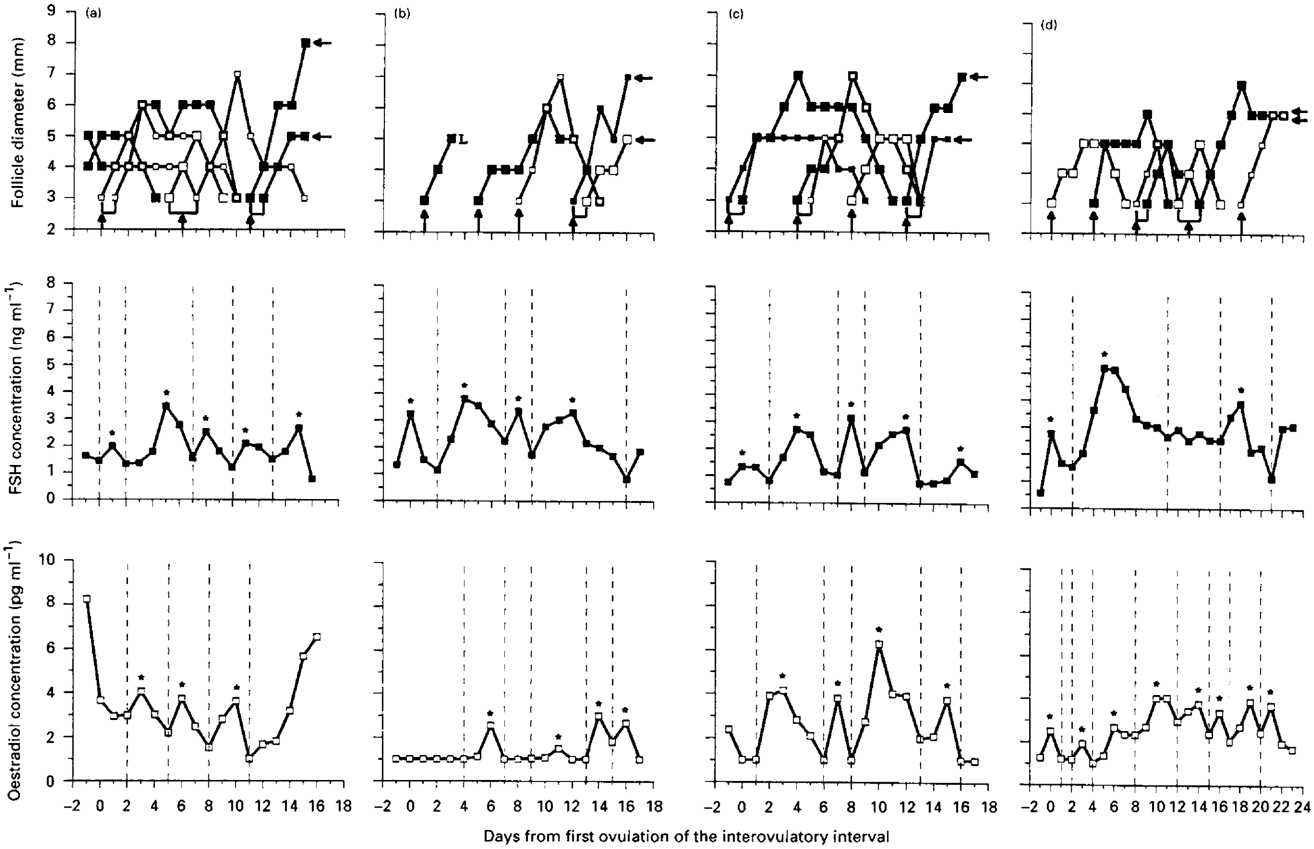

Fig. 1. The diameter profiles of individual antral follicles growing to a size of ≥ 5 mm and recorded during the oestrous cycle in four Western-white faced ewes (a–d), with accompanying serum concentrations of FSH (middle panels; ■) and oestradiol (bottom panels; □). The arrows along the *x*-axis (top panels; ↑) denote the days of follicle wave emergence, and the arrows within the upper chart area (←) indicate the ovulatory follicles on the day before ovulation. L represents a large antral follicle that became luteinized and gradually transformed into a solid luteal structure. The diameters of ovulatory follicles observed at the first ovulation of the interovulatory interval studied are not shown. Asterisks denote the peak values of fluctuations (nadir-to-peak-to-nadir) in circulating concentrations of FSH and oestradiol (the two dashed lines encompass a fluctuation) as determined by the cycle-detection computer program (Clifton and Steiner, 1983).

waves were determined for each ewe: (1) the number of emerging follicular waves; (2) the days of wave emergence; (3) the number of follicles per wave; (4) the maximum diameter attained by the largest follicle of the wave; (5) the duration of the follicle growing, static and regressing phases as well as a total lifespan of the largest follicle; and (6) the number of days between the emergence of sequential follicular waves (interwave intervals). The growing phase was defined as the time taken by a single follicle to grow from 3 mm to its maximum size; the regressing phase was the time taken by that follicle to regress from its maximum size to 3 mm; and the static phase was the time between the end of the growing phase and beginning of the regressing phase or ovulation. If more than one follicle reached the same maximum size, the follicle that first attained the maximum diameter or remained at its maximum size for the longest period of time was regarded as the largest follicle of the wave. Preliminary inspection of follicular data revealed that 13 out of 17 ewes studied had four waves of large follicle growth per oestrous cycle. Therefore, for each of the end points above, a 4 × 2 (number of waves × breed) factorial analysis of variance was used (General Linear Model procedures in the Statistical Analysis System SAS/STAT®, version 6; 1990, Cary, NC). Comparisons between sequential waves, within the oestrous cycle, and between the two breeds, for each follicular wave, were only performed for the ewes that had four waves per cycle, but an additional analysis was performed in which the data for animals with three or four waves per cycle were combined. The characteristics of ovulatory and non-ovulatory antral follicles (follicles growing to ≥ 5 mm in diameter) emerging in the last follicular wave in Western white-faced ewes and in the penultimate wave in Finn ewes were compared using ANOVA. This was not done for the final wave in Finn ewes as all follicles ≥ 5 mm in the wave ovulated, nor for the penultimate wave in Western white-faced ewes, because only one of ten white-faced ewes had an ovulatory follicle that emerged in the penultimate wave of the cycle.

The daily number of emerging 3 mm follicles that did not grow any larger before regression and of 3 mm follicles that subsequently reached 4 or ≥ 5 mm in diameter were normalized to the day of the first ovulation of the interovulatory interval (day 0), for the period from day −1 to day 15 (the entire growing phase could not be determined for

