THE UNIVERSITY OF MANITOBA

HORMONE LEVELS IN PREPUBERTAL EWE LAMBS AND IN MATURE EWES THROUGHOUT THE BREEDING SEASON

by

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ABSTRACT

Hormone Levels in Prepubertal Ewe Lambs and in Mature Ewes Throughout the Breeding Season

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Daily serum samples were collected from five Dorset x "Western White Face" ewe lambs beginning at 163±6.5 days of age and continuing through the first estrous cycle following puberty. More frequent samples were taken during the period of estrus. Serum progesterone (P), luteinizing hormone (LH), follicle stimulating hormone (FSH) and total estrogens (E) were determined by radioimmunoassay (RIA). Four of the five ewe lambs reached puberty during the period of investigation. The remaining animal had not yet shown estrus at an age of 281 days, when observations were stopped. Average age and body weight at first estrus and length of the first cycle of the four ewes which attained puberty was 221.2±10.6 days, 48.2±0.7 kg and 17.2±2.1 days, respectively. First ovulation, as indicated by P levels occurred at 199±5.6 days of age. Three animals had a "silent" ovulation before the first detected estrus while the other one exhibited estrus before first ovulation. Mean concentration of P and LH before first ovulation was 0.6±0.02 and 2.1±0.2 ng/ml, respectively, and minor fluctuations in both hormones were noted in all animals. The mean level of P during the first inter-estrous period was 3.5±0.2 ng/ml and concentrations remained above 1 ng/

ml for 10.7±0.3 days. LH peaks, as determined at estrus, varied from 70 to 103 ng/ml and low levels were seen throughout the luteal phase. The mean FSH level was 33.8 ±2.1 ng/ml and peaks, which occurred about the same time as the LH peak, ranged from 100 to 190 ng/ml. No recognizable pattern was observed from the E determinations. The overall mean was 6.6±0.2 pg/ml and ranged from <2 to 32 pg/ml.

In a second experiment, ten ewes of each of the Line M, Suffolk or Finnish Landrace (Finn) breeds were equally divided into either a control or a treated group. All ewes were given 1.3 kg of hay daily until the second estrus of the breeding season when an additional 0.9 kg of grain (barley) was fed daily to the treated group for two estrous cycle periods. Daily serum samples were collected beginning at the third estrus, with more frequent collections being made during the period of the fourth estrus. Serum P, E and LH were determined by RIA. Fifty percent of the Finn ewes exhibited their first estrus during August, while 55.5 percent of the Line M and 70 percent of the Suffolk ewes did not show estrus until September. Grain feeding significantly ($P<0.05$) increased the body weight in the treated group (control group lost 0.6±1.6 kg while the treated group gained 4.2±1.5 kg), but produced no effect on gestation length (147.1±0.7 vs 148.2±0.6 days), lambing rate (2.1±0.1 vs 2.0±0.1 lambs/ewe) and mean con-

centration of hormones during the estrous cycle (P, 1.3±0.1 vs 1.4±0.1 ng/ml; E, 9.9±1.0 vs 8.9±0.8 pg/ml and LH, 0.9±0.1 vs 1.0±0.1 ng/ml). However, the Finn ewes had a significantly ($P<0.05$) higher lambing rate than the other two breeds (2.5±0.2, 1.7±0.1 and 1.7±0.1 lambs/ewe for Finn, Line M and Suffolk ewes, respectively). The mean LH concentration during the estrous cycle was 1.3±0.2, 1.0±0.1 and 0.6±0.2 ng/ml for Finn, Line M and Suffolk ewes, respectively. Treatment did not appear to produce great differences in terms of the interval from the onset of estrus to the pre-ovulatory LH surge (5.9±1.8 vs 7.1±1.4 h), magnitude of the pre-ovulatory LH surge (181.3±34.5 vs 150.0±15.7 ng/ml), duration of the LH surge when values exceeded 40 ng/ml (5.7±0.5 vs 5.2±0.1 h) or LH secretion rate (63.2±7.3 vs 43.8±3.5 ng/ml/h). However, the Finn ewes had a significantly ($P<0.05$) longer interval from the onset of estrus to the LH surge than the other two breeds; being 6.1±1.6, 2.1±0.1 and 10.4±0.5 h for Line M, Suffolk and Finn ewes, respectively. They also had a significantly ($P<0.05$) longer duration of LH surge (mean values were 4.9±0.5, 4.7±0.4 and 7.0±0.3 h for the Line M, Suffolk and Finn ewes, respectively.

In a third experiment, serum samples were collected at a 20 minute interval for a period of several hours at each estrus throughout the breeding season from five ewes of each of the Finn and the Suffolk breeds. The Finn ewes

experienced a greater number of estrous periods throughout the season (11 vs 5 estrous periods).

The interval from the onset of estrus to the pre-ovulatory LH surge (when LH concentration rose to above 5 ng/ml) was significantly ($P<0.05$) longer in the Finn (13.8±0.6 h) than in the Suffolk ewes (6.4±0.6 h) while the duration of the pre-ovulatory LH surge was significantly ($P<0.05$) longer in the Suffolk (12.4±0.3 h) than in the Finn ewes (11.4±0.3 h). There was no significant difference between breeds in terms of the magnitude of the LH peak (156.7±9.5 vs 145.7±13.5 ng/ml for Finns and Suffolks, respectively) or the LH secretion rate (35.3±2.2 vs 30.6± 2.3 ng/ml/h for the Finn and Suffolk ewes, respectively). Comparisons of the interval from the onset of estrus to the LH surge, duration of the surge, peak magnitude and LH secretion rate between the first and the subsequent estrous periods throughout the breeding season did not show any significant differences. Serum P profiles indicated that the ovaries remained active (ovulations or luteinization occurred) in both breeds for as long as 70 days after the last estrus of the breeding season was observed.

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INTRODUCTION

Since the development of radioimmunoassay (RIA) for steroid as well as polypeptide hormones, a large body of literature on the reproductive hormones in various species has been obtained and the patterns of these hormones throughout the estrous cycle have been quite well established in farm animals. Many hypotheses on the regulation and modes of action of these hormones have been put forward; some of which have been studied in detail. However, our knowledge on reproductive endocrinology in the farm species is far from complete. For instance, what brings about puberty. An understanding of this phenomenon is incomplete and no one theory can clearly explain the initiation of puberty. Thus, study of the changes in reproductive hormones around puberty is an important step for a better understanding of the controlling mechanisms. The patterns of reproductive hormones around puberty have been studied in detail in rats but similar information in the farm species is limited. Therefore, in one of the experiments conducted, an attempt was made to establish profiles of different reproductive hormones in serum before and after puberty in the ewe lamb.

Flushing (high energy feeding) ewes with grain prior to or during the breeding season is generally recommended as a practice to improve lambing rate. Ovulation is known to be induced by luteinizing hormone (LH) alone or in

combination with follicle stimulating hormone (FSH) in a proper ratio. In ewes, increased ovulation rate as a result of flushing may be related to an increase in the magnitude of the pre-ovulatory LH surge. Thus, an experiment was conducted to study the effect of grain feeding on the pre-ovulatory LH surge in the ewe.

Lastly, it is believed that the ovulation rate increases to a plateau and then decreases as the breeding season progresses in sheep. However, at the present time there is no information on whether or not the pre-ovulatory LH surge changes as the breeding season progresses. Therefore, a third experiment was carried out to study the characteristics of the pre-ovulatory LH surge throughout the breeding season in two breeds of sheep (Suffolk and Finnish Landrace) which are known to differ somewhat in ovulation and subsequent lambing rate.

LITERATURE REVIEW

An extensive review of the relationships between the various reproductive hormones (progesterone, estrogen and LH) and the concomitant physiological changes that occur during the estrous cycle of the ewe has previously been done by Yuthasastrakosol (1975a). Therefore, only a brief and general review of these hormones (including FSH) is presented here. A specific review is given separately for each experiment.

Radioimmunoassay (RIA) and competitive protein binding (CPB) procedures permit accurate and precise measurement of blood hormones and the results from the literature being discussed in this review are derived mainly from the above procedures.

Progesterone

Progesterone concentration during the estrous cycle has been reported by many investigators (Thorburn et al., 1969; Stabenfeldt et al., 1969; Obst and Seamark, 1970; Bindon, 1971; Pant et al., 1972; McNatty et al., 1973; Yuthasastrakosol et al., 1975b). Their observations generally agree that at estrus and for a few days after (during the follicular and early luteal phases) progesterone concentration remains below 1 ng/ml then gradually rises to reach a plateau (during the luteal phase) around day 7 or 9 (day 0 = day of estrus) of the estrous cycle. Concentration remains high (2-5 ng/ml) with some minor fluctuation until

one or two days before the next estrus and then drops to begin the next cycle (Fig. 1). Plasma progesterone levels during the estrous cycle reflects corpus luteum (CL) function. A positive correlation between these two parameters has been reported by Smith and Robinson (1969), Stormshak et al. (1963), Plotka et al. (1970) and Thorburn and Mattner (1971).

Estrogen

There appears to be great variation in estrogen concentration in blood as reported by different investigators (Scaramuzzi et al., 1970; Cox et al., 1971a; Cox et al., 1971b; Obst et al., 1971; Bjersing et al., 1972; Pant et al., 1972; Van Der Walt et al., 1972; Yuthasastrakosol et al., 1975b). Variations were reported regardless of the procedure used (RIA or CPB) or source of sample (peripheral or ovarian venous blood). Scaramuzzi et al. (1970) reported a peak of 975 pg/ml on the day before estrus in ovarian blood samples while Bjersing et al. (1972), using the same procedure (RIA), reported a peak of 1807 pg/ml. Cox et al. (1971a and b), using the CPB method, obtained a peak of only 12.4 pg/ml. Daily fluctuation was also reported by Scaramuzzi et al. (1970), Cox et al. (1971b), and Van Der Walt et al. (1972). The peak of estrogens appeared to occur on the day before estrus when samples were collected from the ovarian vein (Scaramuzzi et al., 1970; Cox et al., 1971a; Cox et al., 1971b; Smith and Robinson, 1970; Bjersing et al., 1972)

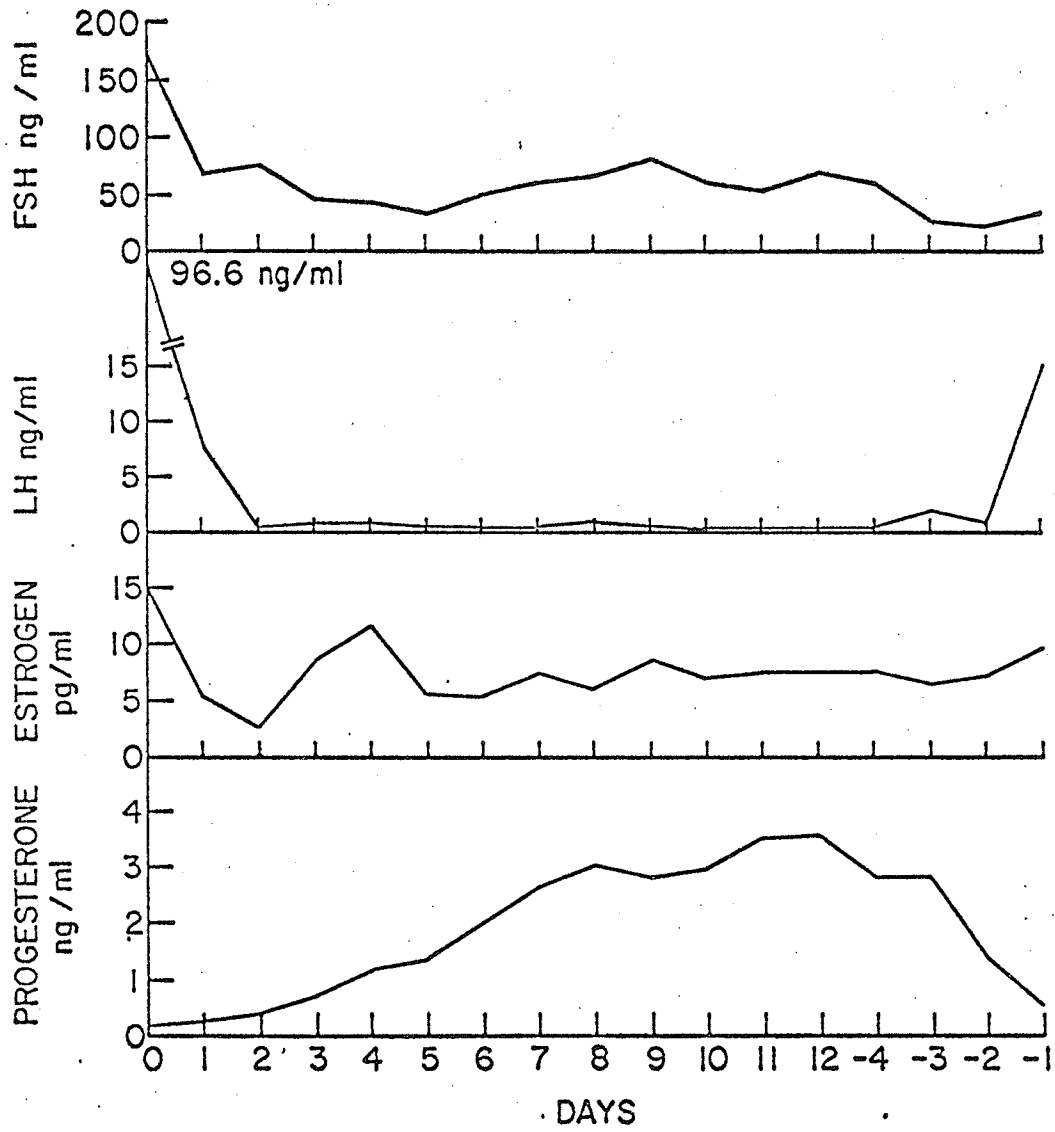

Figure 1. The hormone profile during the estrous cycle in ewes compiled from literature (day 0 = day of estrus).

while in peripheral blood samples the peak occurred on the day of estrus (Van Der Walt et al., 1972; Yuthasastrakosol et al., 1975b). Secondary rises of estrogen were observed on days 3 and 4 (Cox et al., 1971a, 1971b; Holst et al., 1972), days 6 and 9 and days 11 to 15 (Mattner and Braden, 1972) and days 2, 8 and 14 (Scaramuzzi et al., 1970). These investigators believed that the origin of the secondary rises was from follicles. A general profile of the estrogen levels during the estrous cycle compiled from different reports is given in Figure 1.

LH

A pre-ovulatory surge of LH occurring on the day of estrus has been reported by many investigators and its magnitude ranges from 50 ng/ml to more than 200 ng/ml (Pelletier et al., 1968; Geschwind and Dewey, 1968; Niswender et al., 1969; Wheatley and Radford, 1969; Goding et al., 1969; Scaramuzzi et al., 1970; Reeves et al., 1970a; Howland et al., 1971; Nett et al., 1974; Cumming et al., 1972; Yuthasastrakosol et al., 1975b). The wide range in magnitude is due in part to the frequency of sample collection. During the luteal phase, while progesterone levels are high, LH remains at low concentrations (Figure 1). However, evidence of some minor rises during the luteal phase were reported by Scaramuzzi et al. (1971), Wheatley and Radford (1969), Cicmanec and Niswender (1973) and Cumming et al. (1971).

The interval from estrogen rise to LH peak was 24 h, as observed by Scaramuzzi et al. (1970). This was later confirmed by Bjersing et al. (1972), Cox et al. (1971a) and Obst et al. (1971) with the interval ranging from 12 to 24 h. Niswender et al. (1968), Wheatley and Radford (1969) and Goding et al. (1969) reported that the interval from the onset of estrus to the LH peak ranged from 0 to 12 h.

FSH

Information on the FSH concentration during the estrous cycle in ewes is limited. Foster et al. (1975) reported that the FSH level on day 7 of the estrous cycle was 74 ng/ml and was 52 ng/ml on day 15. A more complete FSH profile was reported by Salamonsen et al. (1973). According to these investigators, the FSH peak was observed on the day of estrus, ranged from 80-150 ng/ml and coincided with the LH peak. There were great fluctuations within day as well as between daily samples (ranging from less than 15 to 110 ng/ml). This FSH fluctuation appeared to be independent of the basal variation in plasma LH (Fig. 1).

