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Constancy of Interval Between Luteinizing Hormone Release and Ovulation in the Ewe

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To investigate the time relationships between the preovulatory LH peak and ovulation in the ewe, a "short" radioimmunoassay for measuring plasma LH concentration was developed. This short assay permitted the determination of LH concentration within 6 h of sampling. The interval from LH peak to ovulation was then defined by a single laparotomy on each ewe, at a known interval after the start of the LH surge to determine for each interval the proportion of ewes which had ovulated.

Using this method, the time between LH peak and ovulation was investigated in 47 ewes experiencing normal estrous cycles, in 11 young and 16 old ewes experiencing their second estrus after withdrawal of Cronolone-impregnated sponges, and in 19 ewes experiencing their first estrus after withdrawal of such sponges.

The results indicated that normally cycling ewes ovulated between 21–26 h after the LH peak whilst the young and old ewes and the Cronolone-treated ewes ovulated 22–26 h after the LH surge. At 23 h, 16.6% of ewes from all four groups had ovulated whereas by 24 h, 66.6% of ewes had ovulated, indicating that a high proportion of ewes ovulated between 23–24 h after the start of the LH peak.

It is concluded that the constancy of interval between LH release and ovulation in these experimental situations confirms the importance of the role of LH in the ovulatory mechanism.

As there was a relatively constant interval of 24–25 h between injection of human chorionic gonadotrophin (HCG) and ovulation in ewes (Ortavant, Thibault and Winterberger, 1949; Dzuik, Hinds, Mansfield and Baker, 1964), the possibility existed that a similar time delay existed between the preovulatory release of luteinizing hormone (LH peak) (Goding, Catt, Brown, Kaltenbach, Cumming and Mole, 1969; Wheatley and Radford, 1969) and ovulation.

A study of the time between the LH peak and ovulation was facilitated by the development of a rapid method for determining plasma LH concentration. The interval from LH peak to ovulation was defined by a single laparotomy on each ewe. These studies were carried out in ewes which were experiencing normal estrous cycles, in ewes experiencing their first estrus after withdrawal of 9α-fluoro-11β-hydroxy-17α-acetoxy-progesterone (Cronolone)[1]-impregnated sponges and in young and old ewes experiencing their second estrus after withdrawal of Cronolone-impregnated sponges.

[1] Cronolone: G. D. Searle and Company.

MATERIALS AND METHODS

The experiments were conducted at the S.S. Cameron Laboratory, State Research Farm, Werribee, Victoria.

Experiment 1

In the middle of the breeding season, seven vasectomized rams wearing harnesses fitted with Sire-Sine crayons were joined with a flock of 700 5-yr old Border Leicester-Merino first cross ewes for 3 days. Ninety ewes marked by the rams in this 3-day period were drafted into an experimental flock.

Two days before the next anticipated estrous period, indwelling silastic cannulae were inserted into the jugular veins of 60 ewes. The ewes were housed with vasectomized rams in three 7×7-m group pens and observed continuously to ascertain the time of onset of estrus. Onset of estrus was defined as being the time the ewes first stood for the ram while being mounted.

Blood samples were taken immediately after onset of estrus and at hourly intervals until the LH peak had been identified. A 10-ml sample of jugular venous blood was withdrawn and immediately heparinized and centrifuged. Decision as to the time of laparotomy for each ewe was made on the basis of gradually accumulating data as the experiment progressed.

Laparotomy for the detection of ovulation was performed once on each ewe by a separate group of workers who were not informed as to the interval between the onset of LH peak and time of operation. The operations were carried out under sodium thiamylal anaesthesia.[2]

Experiment 2

Two groups of fine wool Merino ewes from the same flock were used to study the second estrus and ovulation after withdrawal of Cronolone pessaries. In the first group there were twenty-one 1.5-yr old nonparous ewes (Young) with a mean live weight of 36 kg, and in the second group, twenty-two 5-yr-old multiparous ewes (Old) with mean live weight of 42 kg. The pessaries were inserted intravaginally for 14 days and all were removed on the same day in order to synchronize their second estrus following withdrawal in the week May 17–23, 1970.

The procedures were similar to those in Experiment 1, except that six vasectomized rams were used to detect onset of estrus. Blood samples were initially collected at hourly intervals commencing at onset of estrus. However, after 2 days it became obvious that the preovulatory LH peak had preceded the onset of estrus in some ewes. Accordingly, for the remainder of the experiment blood samples were taken from all the remaining ewes at 2-h intervals until onset of estrus, when the frequency of sampling was increased to hourly. After a ewe was detected in estrus, it was removed from the presence of rams for the remainder of the observation period.