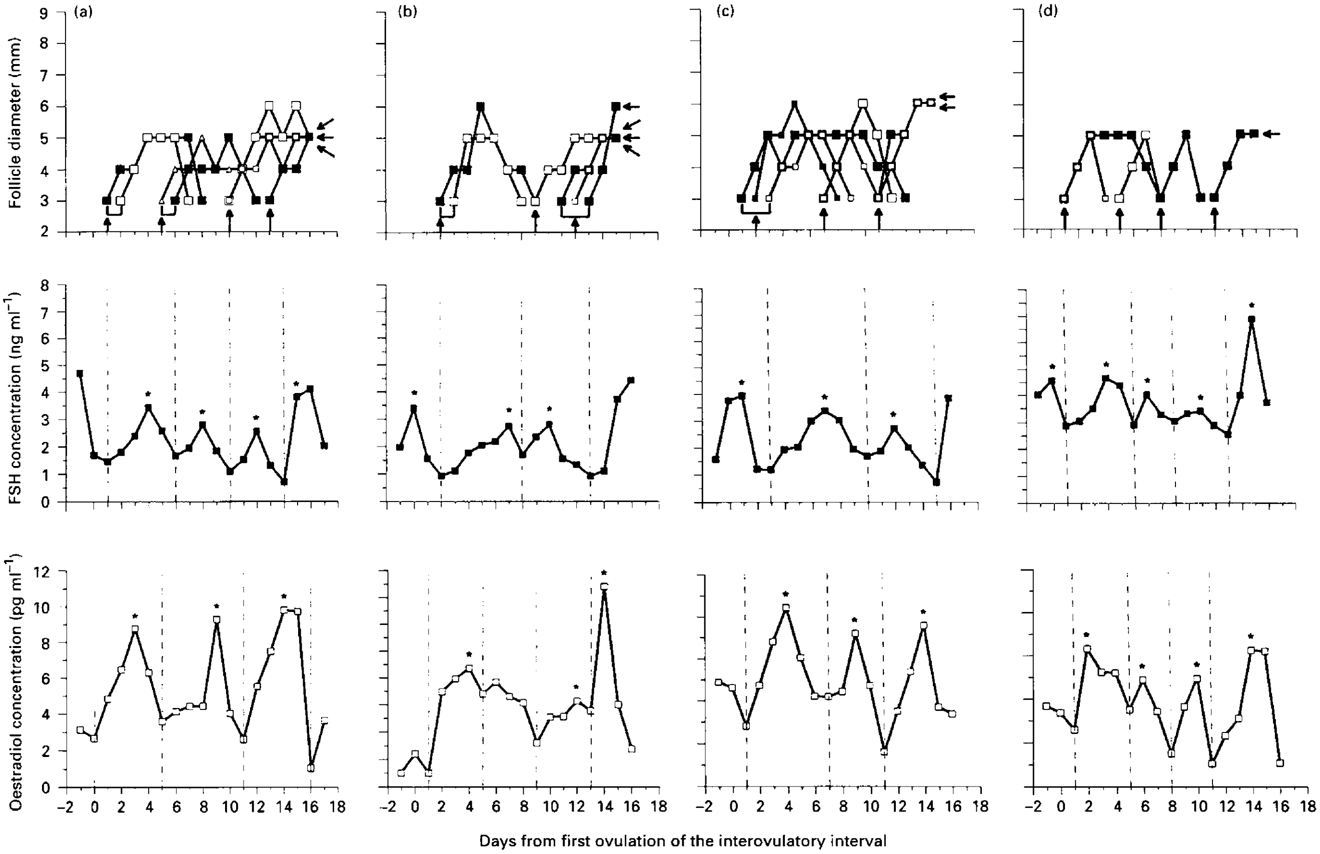

Fig. 2. The diameter profiles of individual antral follicles growing to a size of ≥ 5 mm and recorded during the oestrous cycle in four Finn ewes (a–d), with accompanying serum concentrations of FSH (middle panels; ■) and oestradiol (bottom panels; □). The arrows along the *x*-axis (top panels; ↑) denote the days of follicle wave emergence, and the arrows within the upper chart area (←) indicate the ovulatory follicles on the day before ovulation. The diameters of ovulatory follicles observed at the first ovulation of the interovulatory interval studied are not shown. Asterisks denote the peak values of fluctuations (nadir-to-peak-to-nadir) in circulating concentrations of FSH and oestradiol (the two dashed lines encompass a fluctuation) as determined by the cycle-detection computer program (Clifton and Steiner, 1983).

non-ovulatory follicles emerging on days 16–17 after ovulation) for ewes in each breed that had four waves of follicle growth per cycle. These data were then analysed for day and breed effects and day-by-breed interactions using multivariate analysis of variance (SAS/STAT®, 1990). If the main effect of day was significant ($P < 0.05$), an analysis of variance, and then Fisher's protected least significant difference (LSD) test, were used to compare individual (daily) means within each breed. If there was a significant main effect of breed, or if the day-by-breed interaction was significant, means within each day were compared between breeds by a *t* test using Statistix® Analytical Software (version 4.1, 1997; Tallahassee, FL).

Hormone analysis

Serum samples were analysed by radioimmunoassay for concentrations of LH (Rawlings *et al.*, 1984), FSH (Currie and Rawlings, 1989) and oestradiol (Joseph *et al.*, 1992). Data from the two Western white-faced ewes with abnormally long cycles were excluded from analysis. Concentrations of LH and FSH are given in terms of NIAMDD-oLH-24 and NIDDK.oFSH.RP1, respectively. The sensitivities of assays were 0.1 ng ml^{-1} (LH and FSH) and 1.0 pg ml^{-1} (oestradiol). The range of standards was from 0.0625 to 8.0 ng ml^{-1}, 0.125 to 16.0 ng ml^{-1} and 1.0 to 100 pg ml^{-1} in the LH, FSH and oestradiol assays, respectively. For reference sera with a mean LH concentration of 0.14 or 1.13 ng ml^{-1}, the intra- and interassay coefficients of variation (CV) were 11.3 and 5.0% or 10.1 and 6.9%, respectively. For FSH reference sera with mean concentration of 2.42 or 3.72 ng ml^{-1}, the intra- and interassay CVs were 5.6 and 3.9% or 8.1 and 10.1%, respectively. For oestradiol reference sera with a mean concentration of 9.3 or 18.9 pg ml^{-1}, the intra- and interassay CVs were 15.0 and 10.1% or 16.3 and 10.9%, respectively. Serum concentrations of FSH and oestradiol measured each day from day −1 to day 17 were normalized to the day of the first ovulation of the interovulatory interval (day 0), for ewes of both breeds that had four waves of follicular emergence per oestrous cycle. The main effects of breed, day and the breed-by-day interaction were determined by multivariate analysis of variance.

A cycle-detection program (Clifton and Steiner, 1983) was used to identify peaks in the daily serum concentrations of LH, FSH and oestradiol in individual ewes. This program determines the threshold concentrations of the hormone on the basis of the values of intra-assay coefficients of variation or the variation among sample replicates. Concentrations greater than the threshold values are the peak concentrations of a fluctuation. A cycle, or fluctuation, is defined as a progressive increase and decrease in hormone concentrations that encapsulate a peak concentration (nadir-to-peak-to-nadir). The following characteristics of detected peaks or fluctuations in serum concentrations of FSH and oestradiol measured each day were determined for each breed: (1) the mean number of peaks per ewe per oestrous cycle; (2) the mean duration of sequential fluctuations; (3) the mean peak concentration; and (4) the mean duration of intervals between peaks of adjacent fluctuations. Analyses of variance were used to compare means between the breeds and within the oestrous cycle for ewes with four follicular waves per cycle.

Follicular and hormonal data were analysed for associations between various stages of follicular wave development (follicles growing from 3 to ≥ 5 mm) and peaks in serum concentrations of FSH and oestradiol. For this purpose, the data for ewes with three and with four follicular waves per cycle were combined. The mean number of identified follicular waves and the mean number of FSH or oestradiol peaks per ewe, per oestrous cycle, were compared using a paired t test. Spearman correlations were done between the durations of interpeak intervals for detected FSH and oestradiol fluctuations and the intervals between adjacent days of follicular wave emergence (interwave intervals). Spearman correlations were also done between the interpeak intervals for FSH and oestradiol fluctuations and the intervals between different phases of the growth of the largest ovarian follicles of successive waves, namely: (1) the day of emergence; (2) the beginning of the static phase; (3) the end of the static phase; and (4) the end of the regressing phase (regression to 3 mm in diameter).

Oestradiol fluctuations appeared to begin around the day of follicle wave emergence. Therefore, the duration of the interval between sequential days of onset of oestradiol fluctuations and the duration of interwave intervals were correlated. The distribution of peaks of FSH fluctuations and the onset of oestradiol fluctuations, over the 2 days before or after the day of wave emergence and on the day of emergence, were determined. Similarly, the distribution of oestradiol peaks within 2 days before and after the day that the static phase of the largest follicle of a wave began was calculated. The distribution of FSH peaks and the onset of oestradiol fluctuations in relation to the days of follicular wave emergence were analysed by chi-squared test. A similar analysis was done for the peak values of the daily oestradiol concentrations and the days on which the largest follicle of a wave reached its maximum diameters.

Results

Oestrus detection and cycle duration

At the first observed ovulation of the interovulatory interval studied, all ewes but one were well marked by rams and one animal was poorly marked. At the second ovulation of the interovulatory interval, all ewes were well marked. Vaginal impedance decreased below 40 Ω in all ewes (mean ± SEM 33.2 ± 2.7 and 28.7 ± 2.4 Ω for white-faced and Finn ewes, respectively; $P > 0.05$) on the day they were first marked by rams, at the beginning and end of the oestrous cycle studied. Two Western white-faced ewes had abnormally long interovulatory intervals (both 23 days) associated with a prolonged lifespan of corpora lutea (recorded for approximately 16 consecutive days). Excluding these two ewes, the mean interovulatory interval was 17.0 ± 0.3 and 16.6 ± 0.2 days for Western white-faced and Finn ewes, respectively ($P > 0.05$). In Western white-faced ewes, the duration of the interovulatory intervals in two ewes with three follicular waves per cycle was 17 and 19 days, respectively and, in ewes with four follicular waves ($n = 8$), the range was 16–18 days. Finn ewes with three follicular waves per cycle ($n = 2$) each had 16 day interovulatory intervals and, of the Finn ewes with four emerging follicular waves ($n = 5$), one animal had a 16 day and four ewes had 17 day interovulatory intervals.

Follicular dynamics

Individual follicle profiles (follicles growing from 3 to ≥ 5 mm in diameter) are shown for four different animals, for each breed (including a white-faced ewe with a prolonged cycle), to illustrate the variations in the number of follicular waves per ewe, follicle size and the number and time of emergence of ovulatory follicles (Figs 1 and 2). The mean ovulation rate was 1.8 ± 0.2 and 2.7 ± 0.2, for Western white-faced and Finn sheep, respectively ($P < 0.05$).

For ewes with four waves of follicular growth per cycle, follicular data were aligned to the day of first ovulation of the interovulatory interval studied (day 0). When normalized on this basis, a significant main effect of breed ($P < 0.01$), but not of day or day-by-breed interaction, was seen for mean daily numbers of 3 mm follicles not growing beyond 3 mm in diameter (Fig. 3a). The Western white-faced ewes exceeded ($P < 0.05$) Finn sheep in the number of follicles reaching a maximum size of 3 mm each day from day −1 to day 0, day 4 to day 6, day 9 to day 11 and day 13 to day 15 of the oestrous cycle studied. There was no significant main effect of day, breed or day-by-breed interaction on the mean number of 3 mm follicles growing to a maximum diameter of 4 mm before regression per day.

The number of 3 mm follicles emerging per day that subsequently grew to ≥ 5 mm in diameter differed by day ($P < 0.01$), but there was no significant difference between the two breeds of sheep studied and no significant day-by-breed interaction. Follicles growing to ≥ 5 mm in diameter emerged from the pool of 3 mm follicles on each day of the cycle, except for days 2 and 15 in Western white-faced ewes, and days −1, 7 and 15 in Finn ewes. The number of 3 mm follicles that subsequently reached or exceeded 5 mm in diameter reached peak values on days 1, 5, 8, 10 and 12 of the cycle in Western white-faced ewes, and on days 1, 6, 8, 10, 12 and 14 in Finn sheep (Fig. 3b).