Control of Gonadotrophin Release

An injection of estrogen causes an initial depression in LH levels in both anestrous and ovariectomized ewes (Goding et al., 1969; Scaramuzzi et al., 1971; Howland and Palmer, 1973; Radford and Wallace, 1974; Yuthasastrakosol et al., 1974). However, about 12-24 h after the injection,

blood LH peaks are observed in ovariectomized ewes (Radford et al., 1969; Pelletier and Signoret, 1969; Scaramuzzi et al., 1971; Howland and Palmer, 1973), anestrous ewes (Goding et al., 1969; Beck and Reeves, 1972) and in cyclic ewes (Bolt et al., 1971; Howland et al., 1971).

As reviewed earlier, many investigators have observed high estrogen levels on the day before or on the day of estrus and estrogen appears to modulate the LH release in normal cycling ewes. How estrogen acts upon the hypothalamo-hypophyseal system in bringing about the LH release is not clear (Hauger et al., 1977).

According to hormone levels during the estrous cycle as discussed earlier, progesterone appears to inhibit LH release even in the presence of estrogen. The endogenous progesterone, during the mid and late luteal phase of the estrous cycle, is apparently able to account for the observed absence of LH peaks although endogenous estrogen levels are suggested to rise in mid cycle according to some workers. (This is not obvious in Figure 1 because the data were pooled from different reports). Smith and Robinson (1969) suggested that inhibition of ovulation by progesterone was probably due to a suppression of LH release, but Scaramuzzi et al. (1971) failed to depress plasma LH by the infusion of progesterone. Dial et al. (1977) reported that progesterone implants in ovariectomized ewes augmented the tonic release of LH and that the implants did not raise progesterone in plasma to physio-

logical levels except for a few days in one of the experiments. However, Diekman and Malven (1973) using a progesterone implant, were able to significantly decrease mean levels and rhythmicity of LH in two out of five ovariectomized ewes by 40 h after administering the progesterone implant. In one ewe the mean level of LH rose again at 120 h after implantation and it was suggested that progesterone has a short term inhibitory effect. With a pharmalogical dosage of progesterone, Davis and Borger (1974) could suppress both the magnitude and the frequency of LH pulses in ovariectomized ewes. The negative feedback action of progesterone on tonic LH release has been confirmed by Karsch et al. (1977) when physiological levels of progesterone in serum were established in long-term ovariectomized, immediately ovariectomized or long-term ovariectomized ewes pre-treated with estradiol. The inconsistency of earlier works might have been related to a failure to achieve an effective level of progesterone over a sufficiently long period of time. Similar to estrogen, how progesterone acts on the hypothalamo-hypophyseal system in ewes (or other mammals) is not well understood.

Some workers (Piper and Foote, 1968; Cumming et al., 1971; Scaramuzzi et al., 1971) have indicated that progesterone inhibits estrogen-induced LH release in ewes. However, large doses of estrogen could induce an LH release on days 3, 10 and 11 of the estrous cycle (Bolt et al., 1971; Howland et al., 1971). Differences in dosage of

estrogen required to cause an LH release as compared to anestrous or ovariectomized ewes suggested that progesterone levels might determine the effectiveness of estrogen in bringing about an LH discharge from the pituitary. This hypothesis is difficult to prove since Yuthasastrakosol et al. (1974) found that an injection of progesterone either at 4, 8 or 24 h before or simultaneously with a high dose of estrogen did not consistently block the LH release in ovariectomized ewes.

In 1971, Schally et al. isolated porcine gonadotrophin releasing hormone (GN-RH) which appeared to be structurally identical to a releasing factor obtained from the ovine hypothalamus (Burgus et al., 1969). Since that time many investigators have reported that either crude, purified or synthetic GN-RH (Matsuo et al., 1971) can induce LH release in ovariectomized (Findlay et al., 1973; Rippel et al., 1974b; Radford et al., 1974) anestrus (Gay et al., 1970; Reeves et al., 1971a; 1971b; 1972; Cumming et al., 1972; Crighton et al., 1974; Rippel et al., 1974a; 1974b) and in cycling ewes (Reeves et al., 1970b; 1971a; Foster and Crighton, 1973; Rippel et al., 1974a). Since LH and FSH peaks resulting from estradiol-17B treatment in anestrus ewes are similar to those following GN-RH treatment, Jonas et al. (1973) and Reeves et al. (1971b) concluded that the action of estradiol-17B is mediated via GN-RH. An increased time lag in LH response to estradiol-17B as

compared to GN-RH (9.5 vs 2.5 h) probably represents the time taken for uptake of estradiol-17B and subsequent release of GN-RH by the hypothalamus. It is possible that estrogen may either influence the sensitivity of the pituitary gland to pre-existing levels of GN-RH or stimulate GN-RH release at the hypothalamic level. Evidence for action of estrogen at both levels has been reported by Cumming et al. (1972), Finlay et al. (1973), Rippel et al. (1974b) and Reeves et al. (1971a and 1971b).

It has been shown by Debeljuk et al. (1972) and Przekop et al. (1972) that progesterone alone or in combination with estradiol-17B produced a suppressive effect on GN-RH-induced ovulation in sheep. Thus, LH release may be controlled by changes in the ratio of estradiol to progesterone.

Crighton et al. (1973) reported high hypothalamic GN-RH activity on day 16 of the estrous cycle which declined by the onset of estrus. Decline in pituitary LH content occurred at six h before the onset of estrus which was shortly followed by a peripheral plasma LH peak. This evidence suggests that GN-RH is released from the hypothalamus which in turn stimulates LH release from the pituitary. Results obtained by Roche et al. (1970) and Chakraborty et al. (1974) also support the above theory.

Nett et al. (1974) did not find any changes in peripheral GN-RH levels in estrous, castrated or anestrous ewes in which an LH peak was induced by estradiol-17B.

They concluded that peripheral GN-RH may not reflect the levels present in the hypothalamus. Administration of estradiol-17B augmented the LH release elicited by exogenous GN-RH (Reeves et al., 1971b) and the response to GN-RH treatment differed during various stages of the estrous cycle; being greatest at the time of normal LH peak and least during the luteal phase (Reeves et al., 1971a). These investigators concluded that changes in responsiveness may be caused by estrogen sensitizing the pituitary gland. Clearly, more study is required to resolve the exact relationship between the ovarian steroids and/or the hypothalamus and pituitary.

GENERAL MATERIALS AND METHODS

Experimental animals were maintained in a barn according to each experimental plan of which details will be given. Estrus was detected by using either a vasectomized or aproned ram and the frequency of observation varied in each experiment. Jugular blood was collected by venepuncture using vacutainer tubes. Serum was separated by centrifugation after being kept over night at 5°C. Serum samples were frozen at -20°C until further analysis. Concentrations of progesterone, estrogen, LH and FSH were determined by radioimmunoassay (RIA). Details of the procedures for progesterone and estrogen have been described by Yuthasastrakosol et al. (1975a), for LH by Howland (1972) and for FSH by Cheng (1976). Briefly, the methodology was as follows:

Progesterone

A highly specific progesterone antiserum (#869) was generously provided by Dr. Gordon Niswender, Colorado State University. Among various steroids tested 5 -pregnane 3, 20-dione is the only one which had significant cross reactivity (<3%) Niswender (1973). Mean percentage recovery of ^{3}H- progesterone added to serum samples was 77.4±0.4 (n = 26). Four pooled serum samples from anestrous ewes determined in one assay gave a mean value of 0.18±0.01 ng/ml and on four other separate occasions gave a mean value of 0.18±0.01 ng/ml. The

overall average coefficient of variation when known amounts of progesterone (ranging from 100 to 800 pg) were added to anestrous-ewe serum was 8.1±1.8 percent.

Estrogens

Cross reactivity of the estrogen antiserum used (#029-14) was reported by Wu and Lundy (1971). Only three steroids showed high cross reactivity; percent cross reactivity was 63.7 for estrone, 18.7 for estriol and 5.1 for estradiol-17α. The mean percentage recovery of 6, 7-^{3}H-estradiol-17B when added to serum samples was 62.0± 1.3 (n = 28). Pooled serum from ovariectomized ewes determined at one time gave a mean of 27.8±0.4 pg/ml (n = 4) and on five separate occasions when 12.5 pg of estradiol-17B was added to double distilled water the estimate was 11.0±0.8 pg/ml. The inter-assay coefficient of variation when known amounts of estradiol-17B (ranging from 6.5 to 400 pg) were added to ovariectomized ewe serum in five separate assays was 6.1±0.9 percent.

LH

A double antibody RIA was employed to determine LH concentration using anti-ovine LH serum supplied by Dr. Gordon Niswender (GDN-#15). LH values were expressed in terms of NIH-LH-S14 standard. A detailed description of the procedure has been given by Howland (1972). Serum samples from each animal were assayed in a single assay.

FSH

Details of the procedure, which uses an antibody developed against bovine FSH, are described by Cheng (1976). The values are expressed in terms of NIH-FSH-S6 (ovine) standard, ^{125}I-ovine FSH was used as the trace.

Statistical Analyses

Analysis of variance using a nested design and the Student-Newman-Keul (SNK) test, as described by Snedecor and Cochran (1971) were used whenever the number of observations allowed, to find significance of differences between treatments, breeds and the characteristics of the pre-ovulatory LH release between breeds.

EXPERIMENT I

It has been assumed that the prepuberal period in the ewe lamb is comparable to that of anestrous period in mature ewes and that physiological changes around the time of puberty are similar to the changes that occur at the onset of the breeding season. The hormonal patterns before and during the beginning of the breeding season have been described by Walton et al. (1974), Thorburn et al. (1969) and Yuthasastrakosol et al. (1975b). Limited information of a similar nature is available in ewe lambs which are approaching puberty.

Progesterone levels in anestrous ewes remained low with little variation except for a small rise which occurred several days before the first ovulation. The first ovulatory rise of progesterone was not usually accompanied by estrus (Thorburn et al., 1969; Walton et al., 1974; Yuthasastrakosol et al., 1975b). Fluctuations of the LH level in serum were obvious during anestrus but an LH peak which could be associated with the first ovulation was observed only in few animals (Walton et al., 1974; Yuthasastrakosol et al., 1975b). Fluctuating levels of estrogen during anestrus could not be related to changes in LH and progesterone except that a minor rise occurred around the first estrus (Yuthasastrakosol et al., 1975b).

In ewe lambs, pulsatile LH secretion first appeared around 11 weeks after birth and remained 2 to 5 fold

(2.8±0.8 ng/ml) higher than the levels observed in adults during the anestrous period. LH concentration then decreased to adult levels and adopted a cyclic pattern according to the ovarian cycle after puberty. FSH secretion was more or less constant at around 11 weeks of age and its level was comparable to that of the adult (Foster et al., 1975).

Mansour et al. (1959) reported higher amounts of pituitary gonadotrophins before puberty with a decline occurring at puberty. Whether or not this reflected LH and FSH release at puberty is not known. In cycling ewes, pituitary LH increased gradually from day 1 to 15 and dropped to a low level at estrus while LH in serum did not change much except at estrus (Roche et al., 1970). This suggested the release of LH from the pituitary into the circulatory system around estrus. This relationship between pituitary and serum LH was supported by using GH-RH to induce LH release into blood, thus lowering pituitary LH (Chakraborty et al., 1974). The influence of GH-RH on FSH at the pituitary level appeared to be the same as the influence on LH (Jonas et al., 1973).

MATERIALS AND METHODS

Five Dorset X "Western White Face" ewe lambs, born between February and March 1974, were maintained in the barn under natural lighting conditions. Twice daily observations for estrus began on August 2, by using a raddled vasectomized ram. The ewe lambs were weighed on the day of first estrus. Daily blood collection began at an average age of 163±6.5 days (ranging from 141 to 177 days). Collection ended at the second estrus and more frequent sampling was done during estrus (every 2 h for 16 h). Serum was assayed for progesterone, estrogen, LH and FSH.

RESULTS

Four of the five ewe lambs reached puberty at an average age of 221.2±10.6 days (Table 1). The remaining one was not yet observed in estrus when estrous checking ended in November. Age, weight and date at first estrus and first ovulation (indicated by the progesterone profile) are given in Table 1.

According to the progesterone profiles, three ewes (Nos. 1, 2 and 4) had their first ovulation unaccompanied by estrus, while the remaining one (No. 3) was in estrus shortly before the first ovulation. The interval from first to second estrus in ewe No. 1 was longer than normal (Table 1).

Hormonal Patterns

The hormonal patterns of the individual ewes and their relationship are given in Figures 2, 3, 4 and 5 for ewe Nos. 1, 2, 3 and 4, respectively. The composite pattern for four of the ewes (Nos.1,2,3 and 4) is given in Figure 6, with the day of estrus prior to the second ovulation being normalized as day 0.

Mean progesterone concentration before the first ovulation for ewe Nos. 1, 2, 3 and 4 were 0.60±0.04, 0.74±0.05, 0.49±0.02 and 0.54±0.02 ng/ml, respectively, with an overall average of 0.59±0.02 ng/ml. Fluctuations were observed in all ewes during this period, which ranged from 0.01 to 2.09 ng/ml. Following ovulation, progesterone

Table 1. Age and weight at first estrus and age at first ovulation

Ewe No.	Date of 1st estrus (1974)	Age at 1st estrus (days)	Weight at 1st estrus (kg)	Age at 1st ovulation (days)	Interval from 1st to 2nd estrus (days)
1	Oct. 8	243	48.2	200	24
2	Sept. 24	216	49.2	204	17
3	Sept. 24	194	46.2	183	14
4	Oct. 8	232	49.4	209	16
$\bar{x}$ SE		221.2±10.6	48.2±0.7	199±5.6	17.2±2.1

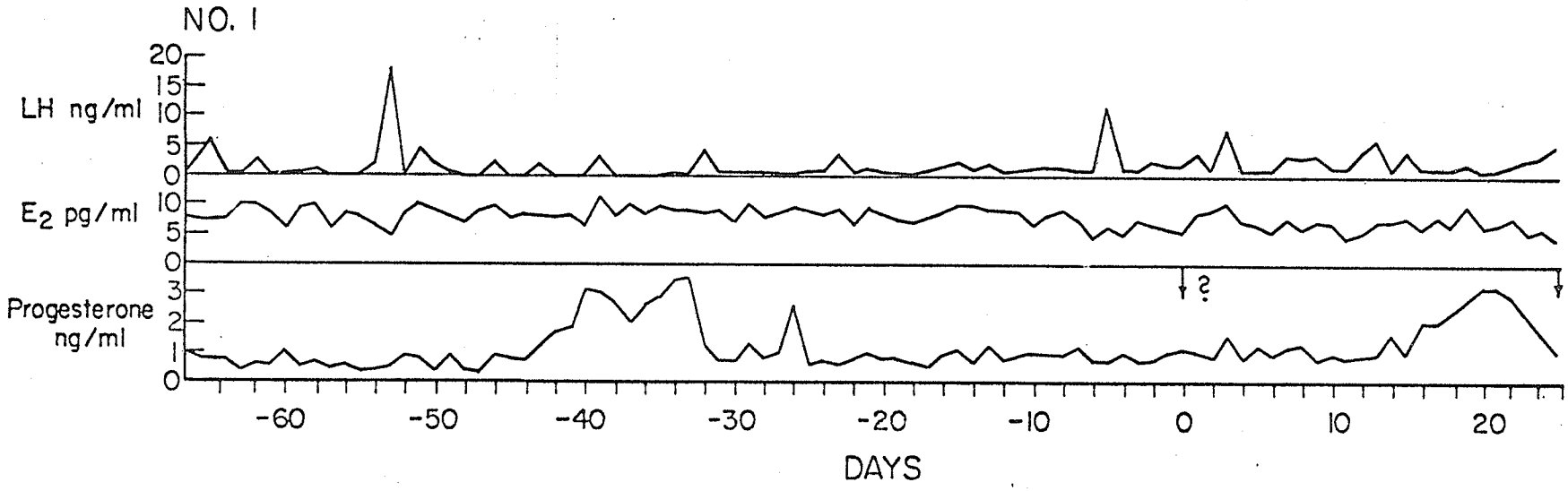

Figure 2. Hormonal pattern prior to and after puberty in ewe lamb No. 1 (day 0 = day of estrus prior to second ovulation).