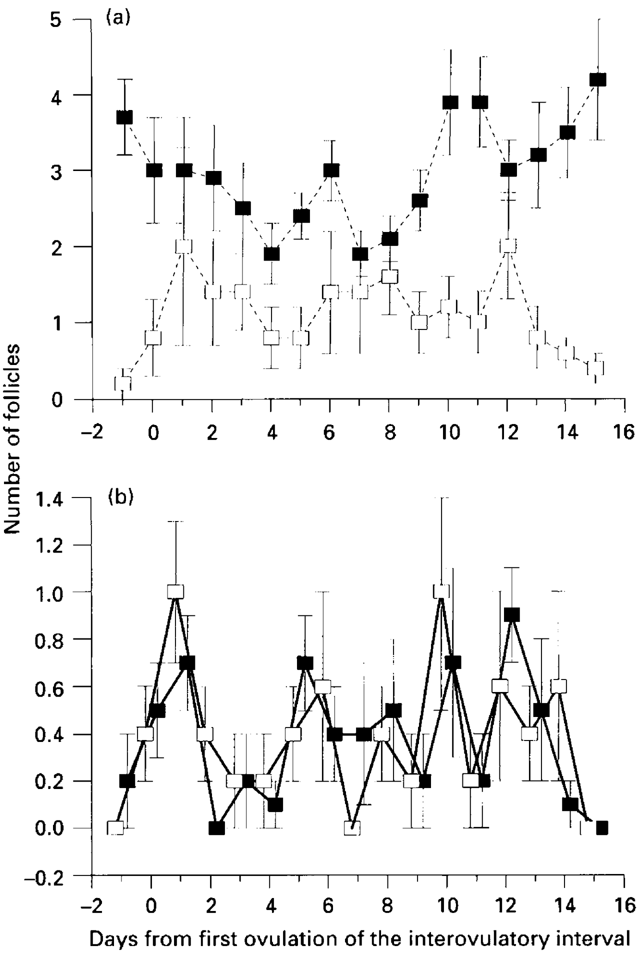

Fig. 3. Mean (± SEM) numbers of 3 mm follicles per day (a) reaching a maximum diameter of 3 mm before regression and (b) that subsequently grew up to ≥ 5 mm in diameter in cyclic Western white-faced (n = 8; ■) and Finn ewes (n = 5; □) that underwent daily transrectal ovarian ultrasonography for one ovulatory cycle during the mid-breeding season (October–December) and had four waves of follicular growth during the cycle. Follicular data were normalized to the day of first ovulation of the oestrous cycle studied (day 0) and analysed from day −1 to day 15.

Characteristics of follicular waves

A total of 64 follicular waves (follicles growing from 3 to ≥ 5 mm in diameter) was obtained in the present study; 38 waves were identified in cyclic Western white-faced ewes and 26 waves in Finn sheep. The characteristics of follicular waves are given for both breeds (Table 1). The data for two Western white-faced ewes with abnormally long interovulatory intervals were excluded. Of ten Western white-faced sheep, two had three follicular waves per oestrous cycle and eight had four waves per oestrous cycle. Two Finn sheep had three waves per cycle and five had four waves per cycle. In all ewes, the last follicular wave of the interovulatory period contained ovulatory follicles. Of all follicles growing from 3 to ≥ 5 mm in diameter in this wave, 77.3 and 100% ovulated, in Western white-faced and Finn ewes, respectively. The penultimate wave of the oestrous cycle bore ovulatory follicles in one white-faced ewe with four waves per cycle and in four Finn sheep (three ewes with four waves of large follicle growth and one with three waves of large follicle growth). In the penultimate follicular wave of the cycle, 8% (1/12) and 50% (6/12) of follicles growing to ≥ 5 mm in diameter were ovulatory in white-faced and Finn sheep, respectively.

The following results are for ewes with four waves of follicular growth per cycle. There was no significant difference in the mean day on which follicular waves emerged between non-prolific white-faced and prolific Finn sheep studied. However, the difference for the last follicular wave (wave 4) approached significance ($P = 0.05$). The mean number of follicles per wave (1.6 ± 0.1) did not vary among white-faced and Finn sheep or among the four follicular waves for each breed. The maximum diameter attained by the largest ovarian follicle of the wave emerging late in the cycle (wave 4) was greater ($P < 0.05$) in Western white-faced than in Finn sheep, and the largest follicle emerging during the early and mid-luteal phase (waves 2 and 3) tended to be larger in non-prolific white-faced than in Finn ewes (wave 2: $P = 0.06$ and wave 3: $P = 0.09$). The mean growth rate of the largest follicles of sequential waves (1.1 ± 0.03 mm day^{-1}) did not differ throughout the oestrous cycle within each breed or between the two breeds studied. The growth phase of large follicles late in the cycle (wave 4) was significantly longer in Western white-faced than in Finn ewes ($P < 0.01$), and it was longer for wave 4 than for the preceding follicular waves ($P < 0.05$) in white-faced ewes. The static phase of the follicular lifespan did not vary between the two breeds studied, but the duration of the static phase of wave 1 was significantly longer relative to subsequent waves (waves 2–4) in both white-faced and Finn ewes. The regressing phase of the first follicular wave was longer ($P < 0.05$) in white-faced sheep than in Finn sheep, and this phase was also longer for wave 1 than for wave 3 in white-faced ewes ($P < 0.01$). Follicular lifespan decreased significantly from wave 1 to wave 2 and from wave 3 to wave 4 in both breeds under study, and it was longer for wave 1 ($P < 0.05$) and wave 4 ($P < 0.01$) in Western white-faced than in Finn ewes. The difference between breeds in the mean lifespan of the largest follicles emerging in mid-cycle (waves 2 and 3) approached significance (wave 2: $P = 0.09$ and wave 3: $P = 0.07$). The duration of interwave intervals did not differ between the two breeds studied, but the interval between the first and the second follicular wave of the cycle in Western white-faced ewes was longer ($P < 0.01$) than the interwave intervals separating the ensuing waves of large follicle growth.

Adding the data for ewes with three follicular waves to those of ewes with four waves per cycle did not alter any of the statistical trends determined for the groups of white-faced and Finn ewes with four waves per oestrous cycle. In ewes with three waves per cycle, wave 3 appeared to emerge 2–4 days later than in ewes with four follicular waves per cycle. Therefore, the mean day of emergence of wave 3 averaged day 9 (8.9 ± 0.5) and day 10 (9.7 ± 0.6) after ovulation when ewes with three or four waves per cycle were combined, as opposed to day 8 (8.2 ± 0.4) and day 9

Table 1. Characteristics of ovarian follicular waves (follicles growing from 3 to ≥ 5 mm in diameter; mean ± SEM) identified in Western white-faced ($n = 8$, regular font) and Finn sheep ($n = 5$, italics) that underwent ovarian ultrasonography each day during one ovulatory cycle in the mid-breeding season (October–December), and had four waves per cycle

End point	Wave 1	Wave 2	Wave 3	Wave 4
Number of waves containing ovulatory follicles	0 (0) *0 (0)*	0 (0) *0 (1)*	1 (2) *3 (2)*	8 (–) *5 (–)*
Mean day of wave emergence	0.2 ± 0.3 (0, 1) *0.8 ± 0.4 (2, 2)*	4.7 ± 0.4 (7, 7) *5.6 ± 0.2 (7, 8)*	8.2 ± 0.4 (11, 12) *9.0 ± 0.4 (11, 11)*	11.8 ± 0.4 (–, –) *13.0 ± 0.4 (–, –)*
Number of follicles per wave	1.6 ± 0.3 (1, 3) *2.0 ± 0.3 (2, 3)*	1.6 ± 0.4 (1, 1) *1.2 ± 0.2 (1, 2)*	1.2 ± 0.2 (1, 2) *1.4 ± 0.2 (2, 4)*	2.4 ± 0.5 (–, –) *1.4 ± 0.3 (–, –)*
Largest follicle Maximum diameter (mm)	6.2 ± 0.3 (5, 7) *5.4 ± 0.2 (6, 6)*	6.0 ± 0.3 (5, 6) *5.2 ± 0.2 (5, 5)*	6.0 ± 0.3 (6, 7) *5.4 ± 0.2 (5, 6)*	6.7 ± 0.2[A] (–, –) *5.6 ± 0.2[B] (–, –)*
Growth rate (mm day^{-1})	1.2 ± 0.08 (0.8, 1) *1.0 ± 0.2 (1, 1)*	1.1 ± 0.1 (1, 1) *1.1 ± 0.2 (0.5, 1)*	1.1 ± 0.09 (0.75, 0.8) *1.1 ± 0.2 (0.7, 1)*	1.0 ± 0.08 (–, –) *1.2 ± 0.2 (–, –)*
Growing phase (days)	3.3 ± 0.4[a] (2, 5) *2.6 ± 0.4 (3, 3)*	3.0 ± 0.4[a] (2, 3) *2.2 ± 0.4 (2, 4)*	3.0 ± 0.5[a] (4, 5) *3.0 ± 0.5 (3, 3)*	3.9 ± 0.1[Ab] (–, –) *2.4 ± 0.2[B] (–, –)*
Static phase (days)	2.9 ± 0.7[a] (2, 5) *3.8 ± 0.4[a] (1, 1)*	1.4 ± 0.3[b] (1, 2) *1.4 ± 0.4[b] (2, 3)*	1.6 ± 0.3[b] (2, 2) *1.8 ± 0.6[b] (2, 2)*	1.4 ± 0.2[b] (–, –) *1.4 ± 0.2[b] (–, –)*
Regressing phase (days)	4.9 ± 0.8[Aa] (3, 4) *2.2 ± 0.4[B] (3, 4)*	4.1 ± 0.8[ab] (2, 3) *2.4 ± 0.6 (–, 4)*	2.7 ± 0.6[b] (–, –) *1.7 ± 0.3 (–, –)*	
Lifespan (days)	11.0 ± 0.7[Aa] (7, 13) *8.6 ± 0.4[Ba] (6, 7)*	8.2 ± 1.0[b] (4, 7) *6.0 ± 0.6[b] (6, 6)*	7.0 ± 0.6[b] (5, 6) *5.8 ± 0.5[b] (4, 4)*	5.3 ± 0.2[Ac] (–, –) *3.8 ± 0.4[Bc] (–, –)*
Interwave interval* (days)	4.5 ± 0.2[a] (7, 7) *4.2 ± 0.2 (5, 6)*	3.5 ± 0.3[b] (4, 5) *3.8 ± 0.4 (4, 4)*	3.5 ± 0.3[b] (–, –) *4.0 ± 0.3 (–, –)*	

For comparison, data for two ewes of each breed that only had three waves of follicle growth per cycle are given in parentheses.
Day 0: day of ovulation.
Means with different superscripts are significantly different ($P < 0.05$): [abc] within rows; [AB] within cells.
*Number of days between emergence of the wave in that column and emergence of the next sequential wave.

(9.0 ± 0.4) after ovulation when only ewes with four waves per cycle were considered, for white-faced and Finn ewes, respectively.

Growth characteristics of ovulatory and non-ovulatory antral follicles

The comparisons between ovulatory and non-ovulatory antral follicles originating from the last follicular wave of the cycle in white-faced ewes and from the penultimate wave in Finn sheep are shown (Table 2). In white-faced ewes, there were no differences in terms of the mean day of emergence or the duration of follicle static phases between ovulatory and non-ovulatory follicles that emerged in the final wave of the interovulatory interval. However, ovulatory follicles were seen to grow over a longer ($P < 0.05$) period of time and reached considerably greater diameters ($P < 0.05$) compared with non-ovulatory follicles in this wave. The mean growth rate was higher ($P < 0.05$) for non-ovulatory than for ovulatory ovarian follicles in the last wave in white-faced ewes. Non-ovulatory follicles that began to grow in the penultimate wave in Finn sheep emerged earlier in the cycle ($P < 0.05$), grew for a shorter duration ($P < 0.05$) to smaller maximum diameters ($P < 0.05$), and had significantly shorter static phases compared with follicles that emerged in the same wave and ovulated subsequently.

Serum concentrations of LH

When data from all ewes with three or four waves of follicular growth per cycle were included, there was a significant main effect of day ($P < 0.01$), but not of breed or day-by-breed interaction for mean serum concentrations of LH per day normalized to the day of first ovulation. In both breeds of sheep, serum concentrations of LH increased before ovulation ($P < 0.05$). Peak values of daily serum concentrations of LH during the luteal phase of the oestrous cycle were only detected in five ewes (three Western white-faced and two Finn ewes), and so no further analysis was done.

Serum concentrations of FSH

When daily serum FSH concentrations were normalized to the day of first ovulation (day 0) for ewes with four follicle waves per cycle, there was a significant main effect of day ($P < 0.01$), breed ($P < 0.01$), and breed-by-day interaction ($P <$

Table 2. Comparisons of the characteristics of ovulatory and non-ovulatory antral follicles (≥ 5 mm in diameter; means ± SEM) originating from the last follicular wave of the oestrous cycle in ten Western white-faced ewes (regular font) and from the penultimate wave in seven Finn ewes (italics)

	Western white-faced ewes last follicular wave		*Finn ewes penultimate follicular wave*	
End point	Ovulatory follicles (n = 17)	Non-ovulatory follicles (n = 5)	*Ovulatory follicles* (n = 6)	*Non-ovulatory follicles* (n = 6)
Mean day of emergence	11.8 ± 0.3	11.6 ± 0.5	*10.0 ± 0.3*^{a}	*8.2 ± 0.5*^{b}
Maximum diameter (mm)	6.2 ± 0.2^{a}	5.0 ± 0.0^{b}	*5.5 ± 0.2*^{a}	*5.0 ± 0.0*^{b}
Growth rate (mm day^{-1})	0.9 ± 0.05^{a}	1.5 ± 0.3^{b}	*0.8 ± 0.08*	*1.1 ± 0.2*
Growing phase (days)	3.6 ± 0.2^{a}	1.6 ± 0.4^{b}	*3.5 ± 0.4*^{a}	*2.0 ± 0.3*^{b}
Static phase (days)	1.4 ± 0.1	1.2 ± 0.2	*3.5 ± 0.8*^{a}	*1.2 ± 0.2*^{b}

Data were combined for ewes with three or four follicular waves. Day 0: day of the first ovulation of the interovulatory interval.
^{ab}Means with different letter superscripts, within each breed, are significantly different (P < 0.05).

0.01; Fig. 4a). Mean daily concentrations of FSH were higher (P < 0.05) in Finn than in white-faced ewes around the day of ovulation, at the beginning and end of the oestrous cycle (days −1, 0 and 16), and on day 7 after ovulation. Distinct increases in mean circulating concentrations of FSH could be seen on days 0, 5 and 16 in Finn and on days 0, 5 and 8 of the oestrous cycle in Western white-faced ewes.

The characteristics of the peak values in serum FSH concentrations in blood samples taken each day during the oestrous cycle in Western white-faced and Finn ewes, as determined by the cycle-detection computer program (Clifton and Steiner, 1983), are given (Table 3). The peak values of the transient increases in daily serum concentrations of FSH were detected in all ewes studied. The mean numbers of identified peak values in FSH per ewe were 3.7 ± 0.2 and 3.6 ± 0.2 for Western white-faced and Finn ewes, respectively (P > 0.05). Further statistical analyses were limited to ewes with four waves of follicular growth per cycle. The amplitude of FSH peak values occurring early in the cycle was higher in Finn than in Western white-faced ewes (P < 0.05). The duration of the fluctuations in daily serum FSH concentrations did not differ significantly throughout the cycle or between the two breeds of sheep studied (P > 0.05). The mean duration of interpeak intervals for daily serum FSH concentrations did not differ significantly between breeds, but the mean interpeak interval between the first two FSH peak values of the cycle was longer (P < 0.05) than the subsequent interpeak intervals in Western white-faced ewes.

Serum concentrations of oestradiol

There were significant main effects of day and breed (P < 0.01) for daily serum concentrations of oestradiol normalized to the day of first ovulation, but the interaction was not significant. In Western white-faced ewes, the mean concentrations of oestradiol increased significantly from day 0 to day 2 (Fig. 4b). During the luteal phase, oestradiol concentrations were variable and there was a sharp decrease (P < 0.05) from day 16 to day 17, at the time of the second ovulation of the interovulatory period. In Finn ewes, the

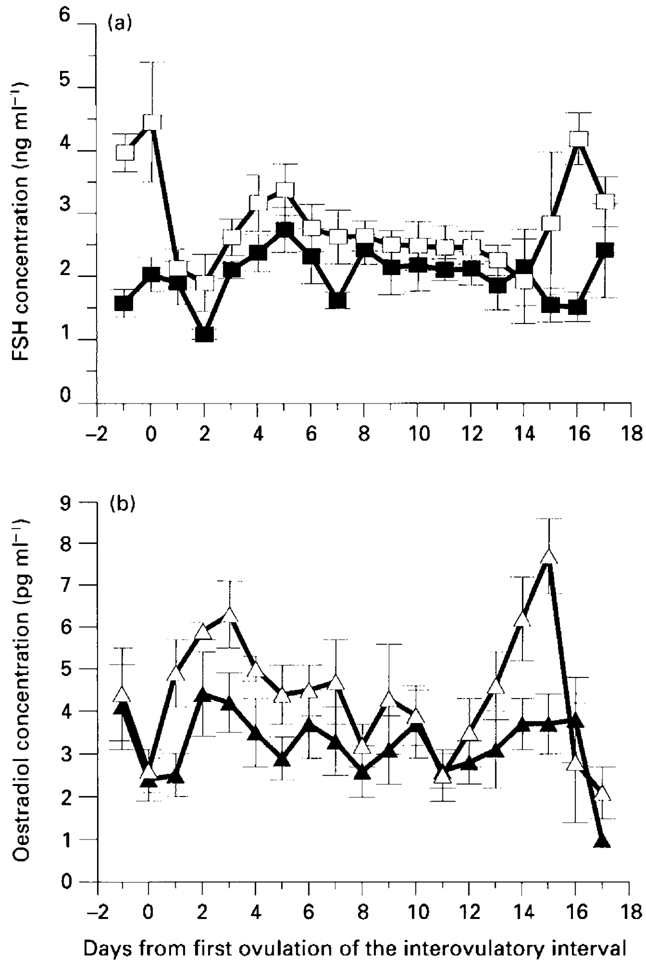

Fig. 4. Mean (± SEM) serum concentrations of (a) FSH and (b) oestradiol from blood samples collected during the oestrous cycle of Western white-faced (n = 8; ■, ▲) and Finn (n = 5; □, △) ewes with four follicular waves per oestrous cycle. Concentrations of the hormones were normalized to the day of first ovulation of the interovulatory interval studied (day 0).