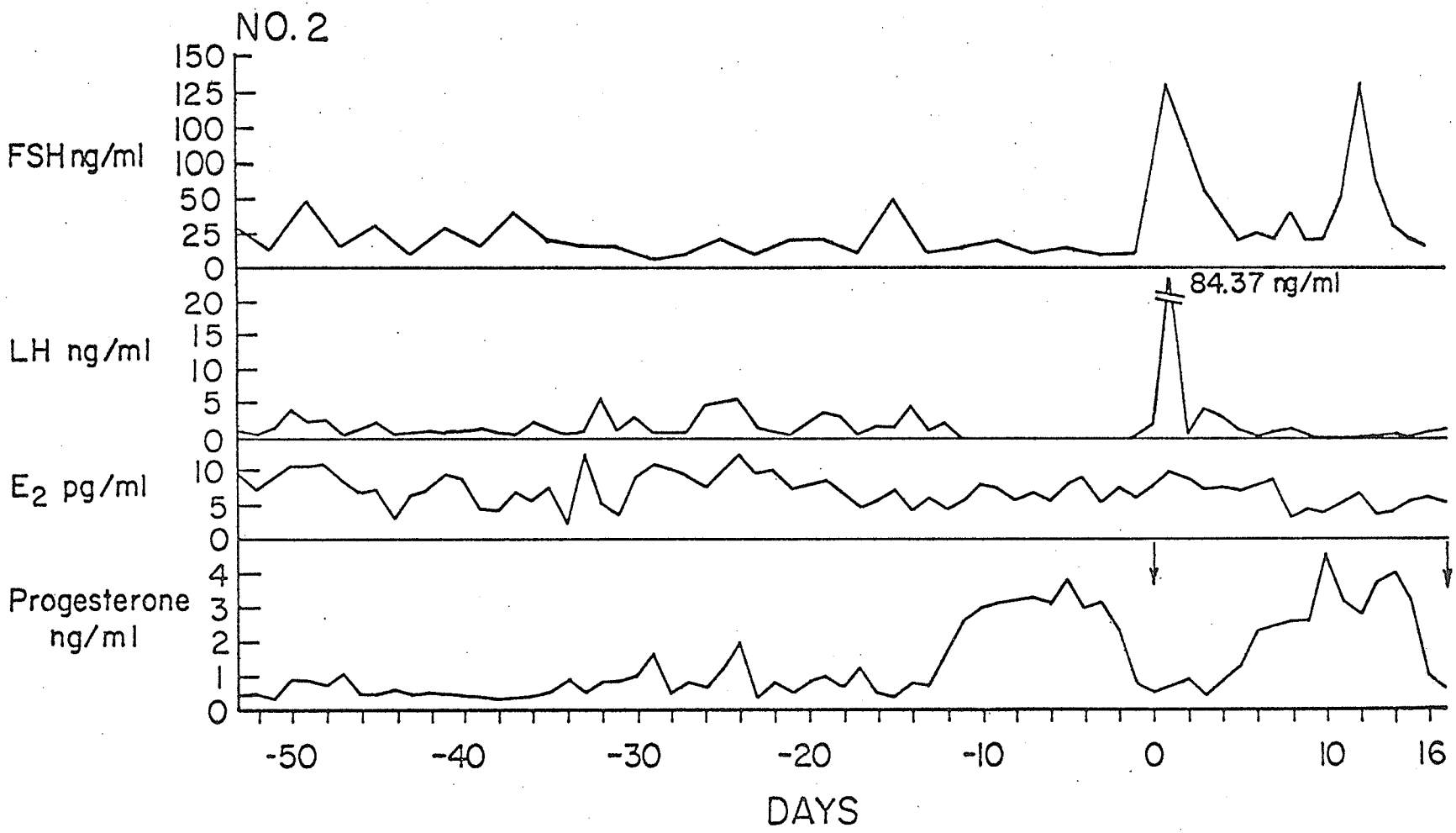

Figure 3. Hormonal pattern prior to and after puberty in ewe lamb No. 2 (day 0 = day of estrus prior to second ovulation).

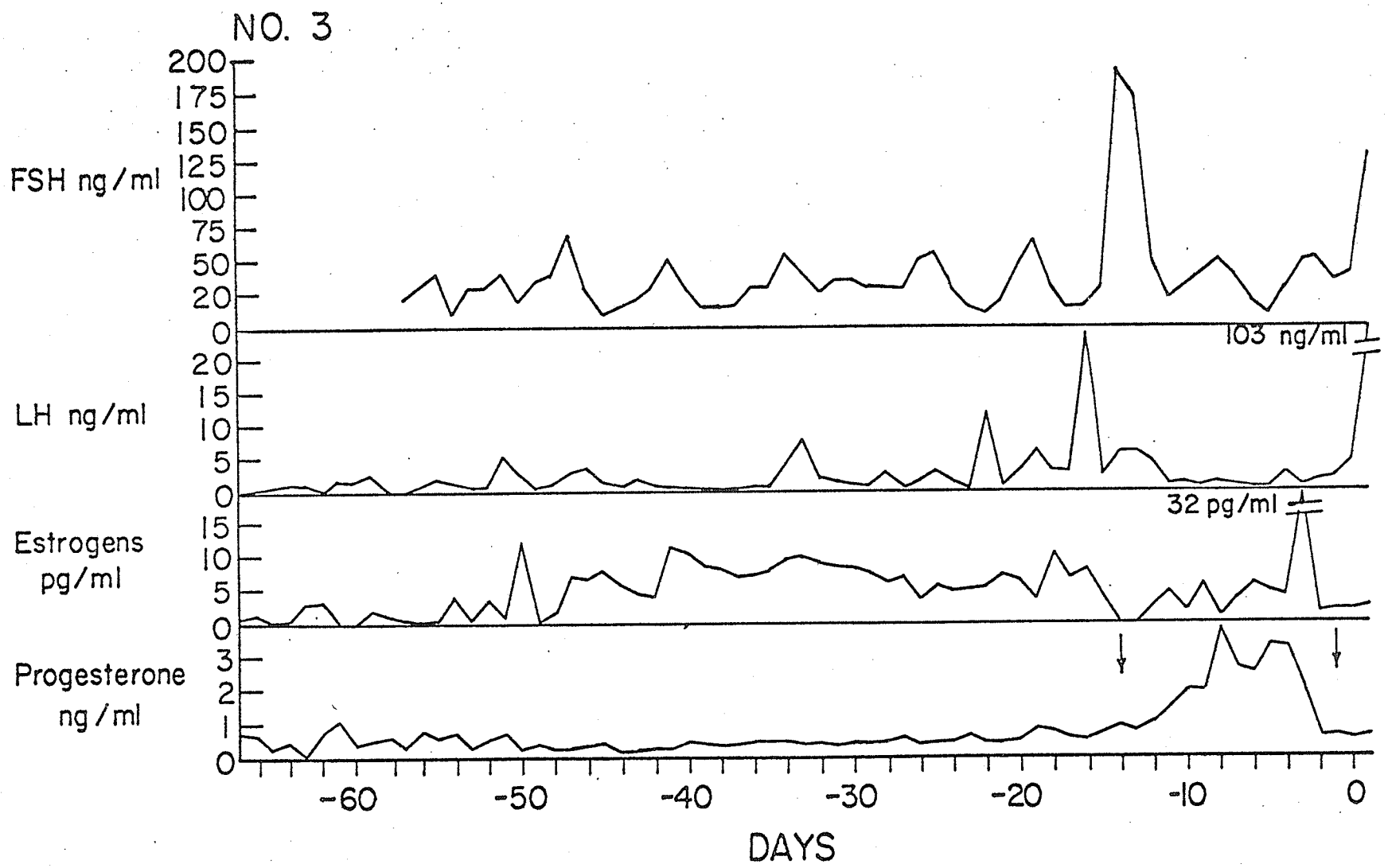

Figure 4. Hormonal pattern prior to and after puberty in ewe lamb No. 3 (day 0 = day of estrus prior to second ovulation).

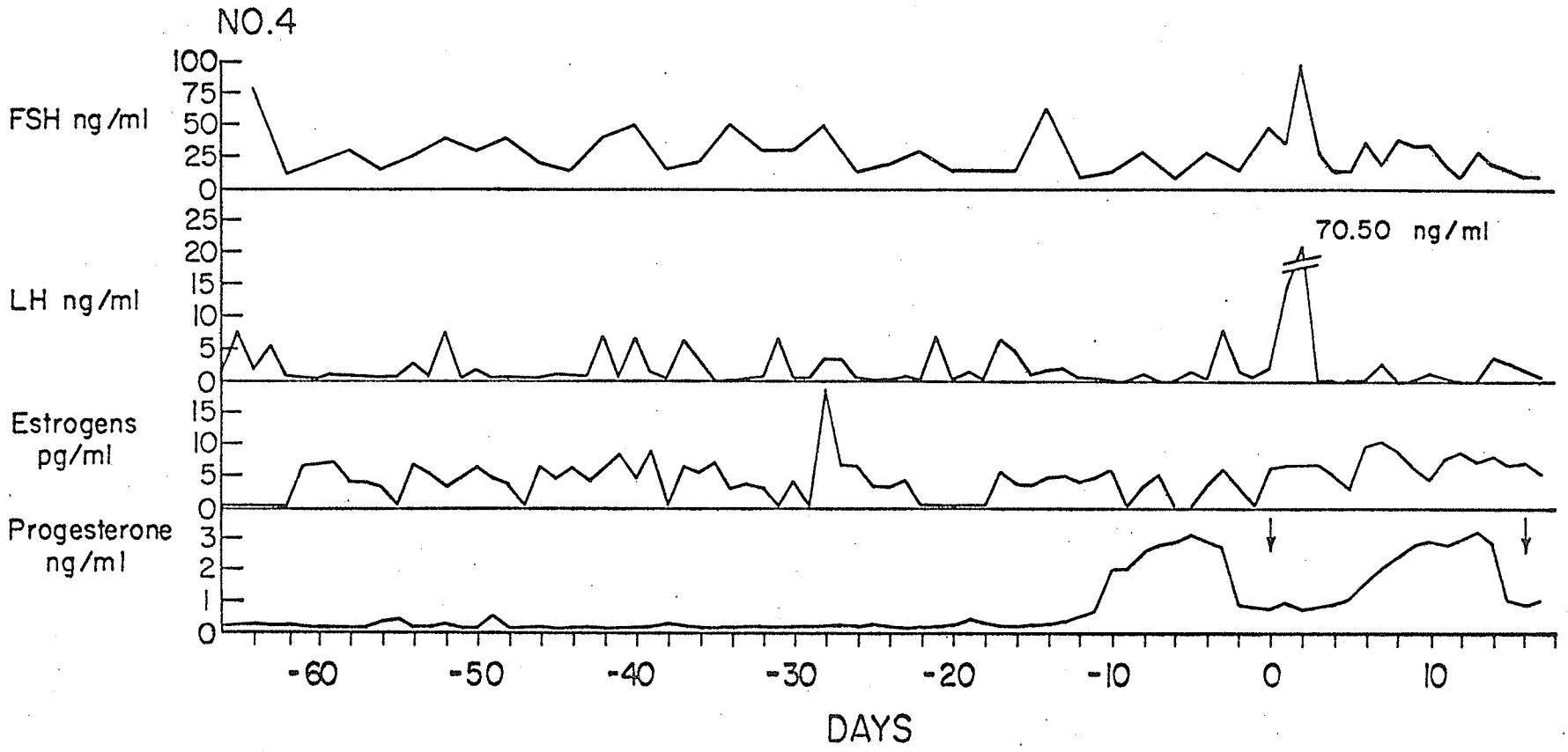

Figure 5. Hormonal pattern prior to and after puberty in ewe lamb No. 4 (day 0 = day of estrus prior to second ovulation).

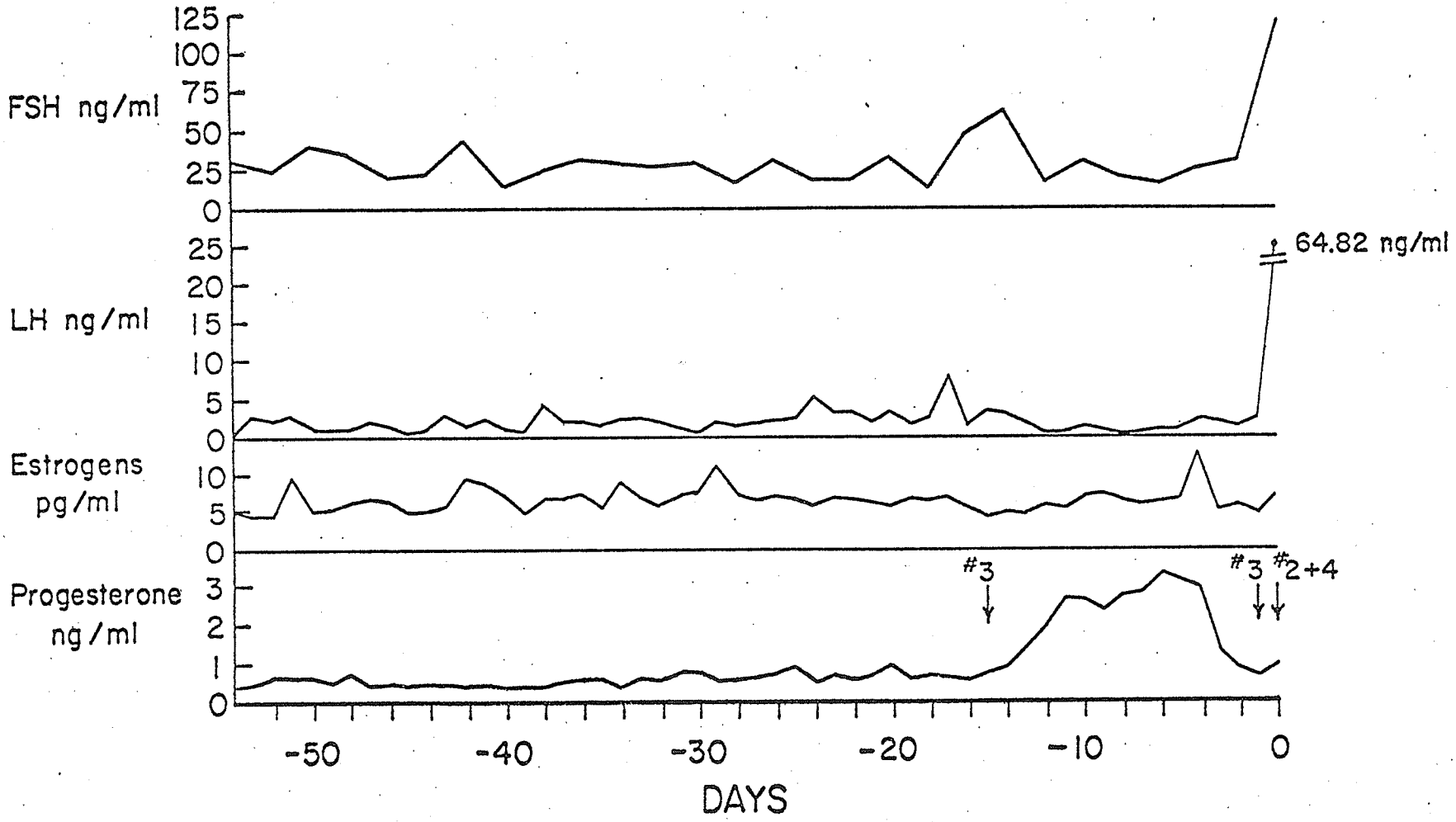

Figure 6. Composite pattern of hormones from ewe lambs.

gradually rose to a mean peak value of 3.5±0.2 ng/ml and remained higher than 1 ng/ml for an average of 10.7±0.3 days (Figure 6). Ewe No. 1 had an unusually long interval between the first and the second ovulation (55 days). During this period a greater progesterone fluctuation was observed (ranging from 0.5 to 2.7 ng/ml).

Daily variations in LH were observed in all ewes before their first ovulation. A mean of 1.8±0.8, 2.1± 0.2, 2.4±0.5 and 1.9±0.3 ng/ml was observed for ewe Nos. 1, 2, 3 and 4, respectively, with the overall mean being 2.1± 0.2 ng/ml. The maximum LH values obtained during the frequent sampling done on the day of estrus are presented for all ewes in Figures 2, 3, 4 and 5. An LH peak of 24.2 ng/ml was detected in only one ewe at the time of the first ovulation (No. 3). This animal was detected in estrus before the first ovulation. However, LH peaks associated with the second ovulation were observed in all ewes except No. 1. During the luteal phase LH concentrations remained low with little variation while progesterone concentrations were elevated.

Average FSH concentration was 29.0±3.9, 40.2± 3.9 and 30.7±2.5 ng/ml for ewe Nos. 2, 3 and 4, respectively. FSH peaks at estrus coincided with the LH peak in all three ewes. In contrast to LH which remained low during the luteal phase, FSH fluctuated throughout the pre- and post-puberal period. FSH was not determined in ewe No. 1 due to the abnormal length of her estrous cycle.

The estrogen pattern, although fluctuating throughout the experimental period, did not show any clear cut relationship to the other hormones. The overall mean was 6.6 ± 0.2 pg/ml and ranged from <2 to 32 pg/ml.

The composite profile (Figure 6) shows relationships between LH, FSH and progesterone after the first ovulation which are similar to that of a cycling mature ewe. There was progesterone fluctuation before the first ovulation which was followed by a characteristic rise during the luteal phase. During this phase LH remained low and rather constant. LH and FSH peaks occurred a few days after progesterone began to decline (Figure 6).

DISCUSSION

The first ovulation in three ewes occurred unaccompanied by estrus. This result is in agreement with a report by Foote et al. (1970) who studied this problem by using a laporatomy technique. Similar results were observed in mature ewes approaching the breeding season (Thorburn et al., 1969; Walton et al., 1974; Yuthasastrakosol et al., 1975b). Age at first estrus in this study (221.2±10.6 days) is in agreement with that for the Hampshire breed reported by McKenzie and Phillips (1930). Foote et al. (1970) found the age of first estrus in the Hampshire, Columbia and Rambouillet breeds to be longer; ranging from 249 to 347 days. However, weight at first estrus was comparable to that seen in this study. This emphasizes the importance of body weight as related to puberty, rather than age.

The first estrus observed in ewe No. 1 was probably a false one since after estrus no progesterone rise indicating ovulation occurred until 12 days later. This may be the reason why the first cycle in this ewe was longer (24 days). False detection is possible since the vasectomized ram was left in the pen with the ewes. During feeding the ram may have jumped over the ewes in order to gain access to the feeder.

The interval between the first and the second ovulation in ewe No. 1 was also unusually long. It is possible that after the first ovulation there was a hormonal

imbalance which caused delay of the second ovulation, or at this time the hypothalamic-pituitary-ovarian axis was not yet coordinated well enough to allow normal cyclic changes. Progesterone fluctuation was observed in all ewes before the first ovulation. In contrast, during the anestrous period in mature ewes progesterone remained low and constant except for a small rise which occurred several days before the first ovulation of the breeding season (Yuthasastrakosol et al., 1975b; Walton et al., 1974; Thorburn et al., 1969) Results from this study suggest that more priming of progesterone is needed in ewe lambs to initiate the characteristic hormonal patterns of the estrous cycle. Perhaps once the cyclic pattern is initiated in the life of the mature ewe only a brief priming may be needed to trigger reinitiation of the cycle each successive breeding season. The mechanism of how progesterone priming initiates ovulation is not known. It is possible that a change in the progesterone to estrogen ratio brings about gonadotrophin release as suggested by Debeljuk et al. (1972) and Przekop et al. (1972) to occur in cycling ewes.

The LH results of this study were similar to those reported by Yuthasastrakosol et al. (1975b) in mature ewes. During the luteal phase, with high progesterone levels, LH levels were depressed indicating a negative feed-back effect of progesterone on the hypothalamo-pituitary system. A suppressive effect of progesterone on GN-RH-induced LH release or on serum LH levels has been demonstrated by

Arimura and Schally (1970) in rats, Hilliard et al. (1971) in rabbits and Przekop et al. (1972) and Karsh et al. (1977) in sheep.

A LH peak on the day of estrus or before ovulation was detected in three ewes (Nos. 2, 3 and 4) confirming the results reported by others. Similar results in cycling ewes were reported by Scaramuzzi et al. (1971), Wheatley and Radford (1969), Cicmanec and Niswender (1973) and Cumming et al. (1971) in which a pre-ovulatory LH peak occurred during estrus. A LH peak prior to the first ovulation was detected in only one ewe (No. 3). In this particular ewe the first estrus occurred before the first ovulation, thus allowing frequent sampling at this stage. The peak of FSH coincided with the LH peak in this study, as also reported by Salamonsen et al. (1973). This supports the view that the release of LH and FSH is controlled by the same releasing factor (Jonas et al., 1973; Reeves et al., 1974b). High levels of LH and FSH during the prepuberal period were not observed in this study as compared to that of Foster et al. (1975).

The estrogen data reported here is similar to that reported by Yuthasastrakosol et al. (1975b) in mature ewes. No obvious relationship of estrogen to other hormones is recognized.

CONCLUSION

The average age and weight at first estrus, and age at first ovulation in four Dorset X "Western White Face" ewe lambs was 221.2±10.6 days, 48.2±0.7 kg and 199±5.6 days, respectively. As indicated by progesterone blood patterns, first ovulation was not accompanied by overt estrus in three of the four lambs observed. The pattern of reproductive hormones (progesterone, estrogen and LH) prior to and after puberty in the ewe lambs was similar to that of the mature ewe prior to and after the onset of the breeding season. The progesterone fluctuations seen prior to puberty in the ewe lambs was not previously observed in the mature ewes prior to the onset of the breeding season (except for the brief rise which occurred several days prior to the first estrus).

EXPERIMENT II

Grain feeding during the beginning of the breeding season increased the percentage of multiple ovulations in ewes (Foote et al., 1959). Energy level and duration of exposure to feeding regime was also shown by Bellows et al. (1963b) to affect ovulation rate. The ovulation rate was affected not only by feeding level but also by the time of the breeding season; being highest at the middle of the season. The effect of feed level on ovulation rate occurred only when ovulation rate had declined towards the end of the season, and to some extent during the early part of the breeding season (Hulet et al., 1974). Recently, Dufour and Wolynetze (1977) have demonstrated that the multiple ovulation response depended more on the feeding regime in effect at that estrus than on the time at which the regime was initiated. Higher ovulation rates were observed in ewes under high energy feeding at the third and fourth estrous periods of the breeding season, but significant differences in ovulation rate between high and low energy feeding groups were observed only at the fourth estrus (after treatment had been initiated three days after the second estrus). Grain feeding in addition to hay for 6 to 8 months before the breeding season produced a greater percentage of multiple ovulations (Foote et al., 1959), but a similar type of feeding during anestrous season did not stimulate ovarian activity or advance the onset of the breeding season according to Gerring (1954).

Energy sources such as glucose and lard in the ration also stimulate higher ovulation rate in gilts (Zimmerman et al., 1960; Brook et al., 1972). Dailey et al. (1972) emphasized that the time at which the higher feed level was initiated during the estrous cycle was critical in producing a higher ovulation rate. Increased feed intake for one day after mating did not affect ovulation rate, pituitary weight and residual LH potency, while increased intake on the day of mating significantly increased ovulation rate but did not affect pituitary weight or residual LH potency (Brook et al., 1972).

Unrestricted feeding for 12 h immediately before expected ovulation increased ovulation rate in underfed female rats (Cooper et al., 1970). Glucose supplement in rats maintained on a restricted feeding regime increased plasma LH concentration while LH concentration in the pituitary gland remained unchanged (Howland, 1972). Pituitary FSH and LH in starved ovariectomized rats were higher than in full fed rats and the concentrations of these hormones in serum were lower (Howland, 1971).

Assay of total gonadotrophin in pituitary glands of underfed rats showed elevated hormone concentration (Rinaldini, 1949; Srebnik and Nelson, 1963). However, Piacsek and Meites (1967), employing a bioassay specific for FSH and LH, found no difference in FSH but a reduced LH concentration in pituitary glands in restricted-fed rats. The results obtained by Piacsek and Meites (1967) on the

effect of restricted feeding suggested that a reduction in ovarian weight may have been due to impairment of gonadotrophin release.

In ewes, grain feeding increased follicular fluid weight and increased pituitary weight and FSH (921 vs 1082 mg) and LH (130 vs 165 mg) potency (Bellows et al., 1963a). It is therefore possible that high energy intake may have something to do with an increased gonadotrophin release which brings about a higher ovulation rate. However, the literature is limited on the effect of energy intake on serum gonadotrophin levels.

In cows, high energy feeding for a period of 68 days did not significantly change LH peak levels (Jones et al., 1976). Lishman et al. (1977) also found that neither energy level in the feed, nor FSH priming, influenced the maximum LH level or the area under the release curve in postpartum cows.

Therefore, at the time that this study was initiated the exact mechanism by which feed intake or energy level in feed affected ovulation rate was not known. Thus, an attempt was made to find out whether grain feeding for a period of approximately one estrous cycle in ewes would have any effect on the status of the pre-ovulatory LH surge during the fourth estrus of the breeding season.

MATERIALS AND METHODS

Ten ewes of each of the Line M, Suffolk and Finnish Landrace (Finn) breeds were used in this experiment. They were kept in an open-front barn and group fed hay at an average of 1.3 kg per day, beginning on June 28, 1974. At their second estrus they were divided into two groups within each breed. One group was individually fed 0.9 kg of grain (barley) daily until their fourth estrus. The amount of grain was gradually increased from 0.2 kg to 0.9 kg within the first four days. The other group (control) was maintained on hay throughout the experiment.

Estrus detection was done by aproned rams; paint was applied on the apron around the chest area. Two rams were used alternately. One of them was allowed to mix with ewes all the time. Observation of estrus was made twice daily beginning on August 1. At the expected fourth estrus the frequency of observation was increased to every 4 or 6 hours.

Daily blood samples were obtained between the third and fourth estrus. When the fourth estrus was observed, blood was collected every 20 minutes for a 16-hour period.

All ewes were weighed at the third and fourth estrus and were bred at the fourth estrus. Serum progesterone, estrogen and LH were determined by RIA.

RESULTS

Onset of Breeding Season

All ewes showed their first estrus of the breeding season during August or September. When these two months were divided into four equal periods, slightly more of the Finn ewes started cycling earlier than the other two breeds (Table 2). Fifty percent of the Finn ewes came into estrus during August, whereas 55.5 percent of the Line M and 70 percent of the Suffolk ewes did not exhibit their first estrus until September.

Weight Changes

A comparison of the weight changes recorded, according to treatment group, is given in Table 3. Control ewes lost an average of 0.6 kg, while grain fed ewes gained an average of 4.2 kg during the experimental period (Table 3). An analysis of variance (Table 3) indicated that there was a significant difference ($P<0.05$) in body weight change between treatments and breeds of ewes. But a further SNK test of the means indicated no significant difference between breeds. When the effect of treatment on individual breeds were subjected to an F test, grain feeding produced significantly ($P<.05$) higher body weight gain in only two breeds (Line M and Finn). Animals which received no grain in these two breeds actually lost weight, while under the same treatment, ewes of the Suffolk breed gained weight. This resulted in a significant ($P<0.05$) interaction between

Table 2. Percentage of ewes showing first estrus during a certain time of the breeding season

	Period			
Breed	First	Second	Third	Fourth
Line M	-	44.4	33.3	22.2
Suffolk	30	-	60	10
Finn	40	10	30	20

First period = 1-15 August

Second period = 16-31 August

Third period = 1-15 September

Fourth period = 16-30 September

Table 3. Effect of Treatment and Breeds on Body Weight Changes (kg)

	Treatment				Analysis of Variance (Anova)			
						DF	MS	F
Breed	Control	No.	Treated	No.	Total	28		
Line M	-1.8±2.7	5	6.0±2.1[a]	5	Treatment (T)	1	380.8	34.3*
Suffolk	2.6±1.1	4	3.7±3.1	5	Breed (B)	2	39.7	3.5*
Finn	-2.0±1.1	5	3.0±1.1[a]	5	T X B	2	53.3	4.8*
Total	-0.6±1.6	14	4.2±1.5[a]	15	Error	23	11.1	

*Significantly different ($P<0.05$).

[a]Significantly different from the control animals ($P<0.05$).

breed and treatment.

Cycle Length

An accurate record of cycle length was obtained from seven Finn, eight Line M and seven Suffolk ewes. Overall cycle length was 17.2±0.5 days (n = 66). The average length of the first, second and third cycles is given in Table 4. There was no significant difference in cycle length ($P>0.05$) between the first, second or third estrous cycle or between breeds. However, the first cycle in the Suffolks and the Finns appeared to be a few days longer than their second or third cycle (Table 4).

Gestation Length

Two ewes died before lambing and two were not pregnant. When gestation length was calculated back from the lambing date to the day of the fourth estrus, it was apparent that two ewes did not conceive at this time, since their gestation lengths were 173 and 182 days. They obviously did not conceive until mating at their next (or fifth) estrus. The remainder of the ewes had a gestation length ranging from 140 to 158 days with an overall mean of 148.2±0.7 days. Although Finn ewes appeared to have a one or two day shorter gestation length than the other two breeds (149.2±1.4, 148.4±0.5 and 147.0±1.3 days for Line M, Suffolk and Finn, respectively), there was no significant difference between breeds (Table 5). Treatment also did not appear to effect gestation length.

Table 4. First, Second and Third Estrous Cycle Length (Days ± S.E.)

Breed[a]	Cycle Length (Days) First	Second	Third
Line M (7)	16.0±1.7	17.0±0.7	17.4±0.7
Suffolk (8)	18.4±4.0	16.7±0.8	16.3±0.3
Finn (7)	19.7±2.0	16.3±0.8	17.1±0.7
Total 22	17.9±1.5	16.7±0.4	16.9±0.3

Anova	df	MS	F
Total	65		
Breed (B)	2	4.8	0.2
Cycle (C)	2	9.9	0.5
B X C	4	12.7	0.6
Error	57	19.7	

[a]Number of animals observed is indicated in parentheses.

Table 5. Gestation Length (Days ± S.E.)

	Treatment		Anova			
				df	MS	F
Breed	Control[a]	Treated[a]	Total	23		
Line M	149.2±2.9 (4)	149.2±0.6 (4)	Treatment (T)	1	0.2	0.01
Suffolk	149.3±0.7 (3)	147.7±0.6 (4)	Breed (B)	2	11.0	0.8
Finn	146.4±2.1 (5)	147.7±1.7 (4)	T X B	2	4.1	0.3
Total	147.2±1.0 (12)	148.2±0.6 (12)	Error	18	13.4	

[a]Number of observations are given in parentheses.

Lambing Rate

Lambing rate was not significantly different between treatments but there was a significant ($P<0.05$) breed difference (Table 6). Ewes of the Finn breed had a significantly greater lambing rate than ewes of the other two breeds (1.7 ± 0.1, 1.7 ± 0.2 and 2.5 ± 0.2 for Line M, Suffolk and Finn, respectively).

Hormonal Profiles

The hormonal profiles during the estrous cycle (from the third to the fourth estrous period of the breeding season) for each breed are given in Figures 7, 8 and 9.

Progesterone

The mean progesterone concentration for the complete estrous cycle obtained from ten Line M, eight Suffolk and nine Finn ewes was 1.4 ± 0.1, 1.3 ± 0.1 and 1.3 ± 0.8 ng/ml, respectively. Mean concentrations for the treated and the untreated groups were 1.4 ± 0.1 and 1.3 ± 0.1 ng/ml, respectively. No significant differences were observed between either breeds or treatment (Table 7). All breeds showed a similar progesterone profile (Figures 7, 8 and 9). Values obtained on the day of estrus were lower than 1 ng/ml. Concentrations gradually rose to more than 1 ng/ml within a few days after estrus, remained high (at approximately 2 ng/ml) for 10 to 12 days and then dropped to baseline levels shortly prior to the next estrus.

Table 6. Lambing Rate (lambs/ewe ± S.E.)