Table 3. Characteristics of sequential (1–4) fluctuations in serum oestradiol and FSH concentrations in blood samples collected each day throughout an oestrous cycle in Western white-faced ($n = 10$, regular font) and Finn sheep ($n = 7$, italics) that underwent transrectal ovarian ultrasonography during the middle portion of the breeding season (October–December) and had four waves of follicle growth per cycle

	FSH				Oestradiol			
End point	1	2	3	4	1	2	3	4
Peak concentration	2.32 ± 0.37[A]	3.13 ± 0.27	2.83 ± 0.33	3.00 ± 0.53	4.9 ± 0.8[A]	4.1 ± 0.7[A]	4.6 ± 0.6	4.2 ± 1.0[A]
(FSH: ng ml^{-1};	(3.69, 4.39)	(1.71, 3.11)	(3.59, 5.86)	(4.60, 7.47)	(3.7, 4.4)	(1.7, 3.1)	(3.6, 5.9)	(–, –)
oestradiol: pg ml^{-1})	*4.43 ± 0.11*[B]	*3.81 ± 0.36*	*3.00 ± 0.28*	*2.91 ± 0.30*	*7.2 ± 0.6*[B]	*6.2 ± 1.0*[B]	*5.6 ± 1.1*	*8.0 ± 0.9*[B]
	(3.38, 3.93)	*(2.74, 3.36)*	*(2.70, 2.80)*	*(–, –)*	*(8.4, 8.7)*	*(6.3, 7.2)*	*(7.6, 14.8)*	*(–, –)*
Duration of	ND	3.7 ± 0.4	3.2 ± 0.4	4.0 ± 0.5	4.0 ± 0.3	3.0 ± 0.3	3.5 ± 0.2	4.0 ± 0.5
fluctuation (days)		(5, 6)	(5, 7)	(ND, ND)	(4, 5)	(3, 3)	(5, 6)	(–, –)
	ND	*5.0 ± 0.3*	*4.0 ± 0.4*	*3.8 ± 0.7*	*4.8 ± 0.3*	*3.4 ± 0.1*	*3.3 ± 0.9*	*4.7 ± 0.3*
		(6, 7)	*(5, 5)*	*(–, –)*	*(4, 6)*	*(4, 4)*	*(2, 5)*	*(–, –)*
Interpeak interval*	4.7 ± 0.2[a]	3.6 ± 0.3[b]	3.7 ± 0.4[b]		4.1 ± 0.3	3.9 ± 0.6	4.5 ± 0.3	
(days)	(6, 8)	(3, 4)	(6, 6)		(3, 4)	(5, 6)	(–, –)	
	4.6 ± 0.4	*3.8 ± 0.4*	*4.0 ± 0.3*		*4.6 ± 0.4*	*4.0 ± 0.5*	*4.5 ± 0.3*	
	(6, 6)	*(4, 5)*	*(–, –)*		*(5, 8)*	*(2, 5)*	*(–, –)*	

Data for two ewes in each breed that had three follicular waves per cycle are given in parentheses. All mean values are mean ± SEM.
Means with different letter superscripts, for each hormone, are significantly different ($P < 0.05$): [AB] within cells; [ab] within rows.
*Number of days between the peak in the indicated column and the next sequential peak.
ND: the duration of fluctuations was not determined as complete fluctuations (nadir-to-nadir) were not seen at the beginning and end of the scanning period.

mean concentrations of oestradiol increased from day 0 to day 3 after ovulation ($P < 0.05$), decreased from day 3 to day 8 ($P < 0.05$), increased again from day 11 to day 15 ($P < 0.05$) and then decreased to its lowest values on days 16–17 of the oestrous cycle ($P < 0.05$), around the time of the second observed ovulation of the interovulatory interval. On days 1–5, days 14–15 and day 17 of the cycle, the mean serum concentrations of oestradiol were significantly higher in Finn than in white-faced ewes ($P < 0.05$).

Peak values in serum oestradiol concentrations were identified in all ewes studied using the cycle-detection program. The mean number of peak values per ewe per oestrous cycle was 3.5 ± 0.2 and 3.4 ± 0.2 for white-faced and Finn ewes, respectively ($P > 0.05$; Table 3). For ewes that had four follicular waves per cycle, the mean peak concentrations of transient increases in serum oestradiol concentrations did not differ throughout the cycle in either breed studied, but were higher in Finn than in white-faced ewes ($P < 0.05$) for peak values 1, 2 and 4 of the cycle. The average duration of fluctuation and interpeak interval for daily serum concentrations of oestradiol did not differ between the two breeds, or throughout the oestrous cycle within each breed, studied.

Relationship between follicle wave development and serum concentrations of FSH

Ewes with three or four waves of follicular emergence per cycle were included in this analysis. The number of emerging follicular waves and the number of identified FSH peak values per ewe did not differ in both breeds of sheep studied (3.7 ± 0.2 versus 3.8 ± 0.1, and 3.6 ± 0.2 versus 3.7 ± 0.2; FSH peaks versus follicular waves, for Western white-faced and Finn ewes, respectively). The duration of the interval between adjacent days of wave emergence (interwave intervals) was positively correlated with the duration of the interpeak interval for FSH fluctuations ($r = 0.76$, $P < 0.01$ and $r = 0.84$, $P < 0.01$, for white-faced and Finn sheep, respectively). The duration of the interpeak interval for FSH concentrations was also correlated with the interval between the emergence ($r = 0.72$, $P < 0.01$), beginning ($r = 0.64$, $P = 0.01$) and end ($r = 0.63$, $P = 0.01$) of the static phase, but not with the end of regression to a 3 mm follicle ($r = 0.45$, $P > 0.05$), for the largest follicles of waves in Finn ewes. However, in white-faced ewes, the duration of the interpeak interval for FSH was only correlated with the interval between the emergence of the largest follicles of waves ($r = 0.73$, $P < 0.01$), but not with the interval between the beginning ($r = 0.24$, $P > 0.05$) and end ($r = -0.1$, $P > 0.05$) of the follicle static phase, or the end of regression ($r = -0.1$, $P > 0.05$). All identified FSH peak values occurred from day −1 to day 2 relative to the nearest day of wave emergence (day 0) in Western white-faced ewes, and from day −2 to day 1 in Finn ewes (Fig. 5a). The highest proportion of FSH peak values occurred on day 0 in white-faced (64.9%, $P < 0.05$) and on day −1 in Finn sheep (43.5%). In Finn sheep, the combined percentage of peaks that occurred on days −1 and 0 (73.9 %) was significantly higher than for the 3 remaining days, before and after the day of wave emergence.

Relationship between follicle wave development and oestradiol secretion

The data for ewes with three or four waves of follicular emergence per cycle were used in this analysis. The number

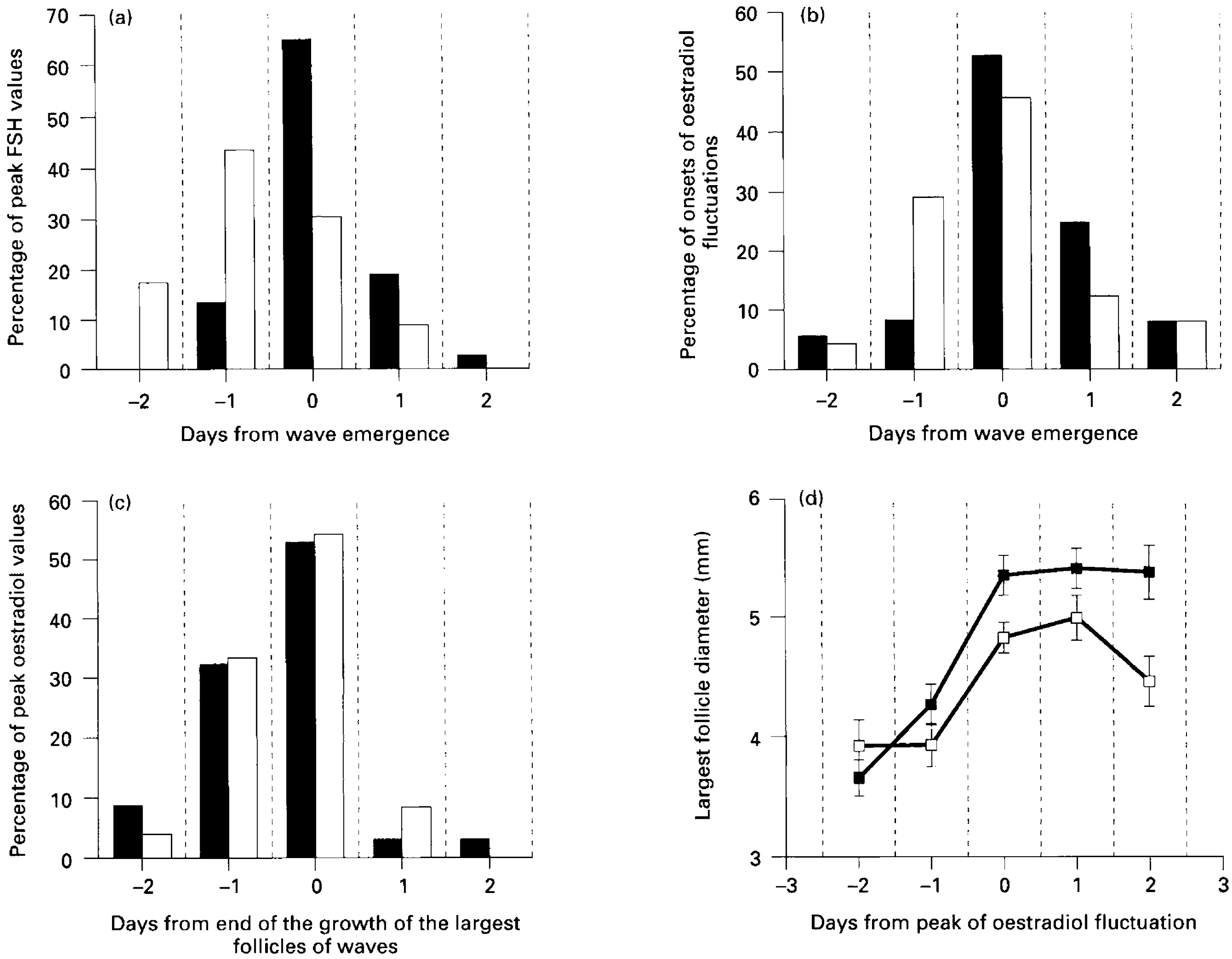

Fig. 5. (a) Relative frequency of the occurrence of peak FSH values ($n = 37$ and $n = 23$, for Western white-faced and Finn ewes, respectively). (b) Distribution of days of the beginning of oestradiol fluctuations ($n = 36$ and $n = 24$, for white-faced and Finn ewes, respectively) within 2 days before and after the day of follicular wave emergence. (c) The percentage of peaks in serum oestradiol concentrations ($n = 34$ and $n = 24$, for white-faced and Finn ewes, respectively), expressed for the 2 days before and after the end of the growing phase of the largest follicle of a wave. (d) Changes in the diameter of the largest follicles of waves on the day of oestradiol peak ± 2 days. The graphs were compiled from follicular and endocrine data obtained in Western white-faced (■; $n = 10$) and Finn ewes (□; $n = 7$) that underwent ovarian ultrasonography each day during one oestrous cycle in the mid-breeding season (October–December), and had three or four waves of follicular development per cycle.

of identified peaks of oestradiol concentration as well as the number of days on which oestradiol fluctuations began did not differ from the number of emerging follicular waves in either breed of sheep studied (3.5 ± 0.2 versus 3.8 ± 0.1 and 3.4 ± 0.2 versus 3.7 ± 0.2; oestradiol fluctuations versus follicular waves for Western white-faced and Finn ewes, respectively). The duration of the interval between the days on which oestradiol fluctuations began was positively and significantly correlated with the interwave interval in both Western white-faced and Finn ewes ($r = 0.42$, $P < 0.05$ and $r = 0.53$, $P < 0.05$, for white-faced and Finn ewes, respectively). When the correlations above were done for days of emergence of the largest follicles of sequential waves, the correlation coefficients were $r = 0.42$, $P < 0.05$ and $r = 0.73$, $P < 0.01$, for white-faced and Finn ewes, respectively. In both breeds, the interval between the onset of oestradiol fluctuations was also significantly correlated with the interval between the ends of the follicle growing phases ($r = 0.38$, $P < 0.05$ and $r = 0.83$, $P < 0.01$, for white-faced and Finn ewes, respectively), but not with the interval between the end of the static phase and the day of regression to 3 mm in diameter ($r = -0.02$, $P > 0.05$ and $r = 0.26$, $P > 0.05$, and $r = 0.21$, $P > 0.05$ and $r = 0.09$, $P > 0.05$, for the end of the static phase and the day of regression, for white-faced or Finn ewes, respectively). The proportion of oestradiol fluctuations that began on the day of the emergence of sequential waves was 52.8% ($P < 0.05$) in Western white-faced and 45.8% ($P < 0.05$) in Finn ewes (Fig. 5b).

In both Western white-faced and Finn ewes, the interval between adjacent peaks of oestradiol increases was strongly and positively correlated with the interwave interval ($r = 0.89$, $P < 0.01$ and $r = 0.67$, $P < 0.01$, respectively), the interval between the days of emergence of the largest follicles (r = 0.93, $P < 0.01$ and $r = 0.79$, $P < 0.01$, respectively), and the interval between the ends of the follicle growing phases (r = 0.49, $P < 0.01$ and $r = 0.83$, $P < 0.01$, respectively). In addition, there was a significant correlation between the interval between the ends of static phases and the interpeak interval for oestradiol concentration in Finn ($r = 0.66$, $P < 0.01$), but not in white-faced ewes ($r = -0.05$, $P > 0.05$), and there was no correlation between the interpeak interval and the day of the largest follicle regression to 3 mm ($r = -0.03$, $P > 0.05$ and $r = -0.12$, $P > 0.05$, for Western white-faced and Finn ewes, respectively). An analysis of the frequency and distribution of the oestradiol peaks within 2 days before and after the end of follicle growing phases revealed that most peak values (85.2%, $P < 0.05$ and 87.5%, $P < 0.05$, for Western white-faced and Finn sheep, respectively) occurred 1 day before and on the day that the largest follicles of waves reached their maximum size (Fig. 5c,d).

Discussion

Previous studies on ovarian follicle dynamics done at slaughter led to contrasting conclusions that follicular growth was either continuous (Hutchinson and Robertson, 1966) or that there were two (Brand and de Jong, 1973) or three (Smeaton and Robertson, 1971) phases of large antral follicle development during the oestrous cycle in ewes. On the basis of studies using ink marking of follicles, waves of follicles were suggested (Driancourt, 1991). Ultrasound scanning studies revealed that the growth of ovarian follicles from a pool of 2 mm follicles could be seen on most days of the interovulatory interval in ewes, with peaks occurring on days 2 and 11 of the cycle (Ravindra *et al.*, 1993). Using laparoscopy, Noel *et al.* (1993) reported three waves of follicles during the cycle but other studies failed to show a wave-like pattern (Schrick *et al.*, 1993; Landau *et al.*, 1996). Ginther *et al.* (1995) used ultrasound imaging of ovaries in cyclic Polypay ewes and noted that follicles only reaching 3 or 4 mm in diameter did not exhibit an organized pattern of growth and demise, but that 3 mm follicles began to grow to ≥ 5 mm approximately every 5 days during the interovulatory interval. The present data obtained in two breeds of sheep with different ovulation rates, strongly support the conclusion that the development of ovarian follicles reaching an ovulatory size occurs in an orderly succession during the oestrous cycle in ewes and that this phenomenon is apparently not influenced by differences in prolificacy.

Ginther *et al.* (1995) reported that the 8% of interovulatory intervals had three waves, 58% had four waves and 34% had five or six waves. Three waves of follicular development were recorded mainly during short interovulatory intervals (9–14 days) that occurred towards the end of the breeding season, and five or six waves occurred during prolonged interovulatory intervals (22–24 days). As the duration of the ovine oestrous cycle in the breeding season is remarkably consistent (17 ± 1 day; Goodman, 1994) and there is little effect of breed and age (Hafez, 1952), the abnormally long interovulatory intervals (23 days each) seen in two Western white-faced ewes in the present study (Fig. 1d) probably reflect aberrant hormonal control of the oestrous cycle. In the remaining ewes of both breeds, the majority of interovulatory intervals (76.5%; 13/17) were associated with the emergence of four follicular waves. On the basis of this and previous observations in Polypay ewes (Ginther *et al.*, 1995), it is concluded that, during the normal oestrous cycle in the ewe, if follicular waves are identified, there are typically four waves of large antral follicle growth. However, on the basis of the results of Ravindra *et al.* (1993) and Noel *et al.* (1993), three waves may be the consistent pattern in some ewes.

In cyclic Polypay ewes, sequential follicular waves emerged, on average, on days 0, 5, 9 and 14 of the oestrous cycle (day 0 = day of ovulation; Ginther *et al.*, 1995). In the present study, the mean days of wave emergence (follicles growing from 3 to ≥ 5 mm in diameter) were days 0, 5, 9 and 12 for Western white-faced ewes or days 1, 6, 10 and 13 for Finn sheep. This breed difference is difficult to explain but, in Finn ewes, follicles achieve smaller maximum sizes, so all phases of follicular development, including follicle recruitment, may occur with smaller follicles than they do in white-faced ewes. If recruitment into the waves occurred on set days of the cycle recruited follicles would be at a smaller follicle diameter in Finn ewes on each day of recruitment. This would mean that follicles in Finn ewes would reach the arbitrary follicle size (3 mm) at which emergence was defined in the present study later than they would in Western white-faced ewes. Previous studies of follicular dynamics in prolific and non-prolific genotypes of ewes indicated that follicles attained maturity at a smaller diameter in prolific animals (Scaramuzzi and Radford, 1983; Webb and Gauld, 1985; Driancourt *et al.*, 1986; Souza *et al.*, 1997). In addition, ovulatory follicles in ewes can be recruited from a wide range of sizes (Tsonis *et al.*, 1984). It is likely that there are differences among breeds of sheep with different ovulation rates in terms of the size of follicles that acquire the ability to respond to hormonal stimuli and grow to the peri-ovulatory size during the oestrous cycle.