Breed	Treatment		Anova			
	Control[a]	Treated[a]		df	MS	F
			Total	25		
Line M	2.0±0.0 (4)	1.6±0.2 (5)	Treatment (T)	1	0.4	0.2
Suffolk	1.7±0.2 (4)	1.7±0.2 (4)	Breed (B)	2	1.8	9.0*
Finn	2.4±0.2 (5)	2.7±0.2 (4)	T X B	2	0.3	1.5
Total	2.1±0.1 (13)	2.0±0.2 (13)	Error	21	0.2	

[a]Number of observations are shown in parentheses.

*(P<0.05).

Table 7. Effect of Treatment and Breed on Progesterone Concentration (ng/ml ± S.E.) during the Estrous Cycle

	Treatment		Anova			
				df	MS	F
Breed	Control[a]	Treated[a]	Total	26		
Line M	1.4±0.1 (5)	1.4±0.3 (5)	Treatment (T)	1	0.07	0.63
Suffolk	1.3±0.1 (4)	1.4±0.2 (4)	Breed (B)	2	0.01	.90
Finn	1.3±0.1 (5)	1.4±0.1 (4)	T X B	2	0.01	0.90
Total	1.3±0.1 (14)	1.4±0.1 (13)	Error	21	0.11	

[a]Number of ewes are indicated in parentheses.

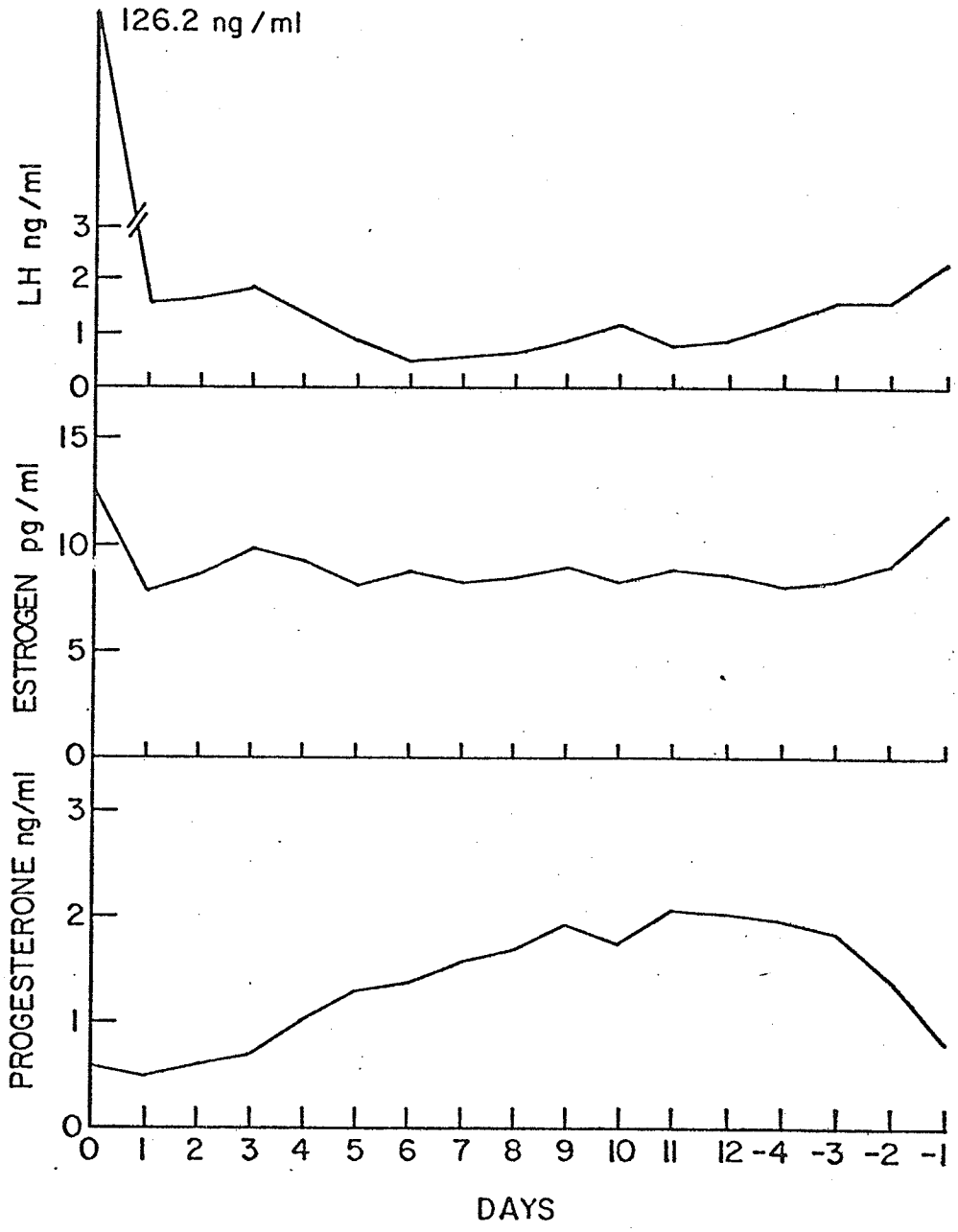

Figure 7. The hormone profile during the estrous cycle in the Line M breed (day 0 = day of estrus).

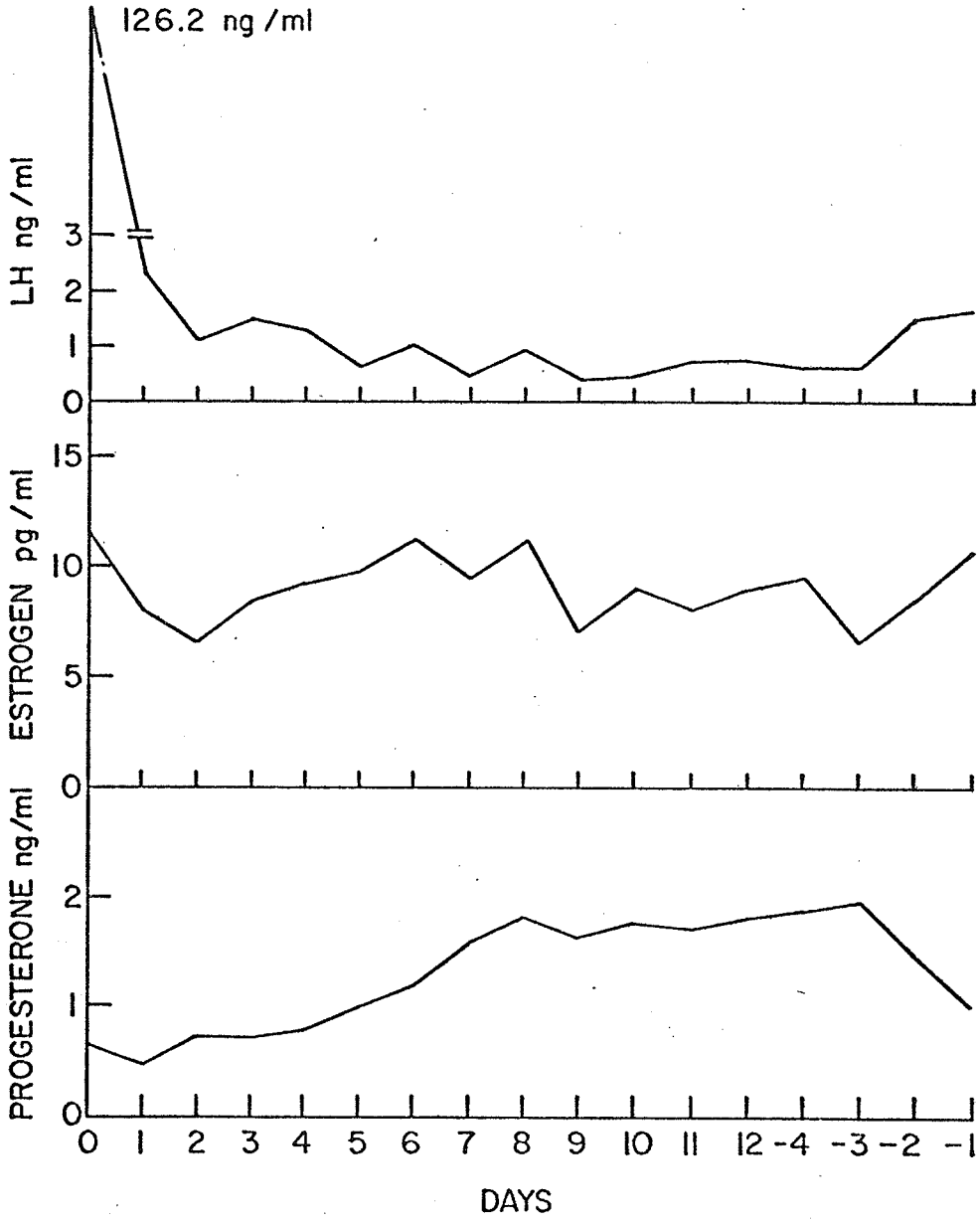

Figure 8. The hormone profile during the estrous cycle in the Suffolk breed (day 0 = day of estrus).

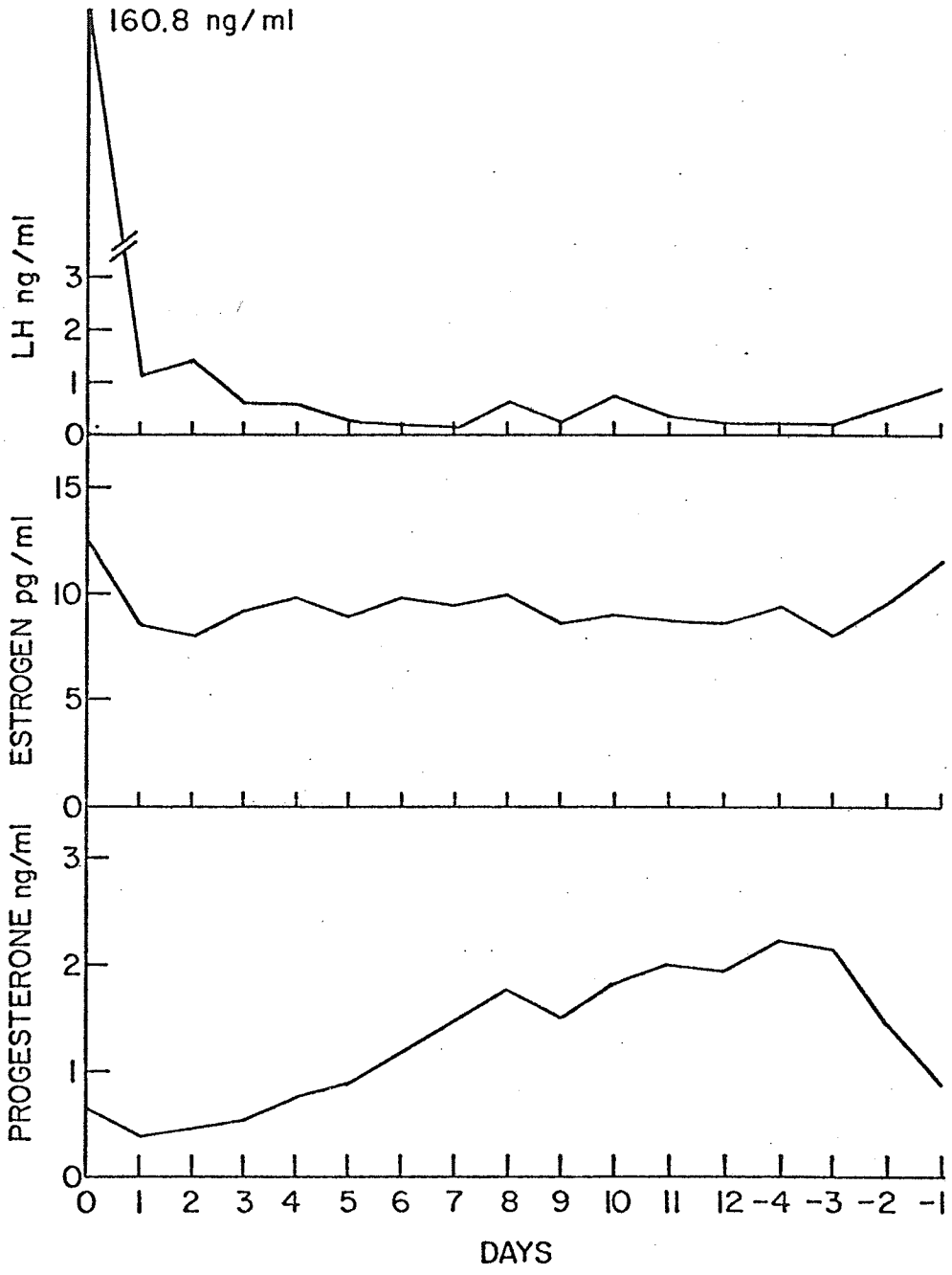

Figure 9. The hormone profile during the estrous cycle in the Finn breed, (day 0 = day of estrus).

Estrogens

Baseline values for estrogens varied from <2 to 25 pg/ml with an overall average of 9.1±1.4 pg/ml. The estrogen peak around estrus ranged from 7 to 29 pg/ml with an overall mean of 16.1±1.2 pg/ml. Pooled values of each breed showed little day to day variation and a mean peak value which was not much higher than the baseline level (Figures 7, 8 and 9). There was no significant consistent difference in mean estrogen concentration throughout the estrous cycle due to breed or treatment (Table 8). However, there was a significant interaction between breed and treatment. Twenty percent of the ewes (regardless of breed) showed the estrogen peak on the day before estrus while in the rest the estrogen peak was seen on the day of estrus.

LH

During the luteal phase (when progesterone levels are high) daily LH concentrations remained low and showed little variation. However, for a few days prior to and after estrus slightly higher LH levels were noticed (Figures 7, 8 and 9). There was no significant difference between treatments in mean LH levels throughout the estrous cycle (Table 9). However, Finn ewes had a significantly lower mean LH concentration than the other two breeds according to the SNK test. Mean LH levels were 1.3±0.1, 1.0±0.1 and 0.6±0.1 ng/ml for the Line M, Suffolk and Finn, respectively.

Table 8. Effect of Treatment and Breed on Mean Concentration (pg/ml ± S.E.) of Estrogens during the Estrous Cycle

	Treatment		Anova			
				df	MS	F
Breed	Control[a]	Treatment[a]	Total	26		
Line M	7.7±0.8 (5)	10.2±0.9 (5)	Treatment (T)	1	6.5	0.7
Suffolk	9.0±1.0 (4)	9.0±2.1 (4)	Breed (B)	2	5.1	0.6
Finn	13.6±2.2 (5)	7.6±1.2 (4)	T X B	2	45.2	5.1*
Total	9.9±1.0 (14)	8.9±0.8 (13)	Error	21	8.8	

[a]Number of ewes observed is shown in parentheses.

*(P<0.05).

Table 9. Effect of Breed and Treatment on Mean LH Concentration (ng/ml ± S.E.) during the Estrous Cycle (not including the Pre-ovulatory Surge)

	Treatment		Anova			
				DF	MS	F
Breed	Control[a]	Treated[a]	Total	26		
Line M	1.3±0.2 (5)	1.3±0.3 (5)	Treatment (T)	1	0.02	0.28
Suffolk	1.0±0.2 (4)	1.1±0.1 (4)	Breed (B)	2	0.95	4.52*
Finn	0.6±0.2 (5)	0.7±0.3 (4)	T X B	2	0.03	0.16
Total	1.0±0.1 (14)	1.0±0.1 (13)	Error	21	0.21	

[a]Number of ewes observed is shown in parentheses.

*($P<0.05$).

Ovulatory LH Surge

Due to the blood collection regime the complete pre-ovulatory surge of LH was obtained from only two ewes from each breed in the control group and from five Line M, three Suffolk and two Finn ewes in the treated group. Therefore statistical analysis was done by comparing breeds only and only treatment means are given. The mean interval from the onset of estrus, magnitude, duration and secretion rate of the LH surges are given in Table 10. The interval from the onset of estrus to when LH concentration rose to above 5 ng/ml is expressed in hours, the magnitude in ng/ml, the duration of secretion in hours and the secretion rate as ng/ml/h (which was derived from computing the area under the curve/h as described by Land et al., (1973). Due to incomplete curves obtained in this experiment it was necessary to measure duration and secretion rate from LH concentrations above 40 ng/ml.

Treatment appeared to have little influence on the above parameters, as indicated by the means in Table 10. However Finn ewes had a significantly ($P<0.05$) longer interval to LH release than the other two breeds. Although the mean magnitude and secretion rate for the Finn ewes was higher than in the other two breeds, statistically there was no difference. Finn ewes had a significantly ($P<0.05$) longer duration of surge than the other two breeds (Table 10). The profile of the pre-ovulatory LH surge is given in Figure 10 for each breed.

Table 10. Mean Interval from the onset of Estrus, Magnitude, Secretion Rate and Duration of the Pre-ovulatory LH Surge

	Breed			Treatment	
	Line M	Suffolk	Finn	Control	Treated
Interval (h ± S.E.)	2.1±0.2	6.1±1.6	*10.5±1.5	5.9±1.8	7.1±1.4
Magnitude (ng/ml ± S.E.)	141.1±29.4	150.6±19.0	209.5±31.0	181.3±34.5	150.0±15.7
Duration (h ± S.E.)	4.9±0.5	4.7±0.4	*7.0±0.3	5.7±0.5	5.2±0.0
Secretion rate (ng/ml/h ± S.E.)	46.5±7.9	48.9±5.8	62.6±7.2	63.2±7.3	43.9±3.5

Interval = time from onset of estrus to beginning of LH discharge (when values exceeded 5 ng/ml) expressed in h.

Secretion rate = derived from area under the $\frac{\text{curve}}{\text{time}}$, from above 40 ng/ml is expressed as ng/ml/h (Land *et al.*, 1973).

Magnitude = highest concentration, expressed as ng/ml.

Duration = time from LH rise from and fall to 40 ng/ml, expressed as h.

*Significantly ($P<0.05$) different from other two breeds.

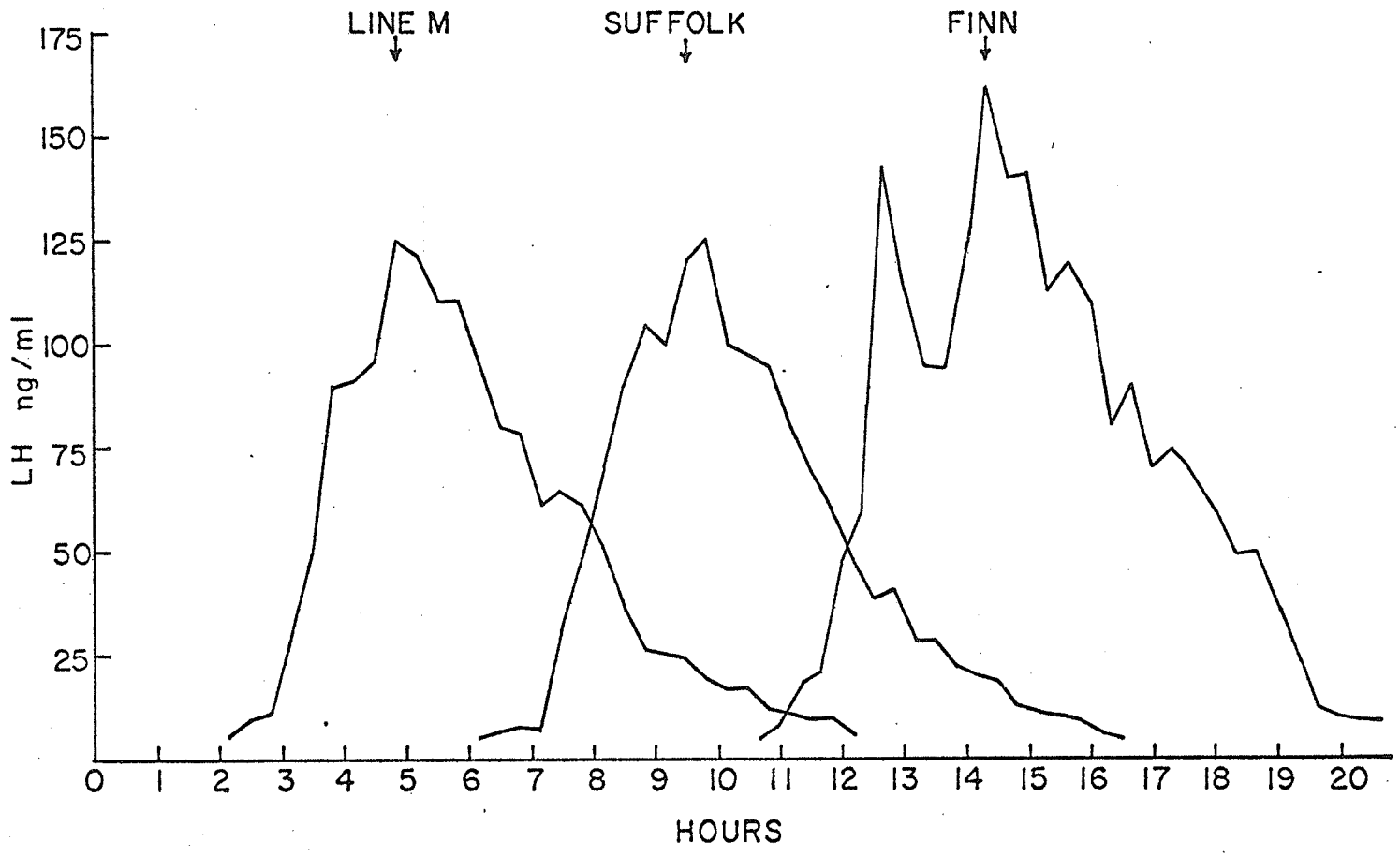

Figure 10. The profile of the pre-ovulatory LH surge in three breeds of ewes (onset of estrus = 0 h).

DISCUSSION

Under the natural conditions allowed the ewes in this study, there was a breed difference in the onset of the breeding season; with more Finn ewes starting to cycle earlier than Line M ewes (Table 2). However, Wheeler and Land (1973) reported that the onset of the breeding season in the Finn breed to be October 7, which is much later than in this study. This may be the result of geographical differences since their work was done in Europe at a different latitude. The difference between the two studies may also be because of differing genetic base in the two flocks, since differences in the duration of the breeding season do exist between breeds and strains within breeds (Cole and Cupps, 1969).

There was no significant difference in cycle length between the first and subsequent estrous cycles. This finding is in agreement with that of Foote et al. (1970). However, abnormal cycle lengths early in the breeding season have been reported (Hafez, 1952). The reason for inaccuracy of estrous detection in eight of the ewes could not be pinpointed, but various factors may have contributed such as:

1) infrequent observation at the beginning of the breeding season,
2) paint may have dried up,

3) wetness of apron (due to urine) caused dirtiness on the apron itself, therefore, mark could not be differentiated from the wool soiled due to rain,

4) too infrequent renewal of paint on the ram's brisket,

5) ram may have had preference for one ewe.

The increase in body weight due to grain feeding appeared to have no influence on estrous cycle length. Grain feeding produced significantly higher body weight gains in the Line M and Finn breeds. This is in agreement with reports by Hulet et al. (1974) and Dufour and Wolynetz (1977). Both control and treated ewes of the Suffolk breed gained weight. Since Suffolk ewes are heavier and more aggressive than the other two breeds, it is possible that these ewes could have competed better for hay during group feeding. Therefore, control ewes from the other two breeds did not receive their full allotment and thus lost weight.

Grain treatment in this study did not produce a difference in lambing rate which is similar to the results reported by Price et al. (1973). Hulet et al. (1974) also reported that level of feeding had no effect on lambing rate except during periods of low lambing rate, i.e., during the early and late portion of the season.

Finn ewes had a greater litter size than the other two breeds. Similar differences in litter size between

Finns and other breeds have been reported by Land (1970) and also by Donald et al. (1968). Hulet and Foote (1967) have shown a positive correlation between lambing rate and ovulation rate. Therefore, Finn ewes probably had a higher ovulation rate than the Line M and Suffolk ewes.

Mean gestation length fell within the normal range for all breeds (Hafez, 1952). Treatment and breed appeared to have no significant effect, but the Finn ewes did, however, have a mean gestation length which was one or two days shorter than the ewes of the other two breeds. This may have been the result of the greater litter size, which is suggested to shorten mean gestation length (Hafez, 1952).

The pattern and concentration of progesterone in this study is in agreement with the results obtained by many other investigators (Stabenfeldt et al., 1969; Thorburn et al., 1969; Obst and Seamark, 1970; Bindon, 1971; Robertson and Sarda, 1971; Pant et al., 1972; McNatty et al., 1973; Yuthasastrakosol et al., 1975b). Mean daily progesterone concentration throughout the estrous cycle was not affected by treatment or breed.

Variable results in the estrogen concentration in plasma or serum have been obtained by different investigators (Cox et al., 1971a and b; Obst et al., 1971; Scaramuzzi et al., 1970; Bjersing et al., 1972; Pant et al., 1972; Van Der Walt et al., 1972; Yuthasastrakosol et al., 1975b) even when similar sources of samples (plasma or

serum) and methods were used. The baseline level in this study was 9.1±1.0 pg/ml which is about twice that reported by Yuthasastrakosol et al. (1975b). However, the mean peak values in the two studies are comparable, but both are lower than those reported by Obst et al. (1971), Pant et al. (1972) and Van Der Walt et al. (1972). Samples taken from the ovarian vein (Scaramuzzi et al., 1970; Bjersing et al., 1972) appeared to have higher values than those from the jugular vein (Pant et al., 1972; Obst et al., 1971; Van Der Walt et al., 1972; Yuthasastrakosol et al., 1975b). Occurrence of the estrogen peak appeared to be related to the source of samples (ovarian vs peripheral blood). Scaramuzzi et al. (1970), Cox et al. (1971a and b) and Bjersing et al. (1972) reported that the estrogen peak occurred a day before estrus when samples were collected from the ovarian vein, while Pant et al. (1972), Van Der Walt et al. (1972) and Yuthasastrakosol et al. (1975b) observed the peak to occur on the day of estrus in peripheral blood samples. Eighty percent of the ewes in this study showed the estrogen peak on the day of estrus. Pant et al. (1972) suggested that the difference in the time of the estrogen peak due to the source (location) of the sample might have been caused by the surgery and anesthesia involved in blood collection from the ovarian venous blood which might have changed the length of the estrous cycle in the ewe.

Pooled values for each breed reduced day to day variation of estrogen concentrations. Individual ewe profiles showed estrogen peaks between day 3 and 7 of the estrous cycle in about 50% of the ewes. Secondary rises of estrogen were also observed by Mattner and Braden (1972), Cox et al. (1971a and b), Holst et al. (1972), Scaramuzzi et al. (1970) and Hauger et al. (1977).

The LH level was low during the luteal phase in all ewes. This supports the hypothesis that progesterone alone, or in combination with estradiol-17B, produces a suppressive effect on GN-RH-induced LH release, (Debeljuk et al., 1972; Przekop et al., 1972). Karsch et al. (1977) demonstrated that progesterone alone could suppress LH level in ovariectomized ewes. Some rise in LH was observed a few days prior to or after estrus, and this was the period when progesterone levels were still low indicating a lesser effect of progesterone on the hypothalamus and/or pituitary. This result is in agreement with Hauger et al. (1977). Whether the hypothalamus or the pituitary is the main site of progesterone negative feedback is not known. Labhsetwar (1971) concluded that in rats progesterone might act at all levels, including the ovaries.

Amounts of LH during days of non-estrus ranged from undetectable levels to 4.1 ng/ml. The findings regarding LH levels in this study are in general agreement with the results obtained by Reeves et al. (1970a), Roche et al.

(1970), Geschwind and Dewey (1968), Niswender et al. (1968), Scaramuzzi et al. (1970), Yuthasastrakosol et al. (1975b) and Land et al. (1973). However, the latter investigator (Land et al., 1973) reported that more prolific breeds had higher (but not statistically significant) mean LH levels on non-estrous days which is the reverse of the results of this study. Occurrence of the pre-ovulatory LH surge on the day of estrus has also been reported by Scaramuzzi et al. (1970), Reeves et al. (1970a), Bjersing et al. (1972) Cumming et al. (1972), Nett et al. (1974), Geschwind and Dewey (1968), Wheatley and Radford (1969), Goding et al. (1969), Land et al. (1972), Yuthasastrakosol et al. (1975b) and Hauger et al. (1977).

A statistical comparison between the two treatment groups regarding the pre-ovulatory LH status could not be made due to insufficient data. However, the mean values obtained did not show great differences due to the treatment on interval from onset of estrus to LH surge, magnitude of the surge, duration and secretion rate (Table 10). Jones et al. (1976) and Lishman et al. (1977) also reported that there was no effect of energy level on the LH peak in cows.

A breed comparison revealed that Finn ewes had a significantly ($P<0.05$) longer interval from estrus to the pre-ovulatory LH surge than the other two breeds. Raabe and Wheaton (1976) obtained similar results when they

compared Finn, Suffolk and Finn X Suffolk ewes. Land et al. (1973) also reported breed differences in ewes, while Thimonier and Pelletier (1971) also reported the longer interval in double-ovulating ewes in French breeds. Since litter size is positively related to ovulation rate (Hulet and Foote, 1967), the present results suggested indirectly that the Finn breed had a greater ovulation rate, possibly due to the longer interval from estrus to the pre-ovulatory LH surge. It is possible that the longer interval may allow more time for the pituitary to recover from the negative feedback of progesterone or may allow for more estrogen to be excreted, thus causing a greater release of LH.

Significant differences in the magnitude of LH peak between breeds was reported by Raabe and Wheaton (1976) and Land et al. (1973). Although the present results were not statistically different the mean value for the Finn ewes was highest (Table 10). This higher value may be physiologically important in terms of ovulation induction. More detailed data is needed to confirm this type of comparison. Frequency of blood sampling is also important, since LH half-life is about 28 minutes according to Geschwind and Dewey (1968). The above investigators only collected blood samples every 2 or 6 h and therefore, their results may not be close to the actual values.

As indicated earlier the insufficient data in this study did not allow statistical comparison of the duration of LH release and secretion rate of LH when LH levels were below 40 ng/ml. Thus results reported here only include that area of the curve when values were above 40 ng/ml. Raabe and Wheaton (1976) did not find any breed difference in the duration of the LH surge, while Land et al. (1973) obtained contrary results with which the present study would agree.

The data on secretion rate from this study is contradictory to that of Land et al. (1973) who reported significant differences between breeds. However, the mean value for the secretion rate in Finn ewes in this study had the highest values. It is possible that data obtained here would have been comparable to that of Land et al. (1973) if comparisons could have been made by using the area of the curve which was lower than 40 ng/ml. One consistent finding between this study and those of Land et al. (1973) and Raabe and Wheaton (1976) is the breed difference in the interval from the onset of estrus to the LH surge.

CONCLUSION

Grain treatment during the third estrous cycle appeared to produce no effect on reproductive performance or endocrine status in mature ewes. There were breed differences in lambing rate, which may have been related to ovulation rate and the pre-ovulatory LH status. Results on the pre-ovulatory LH status were not conclusive. Differences in the interval from the onset of estrus to the pre-ovulatory LH surge in different breeds of ewes was confirmed. Indirect evidence (litter size) suggested that Finn ewes have a greater ovulation rate than Line M and Suffolk ewes. Further study on blood levels of pituitary gonadotrophins and ovulation rate is necessary in order to confirm the relationship between the above parameters and breed differences.

EXPERIMENT III

The blood collection regime in the previous experiment did not provide enough data to make firm conclusions on breed differences in terms of the pre-ovulatory LH surge. However, results from experiment II showed trends toward differences between breeds in the interval from the onset of estrus to the pre-ovulatory LH surge and the duration of LH surge. A difference in secretion rate of LH at estrus was reported by Land et al. (1973). Thimonier and Pelletier (1971) have shown differences in the interval from the onset of estrus to the LH surge between breeds and between single and multiple ovulators in French breeds. Raabe and Wheaton (1976) observed differences in the interval from the onset of estrus to the LH surge in Suffolk, Finn and Suffolk X Finn ewes, but not in the duration of the LH surge. The interval from the onset of estrus to the LH surge has been reported by Geschwind and Dewey (1968), Niswender et al. (1968), Goding et al. (1969), Lishman et al. (1974) and Wheatley and Radford (1969). Their results ranged from 0 to 18 hours. Similarly the duration of the LH surge ranged from 4 to 13 hours.