In a study in cyclic white-faced ewes (Ravindra *et al.*, 1993), the ovulatory follicle that began to grow from 2 mm in diameter at about day 11 of the cycle grew to a significantly larger size and over a longer duration than did any other follicle during the oestrous cycle. In addition, the rate at which the ovulatory follicle grew was significantly higher than that for large antral follicles emerging during the early period of the cycle (around day 2). In the present study, ovulatory follicles (wave 4) only reached greater maximum diameters when compared with the largest non-ovulatory follicles within the same wave, but not when compared with the largest non-ovulatory follicles of other waves.

In the present study, in Western white-faced ewes, the duration of follicular lifespan was greater for follicles that emerged during the early period of the oestrous cycle than for those that emerged during subsequent follicular waves, including waves containing non-ovulatory follicles that emerged during the luteal phase. A similar trend was observed in the prolific Finn sheep. The longer lifespan of the first follicular wave of the cycle was due primarily to the longer static phase. The longer follicular lifespan in Western white-faced compared with Finn sheep may be due, in part, to the larger maximum diameter attained by the ovarian follicles in the white-faced ewes. Growth rates did not differ between the two breeds, but the duration of the growing and regression phases tended to be longer in Western white-faced ewes. However, the reason for the marked decrease in the duration of the static phase between the first and the next two follicular waves of the oestrous cycle in ewes is not clear. The extended static phase of follicles growing early in the cycle may reflect the presence of developing or non-fully functional luteal structures; follicles growing in the mid-luteal phase may have a shorter static phase because of decreased gonadotrophic support under progesterone dominance. The effects of progesterone dominance on gonadotrophic support were also reflected in the shorter interwave intervals seen during the luteal phase in both breeds of ewe. Johnson *et al.* (1996) reported that creation of lower than normal serum concentrations of progesterone (≤ 1 ng ml^{-1}) in ewes resulted in larger follicles than normal. However, prolific Finn ewes had smaller shorter-lived follicles compared with Western white-faced ewes, even though their serum progesterone concentrations were lower (PM Bartlewski, AP Beard and NC Rawlings, unpublished).

The proportion of ovulatory antral follicles that began growing in the penultimate wave of follicular emergence during the oestrous cycle in Finn sheep was 50%. All these follicles were maintained and ovulated along with follicles that emerged after the onset of luteolysis (around day 13 of the cycle). Clearly, large antral follicles are maintained in prolific Finn ewes towards the end of the cycle and, added to the ovulatory follicles emerging just before ovulation, give a higher ovulation rate compared with white-faced ewes. The potential effect of follicular rescue from regression by pregnant mares' serum gonadotrophin has been demonstrated *in vitro* in sheep (Hay and Moor, 1977) and cattle (Monniaux *et al.*, 1984). In Finn sheep, the ovulatory follicles originating from the penultimate wave appeared to emerge approximately 48 h later than non-ovulatory follicles in the same wave. Therefore, it is likely that regression of these follicles in Finn ewes was prevented by the next increase in serum concentrations of FSH. Ovulation of ovarian follicles that began to grow to ≥ 5 mm in diameter before day 10 of the cycle in Western white-faced ewes was sporadic (one out of ten animals) and a proportion (22.7%) of all antral follicles that emerged in the last follicular wave in white-faced ewes became atretic and did not ovulate, compared with 100% ovulation of follicles in this wave in Finn ewes. This finding may be attributed to lower circulating concentrations of FSH just before ovulation in Western white-faced than in Finn ewes. However, there is no agreement as to whether increased ovulation rate in sheep is caused by increased secretion of FSH (Scaramuzzi *et al.*, 1993; Campbell *et al.*, 1995; Fry and Driancourt, 1996; Avdi *et al.*, 1997; Souza *et al.*, 1997). Differences in LH pulsatility during the follicular phase may also be important (Scaramuzzi *et al.*, 1993) but this was not assessed in the present study. In cattle (Pierson and Ginther, 1988; Ginther *et al.*, 1989), goats (Ginther and Kot, 1994) and sheep (Ravindra *et al.*, 1993; Ginther *et al.*, 1995), the follicles that ovulated at the end of a normal cycle originated from the last wave of the cycle but, in Finn ewes, ovulatory follicles can originate not only from the last follicular wave of the oestrous cycle but also from the previous wave. Landau *et al.* (1996) concluded that, with increased ovulation rate, the time width for recruitment of ovulatory follicles increases. This mechanism for increasing ovulation rate was suggested by Scaramuzzi *et al.* (1993).

Analysis of mean daily concentrations of FSH and oestradiol for groups of ewes did not permit the detection of distinct fluctuations in serum concentrations of hormones, as the timing, duration and amplitude of these increases varied among ewes, particularly during the luteal phase. The occurrence of peak values of FSH (Ginther *et al.*, 1995) and oestradiol concentrations was only revealed using a statistical technique based on the assessment of daily concentrations of the hormones in individual animals (Clifton and Steiner, 1983).

The data from the present study confirm the existence of a close temporal relationship between transient increases in circulating concentrations of FSH and follicular growth to peri-ovulatory sizes (follicles growing from 3 to ≥ 5 mm in diameter) throughout the oestrous cycle in Western white-faced and Finn ewes, and indicate that differences in prolificacy do not influence this pattern of follicle emergence. In Western white-faced ewes, the periodicity of fluctuations in daily FSH concentrations was correlated with the emergence of the largest follicle, but later stages of the lifespan of individual follicles were not associated with the occurrence of peak FSH concentrations. In Finn ewes, there was a significant correlation between increases in daily FSH concentration and days of emergence, days on which the static phases commenced, and days on which the static phases of the largest follicle ended. In both breeds studied, there was a tight clustering of days on which FSH peak values were detected around the days of follicle wave emergence. On the basis of these results, it is suggested that rhythmically generated increases in serum FSH concentrations may induce the growth of large follicles or maintain follicles during the static phase, but that follicular demise is independent of the periodicity of FSH secretion. Moreover, as LH secretion is considerably suppressed during the luteal phase of the oestrous cycle (Karsh *et al.*, 1980), the present data confirm that FSH is the primary gonadotrophin that stimulates ovarian antral follicle development up to peri-ovulatory size in cyclic ewes (McNeilly *et al.*, 1991).

At any stage of the ovine oestrous cycle, the largest non-atretic ovarian follicles are the main source of oestradiol (Bjersing *et al.*, 1972). A peak value of oestradiol has been shown to occur on the day preceding ovulation (Scaramuzzi *et al.*, 1970; Cox *et al.*, 1971), but also during mid-cycle, on days 3–4 (Cox *et al.*, 1971) and days 6–9 (Scaramuzzi *et al.*, 1970; Mattner and Braden, 1972), suggesting that there are periods of increased oestradiol secretion throughout the oestrous cycle in ewes (Campbell *et al.*, 1995). Considerable variability among ewes in the production of oestradiol during the luteal phase has been noted by Baird (1978). In the present study, using the cycle-detection program, peaks in daily serum concentration of oestradiol were identified that appeared to coincide with the culmination of the growth of the largest ovarian follicles, in all ewes. This finding confirms the observations of Souza *et al.* (1997). There was a temporal relationship between the beginning of consecutive oestradiol fluctuations and the day of follicular wave emergence (follicles growing from 3 to ≥ 5 mm in diameter). These results imply that the largest antral follicles (growing from 3 to ≥ 5 mm in diameter) are capable of producing significant amounts of oestradiol at different stages of the sheep oestrous cycle. Therefore, the peri-ovulatory pattern of gonadotrophin release is not critical for the establishment of oestradiol production by the large antral follicles in cyclic ewes. In addition, the net production of oestradiol was significantly higher for most of the cycle in the prolific Finn ewes than it was in the non-prolific white-faced ewes, despite a lack of significant differences in serum concentrations of gonadotrophic hormones, except for FSH, during the peri-ovulatory stage of the cycle. However, more frequent sampling may reveal differences. On the basis of the results of previous studies (Driancourt, 1991), it was concluded that increased ovulation rate resulted in decreased oestradiol production from follicles due to decreased granulosa cell numbers. However, Driancourt *et al.* (1996) found that oestradiol production from granulosa cells was higher in Finn ewes selected for ovulation rate than in Finn ewes with lower ovulation rates. Serum concentrations of oestradiol in some Western white-faced ewes, but not in Finn sheep, were lower than the assay sensitivity on many days (1 pg ml^{-1}, Fig. 1b) and, in some of these ewes, luteinization of large follicles was detected by ultrasonography (PM Bartlewski, AP Beard and NC Rawlings, unpublished). On the basis of this observation, it is suggested that ovarian follicles in ewes of different genotypes differ in their physiological properties and responsiveness to FSH and LH. The nature of these divergent patterns of follicular secretion and morphology remains to be elucidated.

In summary, the growth of ovine antral follicles reaching ovulatory sizes exhibits a distinct wave-like pattern throughout the oestrous cycle in both prolific and non-prolific genotypes of sheep. Ovarian follicular emergence from the pool of 3 mm follicles and the initial stages of the growth of the largest follicles appear to be controlled primarily by increases in FSH secretion. The largest ovarian follicles acquire the ability to produce large amounts of oestradiol from the day of emergence (beginning of growth from 3 to ≥ 5 m in diameter) and a peak value of oestradiol release occurs about the time they reach their maximum diameter. The higher ovulation rate in the prolific Finn ewes appears to be due to the maintenance of follicles emerging in the penultimate follicular wave and their addition to ovulatory follicles emerging in the last wave of the interovulatory interval. The dissimilar patterns of ovulatory follicular recruitment in prolific Finn and non-prolific Western white-faced sheep may be caused by differences in circulating concentrations of FSH, but may also reflect different intrinsic properties of ovarian antral follicles.