Varying results obtained by the above investigators may be due to three main factors:

1) frequency of estrus observation,

2) frequency of sample collections, and

3) differences between breeds of ewes.

Since the half-life of LH is about 30 minutes (Geschwind and Dewey, 1968) frequent sampling is needed.

The present experiment was designed not only to clarify results of the previous experiment, but also to determine if there are changes in the characteristics of the pre-ovulatory LH surge during a breeding season. The second objective was undertaken since at the time that the study was initiated there were no reports on possible changes in the pre-ovulatory LH surge in relation to changes in ovulation rate during the breeding season. Several previous investigators have suggested that the ovulation rate increases as the breeding season progresses (Dermody et al., 1966; Hulet and Foote, 1967; Hulet et al., 1969; Land et al., 1973 and Hulet et al., 1974).

MATERIALS AND METHODS

Five ewes of each of the Suffolk and Finn breeds were maintained indoors under natural lighting conditions. Estrus detection began in the middle of August 1976, using vasectomized rams. Rams were kept in a separate pen next to the ewes. Detection was made by putting rams into the ewe pen and observing for 15 to 20 minutes. Frequency of checking was every 4 h or less. Blood collection began as soon as estrus was detected and continued at 20 minute intervals for 20 to 24 h. Towards the end of the breeding season, blood samples were collected every 3 or 4 days for a period of a few weeks after the last estrus was observed. Serum was analysed for LH and progesterone as described previously.

RESULTS

Breeding Season

The breeding season for Finn ewes started earlier and lasted longer as compared to Suffolk ewes (Table 11). The average number of estrus periods observed during the whole breeding season was 11.6±1.0 for the Finn and only 5.2±0.4 for the Suffolk (Table 13).

Estrous Cycle Length

There was no significant difference in estrous cycle length between breeds or between first, second and subsequent cycles within the same breed (Table 12a and b). Overall estrous cycle length for the Finn ewes was 17.0± 0.1 days (range from 14 to 21 days) and for the Suffolk ewes 17.1±0.1 days (range from 16 to 19 days).

Table 13 shows the record of blood collections done throughout the breeding season. In Finn ewes, attempts were made to collect blood at 41 out of the 62 estrous periods observed. Of the 41 estrous periods, only 22 yielded complete (C) pre-ovulatory LH peaks. The other 19 were incomplete (I). In Suffolk ewes, blood was collected at 25 out of 30 estrous periods and 19 complete pre-ovulatory LH peaks were obtained.

Although taking of blood samples did not permit an accurate record of the length of estrus, Finn ewes appeared to have a somewhat longer period of estrus than Suffolk ewes.

Table 11. Length of Breeding Season in Finn and Suffolk Ewes

Breed	Ewe No.	Breeding Season: Date of 1st Estrus	Breeding Season: Date of Last Estrus	Length of Breeding Season (days)
Finn	3008	20 Sept./76	4 Apr./77	197
	3009	14 Sept./76	4 May/77	263
	3086	30 Aug./76	19 Mar./77	202
	3249	23 Sept./76	27 Mar./77	186
	3001	1 Oct./76	3 Mar./77	154
Mean ± S.E.				200.4±17.3
Suffolk	1007	14 Sept./76	26 Dec./76	120
	4037	17 Sept./76	10 Dec./76	85
	4039	17 Sept./76	28 Dec./76	103
	3051	20 Sept./76	15 Dec./76	87
	1017	16 Sept./76	17 Dec./76	110
Mean ± S.E.				101.0±6.7

Table 12. Estrous cycle length (days)

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	17.0 ±0.1	57
Suffolk	17.1 ±0.1	26

ANOVA

	df	MS	F
Total	82	-	-
Breed	1	0.5	0.3
Animal/Breed	8	1.5	1.5
Error	73	1.0	-

(b) Comparison of the estrous periods during the breeding season within the same breed

FINN

Cycle length	$\bar{X}$ ± SE	No.
1	16.2 ±0.3	5
2	16.6 ±0.2	5
3	16.8 ±0.4	5
4	17.2 ±0.3	5
5	17.0 ±0.3	5
6	17.4 ±0.5	5
7	16.8 ±0.2	5
8	18.0 ±0.9	5
9	16.8 ±0.9	5
10	17.2 ±0.5	4
11	17.0 ±0.4	4

ANOVA

	df	MS	F
Total	52	-	-
Cycle length	10	1.1	1.0
Error	42	1.2	-

SUFFOLK

Cycle length	$\bar{X}$ ± SE	No.
1	16.6 ±0.2	5
2	16.8 ±0.2	5
3	17.6 ±0.4	5
4	17.2 ±0.4	5
5	17.5 ±0.5	4

ANOVA

	df	MS	F
Total	23	-	-
Cycle length	4	0.9	1.5
Error	19	0.6	-

Table 13. Record of blood collection at estrus throughout breeding season

(a) FINN

Animal No.		Number of estrus period														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3008	Date	20/9/76	7/10/76	24/10/76	11/11/76	29/11/76	16/12/76	4/1/77	21/1/77	9/2/77	28/2/77	18/3/77	4/4/77			
		-	A	A	A	A	A	-	A	-	A	-	-			
		-	C	I	I	C	I	-	C	-	C	-	-			
3009	Date	14/9/76	30/9/76	16/10/76	1/11/76	18/11/76	5/12/76	23/12/76	9/1/77	26/1/77	12/2/77	1/3/77	18/3/77	4/4/77	20/4/77	4/5/77
		A	A	A	A	A	A	A	-	A	-	A	-	-	-	-
		I	C	I	I	I	I	C	-	C	-	I	-	-	-	-
3086	Date	30/8/76	14/9/76	30/9/76	17/10/76	3/11/76	19/11/76	6/12/76	23/12/76	9/1/77	25/1/77	12/2/77	28/2/77	19/3/77		
		-	A	A	A	A	A	A	A	-	A	-	A	-		
		-	I	C	C	C	C	I	I	-	C	-	I	-		
3249	Date	23/9/76	10/10/76	27/10/76	12/11/76	29/11/76	16/12/76	2/1/77	18/1/77	3/2/77	21/2/77	9/3/77	27/3/77			
		A	A	A	A	A	A	-	A	-	-	A	-			
		I	C	I	I	C	C	-	C	-	-	C	-			
3001	Date	1/10/76	17/10/76	3/11/76	20/11/76	7/12/76	25/12/76	10/1/77	27/1/77	17/2/77	3/3/77					
		A	A	A	A	A	-	A	A	-	-					
		C	C	C	I	C	-	I	C	-	-					

(b) SUFFOLK

1007	Date	14/9/76	1/10/76	18/10/76	4/11/76	21/11/76	9/12/76	26/12/76
		A	A	A	A	A	A	-
		C	C	C	C	C	C	-
4037	Date	17/9/76	3/10/76	19/10/76	5/11/76	22/11/76	10/12/76	
		A	A	A	-	A	A	
		I	C	C	-	C	C	
4039	Date	17/9/76	3/10/76	20/10/76	6/11/76	22/11/76	10/12/76	28/12/76
		A	A	A	-	A	A	A
		I	C	I	-	C	C	I
3051	Date	20/9/76	7/10/76	24/10/76	11/11/76	29/11/76	15/12/77	
		-	A	A	A	A	A	
		-	C	C	C	I	I	
1017	Date	16/9/76	3/10/76	20/10/76	8/11/76	26/11/76		
		-	A	-	A	A		
		-	C	-	C	C		

A = Collection made at that estrus.
C = Complete pre-ovulatory LH surge was obtained.
I = Incomplete LH curve.
- = No collection.

Interval to Pre-ovulatory LH Surge

The interval from onset of estrus to the pre-ovulatory LH surge (when LH concentration rose to above 5 ng/ml), was significantly different between breeds, being 13.8±0.6 h for Finn and 6.4±0.6 h for Suffolk ewes (Table 14a). A comparison of the interval length at each estrus within the same breed throughout the breeding season showed no significant difference (Table 14b).

Similar results were obtained for the time interval from the onset of estrus to the time of the maximum point of the pre-ovulatory LH peak (Table 15a and b). Finn ewes took about 3.3 h from the beginning of the LH surge to the peak while Suffolk ewes took 5.0 h. These data were calculated by subtracting the interval from the onset of estrus to the pre-ovulatory LH surge from the interval from the onset of estrus to its peak (Table 14a and 15a).

Magnitude

Although Finn ewes had a somewhat higher peak magnitude of LH release (156.7±9.5 vs 145.7±13.5 ng/ml) there was no significant difference between breeds (Table 16a). The same is true for the comparison made between estrous periods within the same breed (Table 16b). However, there was significant ($P<0.05$) variation between animals within the same breed (Table 16a).

Table 14. Interval from the onset of estrus to the pre-ovulatory LH surge (h).

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	13.8 ±0.6	39
Suffolk	6.4 ±0.6	18

ANOVA	df	MS	F
Total	57	-	-
Breed	1	674.8	50.7*
Animal/Breed	8	13.3	1.2
Error	48	11.0	-

*P<0.05

(b) Comparison of the estrous periods during a breeding season within the same breed

FINN

Cycle	$\bar{X}$ ± SE	No.
2	13.1 ±1.6	5
3	13.4 ±1.6	5
4	17.6 ±1.6	5
5	14.1 ±1.8	5
6	13.4 ±3.1	4
7	13.0 ±1.3	3
8	11.4 ±0.3	4

ANOVA	df	MS	F
Total	30	-	-
Cycle	6	16.9	1.2
Error	24	14.1	-

SUFFOLK

Cycle	$\bar{X}$ ± SE	No.
2	6.0 ±1.1	5
3	6.0 ±0.9	3
4	5.2 ±3.0	3
5	6.4 ±0.4	4
6	7.4 ±2.4	

ANOVA	df	MS	F
Total	17	-	-
Cycle	4	2.4	0.3
Error	13	9.1	-

Table 15. Interval from onset of estrus to the pre-ovulatory LH peak (h)

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	17.1 ±0.5	29
Suffolk	11.4 ±0.6	16

ANOVA

	df	MS	F
Total	46	-	-
Breed	1	409.3	66.0*
Animal/Breed	8	6.2	0.8
Error	37	7.4	-

(b) Comparison of the estrous periods during a breeding season within the same breed

FINN

Cycle	$\bar{X}$ ± SE	No.
2	17.4 ±1.6	4
3	17.0 ±1.3	3
5	18.4 ±0.7	4
6	15.0 ±2.0	3
8	16.1 ±0.7	4

ANOVA

	df	MS	F
Total	17	-	-
Estrus	4	5.8	0.9
Error	13	6.1	-

SUFFOLK

Cycle	$\bar{X}$ ± SE	No.
2	9.7 ±1.1	5
3	11.5 ±1.4	3
5	11.2 ±0.6	4
6	11.4 ±2.6	3

ANOVA

	df	MS	F
Total	14	-	-
Estrus	3	3.2	0.4
Error	11	7.4	-

Table 16. Magnitude of the pre-ovulatory LH surge (ng/ml)

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	156.7 ±9.5	29
Suffolk	145.7 ±13.5	23

ANOVA

	df	MS	F
Total	51	-	-
Breed	1	2160.3	0.2
Animal/Breed	8	12907.4	8.7*
Error	42	1484.4	-

*P<0.05

(b) Comparison of the estrous periods during the breeding season within the same breed

FINN

Estrus	$\bar{X}$ ± SE	No.
2	137.6 ±24.8	4
3	96.9 ±14.8	3
5	131.8 ±21.8	4
6	187.3 ±34.6	3
8	189.8 ±13.0	4

ANOVA

	df	MS	F
Total	17	-	-
Estrus	4	522.4	2.9
Error	13	179.5	-

SUFFOLK

Estrus	$\bar{X}$ ± SE	No.
2	160.0 ±41.7	5
3	138.1 ±26.1	4
4	91.4 ±14.4	3
5	161.7 ±44.7	4
6	162.0 ±28.1	4

ANOVA

	df	MS	F
Total	19	-	-
Estrus	4	304.2	0.6
Error	15	517.9	-

Duration of LH Release

Duration of release is defined as the time period from when LH concentration rose above 5 ng/ml to the time when it fell back to 5 ng/ml. Suffolk ewes had a significantly ($P<0.05$) longer duration than Finn ewes (Table 17a). However, there was no significant difference between estrous period during the breeding season in either breed (Table 17b).

Area Under the Pre-ovulatory LH Curve and Secretion Rate

Calculation of the area under the pre-ovulatory LH curve was similar to that used in Experiment II except a computer program (developed by Dr. F.S. Chebib, Dental School, University of Manitoba) was employed instead of manual estimations. The values are expressed as ng/ml x minutes. Secretion rate was calculated by dividing the area under the curve by the duration of the pre-ovulatory LH surge and expressed as ng/ml/h. There was no significant difference in either of these characteristics whether the comparison was made between breeds or between estrous periods within the same breed during the breeding season (Table 18a and b; Table 19a and b). There was however, significant ($P<0.05$) variation between animals within the same breed (Table 18a and Table 19a).

Table 17. Duration of pre-ovulatory LH surge (h)

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	11.4 ±0.3	22
Suffolk	12.4 ±0.3	19

ANOVA

	df	MS	F
Total	40	-	-
Breed	1	10.3	20.6*
Animal/Breed	8	0.5	0.2
Error	31	2.8	-

*P<0.05

(b) Comparison of the estrous periods during the breeding season within the same breed

FINN

Estrus	$\bar{X}$ ± SE	No.
2	10.8 ±0.4	4
5	10.6 ±0.9	4
8	11.0 ±0.3	3

ANOVA

	df	MS	F
Total	10	-	-
Estrus	2	.7	0.4
Error	8	1.7	-

SUFFOLK

Estrus	$\bar{X}$ ± SE	No.
2	12.0 ±0.3	5
3	13.5 ±0.9	3
4	12.5 ±1.2	3
5	11.9 ±0.7	4
6	11.9 ±0.6	3

ANOVA

	df	MS	F
Total	17	-	-
Estrus	4	1.6	0.9
Error	13	1.8	-

Table 18. Area under pre-ovulatory LH curve expressed as ng/ml x minute

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	3999.4 ±2.8	22
Suffolk	3771.5 ±2.8	19

ANOVA

	df	MS	F
Total	40	-	-
Breed	1	52.9	0.1
Animal/Breed	8	390.1	3.7*
Error	31	104.3	-

*P<0.05

(b) Comparison of the estrous periods during the breeding season within the same breed

FINN

Estrus	$\bar{X}$ ± SE	No.
2	4336.6 ±8.3	4
3	3247.5 ±6.7	4
8	4179.8 ±2.4	3

ANOVA

	df	MS	F
Total	11	-	-
Estrus	2	135.0	0.9
Error	9	156.4	-

SUFFOLK

Estrus	$\bar{X}$ ± SE	No.
2	4076.3 ±7.6	5
3	3790.3 ±4.0	3
4	2848.1 ±2.9	3
5	3939.9 ±9.0	4
6	3976.3 ±5.5	3

ANOVA

	df	MS	F
Total	17	-	-
Estrus	4	81.6	0.4
Error	13	188.7	-

Table 19. Pre-ovulatory LH secretion rate expressed as ng/ml/hr

(a) Comparison between breeds

Breed	$\bar{X}$ ± SE	No.
Finn	35.3±2.2	22
Suffolk	30.6±2.3	19

ANOVA

	df	MS	F
Total	40	-	-
Breed	1	2.3	0.8
Animal/Breed	8	2.7	4.5*
Error	31	0.6	-

*P<0.05

(b) Comparison of the estrous periods during the breeding season within the same breed

FINN

Estrus	$\bar{X}$ ± SE	No.
2	42.2±6.4	4
5	30.1±4.8	3
8	37.9±1.1	3

ANOVA

	df	MS	F
Total	10	-	-
Estrus	2	14.9	1.5
Error	8	9.6	-

SUFFOLK

Estrus	$\bar{X}$ ± SE	No.
2	33.7±6.1	5
3	27.9±1.9	3
4	23.1±2.4	3
5	32.8±7.0	4
6	34.0±6.2	3

ANOVA

	df	MS	F
Total	17	-	-
Estrus	4	7.3	0.6
Error	13	2.5	-

Anestrus

According to progesterone levels, ovaries remained active in ewes of both breeds for quite some time after the last overt estrus of the breeding season (Figure 11 and 12). Progesterone profiles showed the characteristic pattern of normal ovulation in three Suffolk and four Finn ewes. The period of fluctuating progesterone was observed as long as 71 days in the Finn and for 73 days in the Suffolk ewes after the last observed estrus.

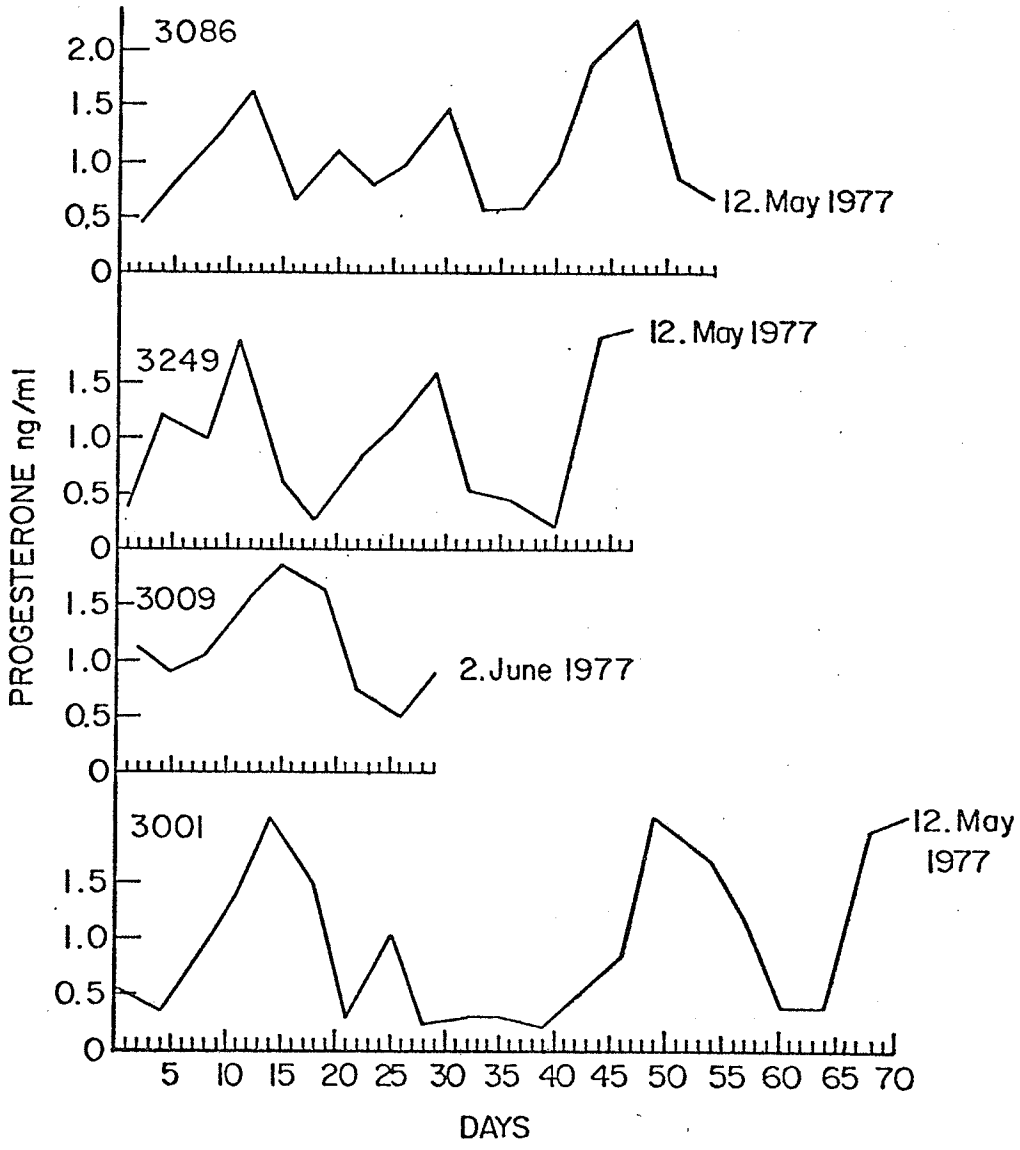

Figure 11. Progesterone profile of the last estrus of the breeding season in four Finn ewes (day 0 = day of the last observed estrus).

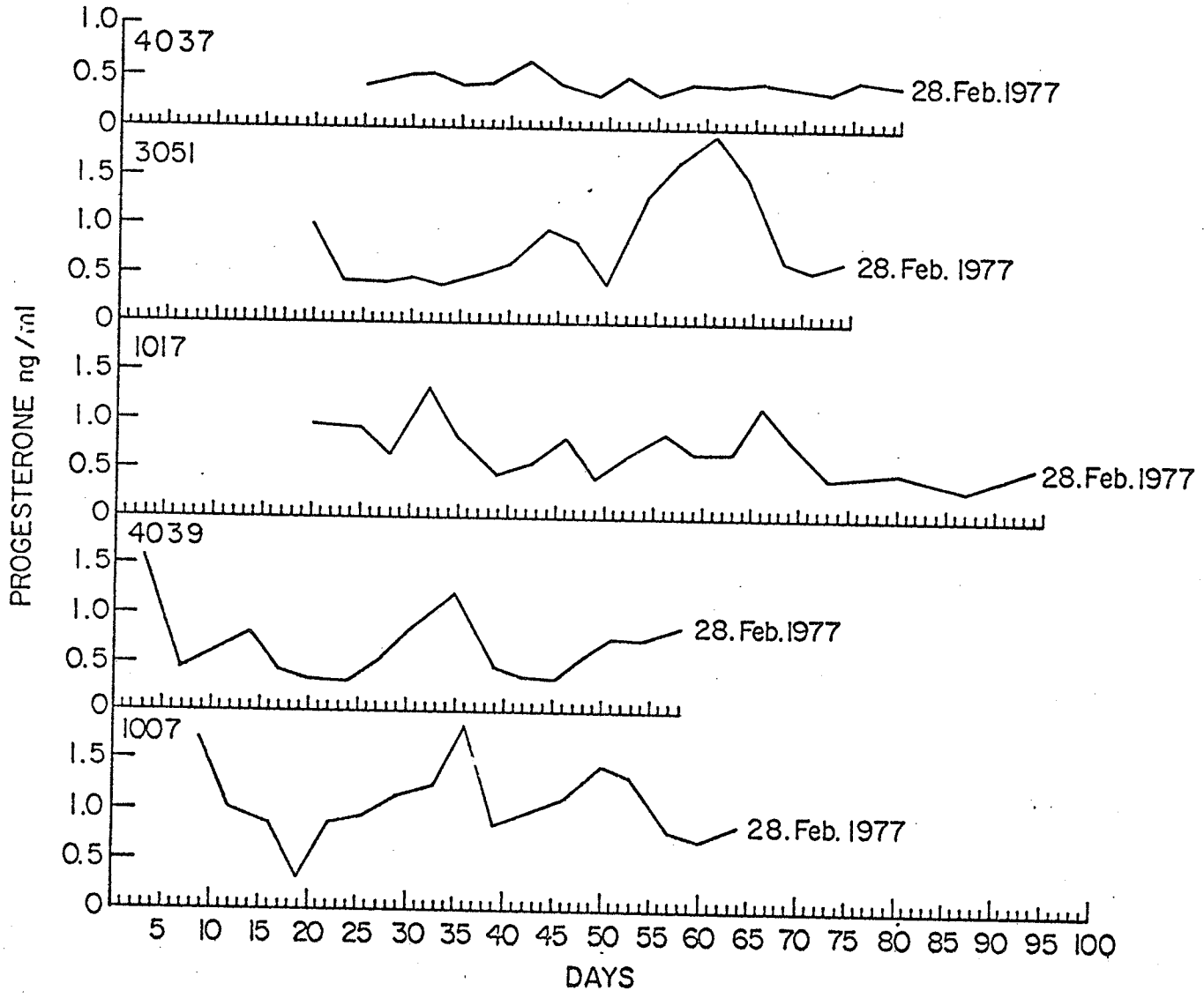

Figure 12. Progesterone profile after the last estrus of the breeding season in five Suffolk ewes (day 0 = day of the last observed estrus).

DISCUSSION

Onset of the breeding season in Finn and Suffolk ewes occurred in August which was similar to that seen in the second experiment. However, the breeding season in the Finn breed was longer than that of the Suffolk breed. This result is in agreement with that of Wheeler and Land (1973) who compared the length of the breeding season between Finn, Scottish Black Face and Tasmanian Merino breeds. The Finn breed had a season which extended from 7th October to 24th April. Land et al. (1973) reported that the longer breeding season was not due to a difference in the onset of the breeding season but in the time of onset of anestrus.

Estrous cycle lengths appeared to be regular from the first to the last estrus observed in both breeds. Normal cycle length at the onset of the breeding season was reported by Yuthasastrakosol et al. (1975b) in mature ewes, Foote et al. (1970) in ewe lambs and by Rawlings et al. (1977) in mature ewes toward the end of the breeding season. According to Hafez (1952) abnormal cycle length is more common early and late in the breeding season. The present experiment also agrees well with the second experiment of this study with respect to cycle length. The longer cycle length reported by Hafez (1952) at the onset of the breeding season may have resulted from inadequate estrus detection.

The interval from onset of estrus to the preovulatory rise of LH was significantly longer in Finn than

Suffolk ewes. Similar results were obtained when comparing Finn with Suffolk and Line M ewes in the second experiment of this study. This was also reported by Raabe and Wheaton (1976) when the comparison was made between Finn, Suffolk and Finn x Suffolk ewes. A significantly longer interval in more prolific breeds (European breeds) was also reported by Land et al.(1973) and Thimonier and and Pelletier (1971). However, the interval obtained in this experiment is longer than in the previous experiment and that of Raabe and Wheaton (1976). Frequency of estrus observation may be a contributing factor. Finn ewes took 1.7 h longer on the average than Suffolk ewes to reach the LH peak from the time that LH rose to more than 5 ng/ml.

Although there was no significant difference in terms of magnitude of the LH peak, Finn ewes had a higher mean peak LH concentration than Suffolk ewes. This is in agreement with the second experiment and with the report of Raabe and Wheaton (1976) who reported a non-significant difference. Land et al. (1973) reported significant differences between European breeds. Whether the non-significantly higher magnitude of LH contributed to higher ovulation rate is not known.

Suffolk ewes had a significantly longer duration of the pre-ovulatory LH surge than that of the Finn ewes which does not agree with the results of the second experiment. However, in the second experiment, duration was defined at the interval during which levels exceeded

40 ng/ml. The present results are similar to those of Land et al. (1973) who reported a significant difference between breeds. But Raabe and Wheaton (1976) did not find any difference between Finns and Suffolks.

The data on area under the LH curve and secretion rate obtained here is similar to the second experiment in which there was no significant difference between breeds or between estrous periods. However, Finn ewes showed greater mean values for both area and secretion rate. This non-significant difference may be physiologically important in the induction of multiple ovulation in the more prolific breeds of sheep. Results obtained by Land et al. (1973) indicated that more prolific breeds had a significantly greater interval from estrus to the pre-ovulatory LH surge. They also reported a significantly greater duration and secretion rate of the pre-ovulatory LH surge and suggested that these characteristics are genetically controlled. Thimonier and Pelletier (1971) associated the longer interval from estrus to LH release with sheep shedding two eggs as compared to single ovulators.

Results from the present experiment together with the results from other investigators mentioned above confirm the relationship between the interval from the onset of estrus to pre-ovulatory LH surge with breeds of ewes with higher ovulation rate. How the longer interval relates to greater ovulation is not known. Perhaps the longer in-

terval allows more time for the hypothalamus or pituitary to recover from progesterone negative feedback; therefore, it releases more LH.

It has been suggested that ovulation rate increases as the breeding season progresses (Land et al., 1973; Hulet and Foote, 1967; Dermody et al., 1966; Hulet et al., 1969; Hulet et al., 1974). However, this experiment did not show differences in the interval from onset of estrus to the pre-ovulatory LH surge as the breeding season progressed (although data from first estrus was inadequate, it also did not show a tendency to be different). Therefore, the effect of the longer interval on ovulation rate is still inconclusive. It is possible that this relationship may be secondary mechanism to other factors.

It is also possible that the longer interval may allow progesterone and estrogen to change to such a ratio that favours greater stimulation of the nervous system leading to the release of more releasing hormone, which in turn allows more LH and/or FSH release. A greater gonadotrophin release may not be statistically significant but still great enough to be physiologically important in inducing more ovulations.

Further study in detail on levels of progesterone and estrogen during this interval and comparison between breeds and estrous period during a breeding season is needed to make further conclusions.

FSH alone or in combination with LH may play a greater role in bringing about more ovulations. Information on FSH levels is also necessary in order to arrive at a better conclusion.

Besides concentration of hormones, hormone receptors in ovarian tissue may be an important factor too. It has been reported that estrogen priming increases progesterone receptors in the chicken oviduct (O'Malley and Means, 1974). It is possible that a difference in quantity of receptors is genetically controlled. Koligian and Stormshak (1977) concluded that suppression of uterine estrogen receptor concentrations during the luteal phase was due to progesterone interfering with cytoplasmic estrogen receptor replenishment. It is possible that a similar change in receptors due to hormonal influence may occur in follicular or luteal tissue.

Following estrous activity, the ovaries did not stop functioning abruptly, as indicated by the progesterone levels. There appeared the possibility that ovulation continued during early anestrus. This would agree well with results obtained by Foote et al. (1970) who also reported CL (indicative of ovulation) after the last estrus of the breeding season. However, Rawlings et al. (1977) were able to detect the elevated progesterone during this period from only one of six ewes.

CONCLUSION

Results obtained from this study and other different investigators pertaining to duration and secretion rate of the pre-ovulatory LH surge in relation to higher prolificacy in different breeds of ewes are still not conclusive. There is a definite relationship between a longer interval from the onset of estrus to the LH rise in breeds of ewes with a greater ovulation rate. How the longer interval causes more ovulations is not known at present; more investigation is needed. There is no difference in terms of interval, duration magnitude and secretion rate of the pre-ovulatory LH surge between estrous periods throughout a breeding season. There was a significant variation of the magnitude, area under the curve and secretion rate of the pre-ovulatory LH surge between animals within the same breed. After the onset of anestrus at the end of the breeding season the ovaries remained active for quite some time, as indicated by progesterone levels.

SUMMARY

Four out of five ewe lambs, (Dorset x "Western White Face") born between February and March 1974, reached puberty at an average age of 221.6±10.6 days. Progesterone profiles suggested that three of the four ewes had the first ovulation unaccompanied by overt estrus while one ewe had her first overt estrus before the first ovulation. There was fluctuation of progesterone during the period prior to the first ovulation. Following the first ovulation the progesterone pattern was similar to that of mature cycling ewes. In one ewe an LH peak occurred before the first ovulation and the LH peak associated with the second ovulation was observed in three ewes. FSH peaks associated with the second ovulation, occurred about the same time as the LH peak. LH concentration remained low during the luteal phase while fluctuating FSH levels were observed during the same period. Estrogen showed no obvious relationship to other hormones.

Grain treatment starting at the second estrus of the breeding season and lasting for two cycle lengths appeared to produce no effect on gestation length, lambing rate, interval from the onset of estrus to the pre-ovulatory LH surge, duration of the pre-ovulatory surge and its secretion rate. There were significant breed differences in the interval from the onset of estrus to the pre-ovulatory LH surge and its duration, between the Line M, Suffolk and Finn

breeds. Finn ewes had the longest interval and duration. However, when the comparison between the Suffolk and Finn ewes were studied in more detail in a further experiment the Suffolk ewes had a significantly longer duration than the Finn ewes. A significantly longer interval from onset of estrus to the pre-ovulatory LH surge in the Finn ewes was confirmed in this experiment. There was no significant difference in estrous cycle length, the interval from the onset of estrus to the pre-ovulatory LH surge, its duration and secretion rate between the first estrous period and subsequent estrous periods throughout the breeding season. Blood progesterone levels suggested that one or more ovulations or luteinization of follicles occurred in both breeds studied (Finn and Suffolk) after the last observed estrus of the breeding season.

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