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References

Avdi M, Chemineau P and Driancourt MA (1997) Alterations in follicular maturation associated with within-breed variation in ovulation rate in Chios sheep *Animal Reproduction Science* **46** 223–235

Baird DT (1978) Pulsatile secretion of LH and ovarian oestradiol during the follicular phase of the sheep estrous cycle *Biology of Reproduction* **18** 359–364

Baird DT, Land RB, Scaramuzzi RJ and Wheeler AG (1976) Gonadotrophic control of follicular development and function during the oestrous cycle of the ewe *Journal of Endocrinology* **69** 275–286

Bjersing L, Hay MF, Kann G, Moor RM, Naftolin F, Scaramuzzi RJ, Short RV and Younglai EV (1972) Changes in gonadotrophins, ovarian steroids and follicular morphology in sheep at oestrus *Journal of Endocrinology* **52** 465–479

Brand A and de Jong WHR (1973) Qualititative and quantitative micromorphological investigations of the tertiary follicle population during the oestrous cycle in sheep *Journal of Reproduction and Fertility* **33** 431–439

Campbell BK, McNeilly AS, Mann GE and Baird DT (1991) The effect of stage of oestrous cycle and follicular maturation on ovarian inhibin production in sheep *Biology of Reproduction* **44** 483–490

Campbell BK, Scaramuzzi RJ and Webb R (1995) Control of antral follicle development and selection in sheep and cattle *Journal of Reproduction and Fertility Supplement* **49** 335–350

Clifton DK and Steiner RA (1983) Cycle detection: a technique for estimating the frequency and amplitude of episodic fluctuations in blood hormone and substrate concentrations *Endocrinology* **112** 1057–1064

Cox RI, Mattner PE and Thorburn GD (1971) Changes in ovarian secretion of oestradiol-17β around oestrus in sheep *Journal of Endocrinology* **49** 345–346

Cummins LJ, O'Shea T, Bindon BM, Lee VWK and Findlay JK (1983) Ovarian inhibin content and sensitivity to inhibin in Booroola and control strain Merino ewes *Journal of Reproduction and Fertility* **67** 1–7

Currie WD and Rawlings NC (1989) Prolonged infusion of morphine and naloxone in the ewe: fluctuation in responsiveness of LH and lack of responsiveness of FSH *Journal of Reproduction and Fertility* **86** 359–366

Driancourt MA (1991) Follicular dynamics in sheep and cattle *Theriogenology* **35** 55–77

Driancourt MA, Gauld IK, Terqui M and Webb R (1986) Variations in patterns of follicle development in prolific breeds of sheep *Journal of Reproduction and Fertility* **78** 565–575

Driancourt MA, Webb R and Fry RC (1991) Does follicular dominance occur in ewes? *Journal of Reproduction and Fertility* **93** 63–67

Driancourt MA, Hermier D and Hanrahan JP (1996) Alterations in follicular function associated with selection on ovulation rate in Finn ewes *Journal of Animal Science* **74** 199–210

Fry RC and Driancourt MA (1996) Relationship between follicle-stimulating hormone, follicle growth and ovulation rate in sheep *Reproduction, Fertility and Development* **8** 279–286

Ginther OJ and Kot K (1994) Follicular dynamics during the ovulatory season in goats *Theriogenology* **42** 987–1001

Ginther OJ, Kastelic JP and Knopf L (1989) Composition and characteristics of follicular waves during the bovine estrous cycle *Animal Reproduction Science* **20** 187–198

Ginther OJ, Kot K and Wiltbank MC (1995) Associations between emergence of follicular waves and fluctuations in FSH concentrations during the oestrus cycle in ewes *Theriogenology* **43** 689–703

Goodman RL (1994) Neuroendocrine control of the ovine estrous cycle. In *The Physiology of Reproduction* pp 660–693 Eds E Knobil and JD Neill. Raven Press, New York

Hafez ESE (1952) Studies on the breeding season and reproduction of the ewe *Journal of Agricultural Science* **42** 189–265

Hay MF and Moor RM (1977) Changes in the Graafian follicle population during the follicular phase of the oestrous cycle. In *Control of Ovulation* pp 177–196 Eds DB Crighton, NB Haynes, GR Foxcroft and GE Lamming. Butterworth, London

Hutchinson JSM and Robertson HA (1966) The growth of the follicle and corpus luteum in the ovary of the sheep *Research in Veterinary Science* **7** 17–24

Johnson SK, Dailey RA, Inskeep EK and Lewis PE (1996) Effect of peripheral concentrations of progesterone on follicular growth and fertility in ewes *Domestic Animal Endocrinology* **13** 69–79

Joseph IBJK, Currie WD and Rawlings NC (1992) Effects of time after ovariectomy, season and oestradiol on luteinizing hormone and follicle-stimulating hormone secretion in ovariectomized ewes *Journal of Reproduction and Fertility* **94** 511–523

Karsh FJ, Legan SJ, Ryan KD and Foster DL (1980) Importance of estradiol and progesterone in regulating LH secretion and estrous behavior during the sheep estrous cycle *Biology of Reproduction* **23** 404–413

Landau S, Houghton JAS, Mawhinney JR and Inskeep EK (1996) Protein sources affect follicular dynamics in ewes near the onset of the breeding season *Reproduction, Fertility and Development* **8** 1021–1028

Lopez-Sebastian A, de Bulnes AG, Moreno JS, Gomez-Brunet A, Townsend EC and Inskeep EK (1997) Patterns of follicular development during the oestrous cycle in monovular Merino del Pais ewes *Animal Reproduction Science* **48** 279–291

McNeilly AS, Picton HM, Cambell BK and Baird DT (1991) Gonadotrophic control of follicle growth in the ewe *Journal of Reproduction and Fertility Supplement* **43** 177–186

Mattner PE and Braden AWH (1972) Secretion of oestradiol-17β by the ovine ovary during the luteal phase of the oestrous cycle in relation to ovulation *Journal of Reproduction and Fertility* **28** 136–137

Monniaux D, Mariana JC and Gibson WR (1984) Action of PMSG on follicular populations in the heifer *Journal of Reproduction and Fertility* **70** 243–253

Noel B, Bister JL and Paquay R (1993) Ovarian follicular dynamics in Suffolk ewes at different periods of the year *Journal of Reproduction and Fertility* **99** 695–700

Pierson RA and Ginther OJ (1988) Follicular populations during the oestrous cycle in heifers III. Time of selection of the ovulatory follicle *Animal Reproduction Science* **16** 81–95

Ravindra JP (1993) *Gonadotrophins and Ovarian Function in Ewes* PhD Thesis, University of Saskatchewan

Ravindra JP, Rawlings NC, Evans ACO and Adams GP (1993) Ultrasonographic study of ovarian follicular dynamics in ewes during the oestrous cycle *Journal of Reproduction and Fertility* **101** 501–509

Rawlings NC, Jeffcoate IA and Howell WE (1987) Response of purebred and crossbred ewes to intensified management *Journal of Animal Science* **65** 651–657

Rawlings NC, Jeffcoate IA, Currie WD and Cook SJ (1988) Control of the surge release of LH and FSH in oestradiol- and progesterone-treated ovariectomized ewes *Canadian Journal of Animal Science* **68** 1089–1096

Scaramuzzi RJ and Radford HM (1983) Factors regulating ovulation rate in ewes *Journal of Reproduction and Fertility* **69** 353–367

Scaramuzzi RJ, Caldwell BV and Moor RM (1970) Radioimmunoassay of LH and oestrogen during the oestrous cycle of the ewe *Biology of Reproduction* **49** 1133–1140

Scaramuzzi RJ, Adams NR, Baird DT *et al.* (1993) A model for follicle selection and the determination of ovulation rate in the ewe *Reproduction, Fertility and Development* **5** 459–478

Schrick FN, Surface RA, Prichard JY, Dailey RA, Townsend EC and Inskeep EK (1993) Ovarian structures during the estrous cycle and early pregnancy in ewes *Biology of Reproduction* **49** 1133–1140

Smeaton TC and Robertson HA (1971) Studies on the growth and atresia of Graafian follicles in the ovary of the sheep *Journal of Reproduction and Fertility* **25** 243–252

Souza CJH, Campbell BK, Webb R and Baird DT (1997) Secretion of inhibin A and follicular dynamics throughout the estrous cycle in the sheep with and without Booroola gene (*Fec-B*) *Endocrinology* **138** 5333–5340

Sutama IK, Edey TN and Fletcher IC (1988) Oestrous cycle dynamics in peri-pubertal and mature Javanese thin-tail sheep *Animal Reproduction Science* **16** 61–70

Szczepanski W, Milewski S, Brzostowski H, Tanski Z and Czerniawska-Zajac S (1994) Detection of oestrus and pregnancy in sheep basing on electrical resistance of vaginal mucus (in Polish with English abstract) *Acta Academiae Agriculturae ac Technicae Olstenensis* **41** 127–134

Tsonis CG, Cahill LP, Carson RS and Findlay JK (1984) Identification at the onset of luteolysis of follicles capable of ovulation in the ewe *Journal of Reproduction and Fertility* **70** 609–614

Webb R and Gauld RB (1977) Folliculogenesis in sheep: control of ovulation rate. In *Genetics of Reproduction* pp 261–274 Eds RB Land and DW Robinson. Butterworth, London