[2] Surital: Parke Davis.

Experiment 3

A group of 22 multiparous 5-yr-old Merino ewes (Cronolone-treated ewes) from a different flock than those ewes of Experiment 2 and of a mean live weight of 45 kg, were used to study the first estrus and ovulation after synchronization with Cronolone pessaries. The pessaries were inserted intravaginally for 14 days and withdrawal was timed so that the estrous cycle to be studied coincided with the second estrus of the ewes in Experiment 2, i.e., in the week May 17–23, 1970. The ewes were kept in the same pens as those in Experiment 2. The procedures were similar to those in the above experiments except that blood samples were taken every 3 h from all ewes commencing 2 days before the first expected onset of estrus. This procedure was adopted as previous work carried out in this laboratory (Cumming, Blockey, Brown, Catt, Goding and Kaltenbach, 1970) indicated that the LH peaks begin before the onset of behavioural estrus in a large proportion of Cronolone-treated ewes. As soon as a ewe was found to be in estrus, the frequency of blood sampling was increased to once hourly.

Development of the "Short" Assay for Luteinizing Hormone

The solid-phase radioimmunoassay for ovine LH used in this laboratory had a total incubation period of 48 h (Goding *et al.*, 1969). Tests were undertaken to determine the shortest incubation period that would produce an assay sufficiently precise to distinguish between the normal resting levels of LH (2–5 ng/ml) and the LH peak levels obtained prior to ovulation (10–200 ng/ml).

The materials used were the same as those used in the conventional assay. Standard solutions of 0, 10, 25 and 50 ng/ml were set up with 12 tubes per concentration. Approximately 100,000 cpm of ovine LH-^{125}I in 0.2 ml was added to all tubes immediately and then the tubes were placed in the incubator at 37°C. Two tubes of each concentration were aspirated, washed and counted after 1, 2, 4, 8 and 24 h. As the incubation time increased, the number of counts bound at 0 ng/ml increased as did the difference in counts between the standards (Fig. 1). However, using an incubation period of as little as 4 h, unknown samples could be placed in categories of <10,

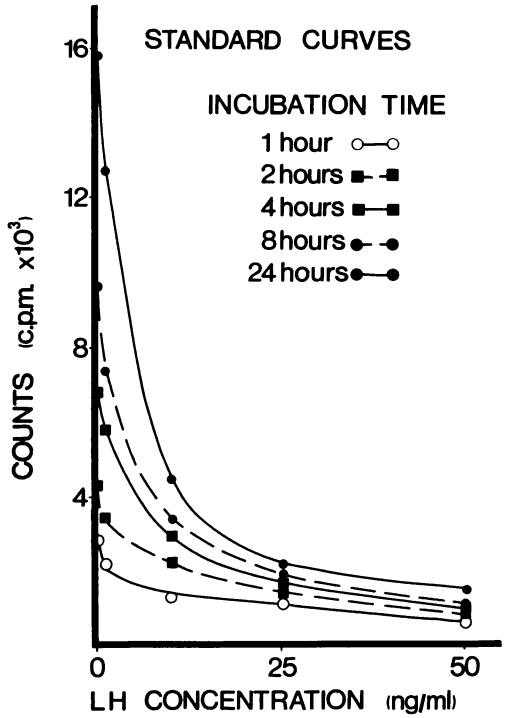

FIG. 1. Standard curves of radioimmunoassay for ovine LH using incubation times of 1, 2, 4, 8 and 24 h.

10–20, 20–50 or >50 ng/ml. Samples which had previously been assayed by the conventional method were assayed by the "short" assay and these results confirmed the validity of the assay.

During the experiment, plasma samples were brought to the laboratory every hour and assayed with standards of 0, 10, 20 and 50 ng/ml. Tracer was added and the tubes were incubated at 37°C for 4 h then aspirated, washed and counted.

When two consecutive samples of >20 ng/ml had been obtained for any ewe it was assumed that the LH peak had commenced and no further blood samples were taken. The onset of the LH peak was taken at the time of the first value of >10 ng/ml.

RESULTS

In Experiment 1, 47 of the 60 ewes with jugular catheters showed estrus and had an LH release during the sampling period. In Experiment 2, seven Young and three Old ewes failed to show estrus and an LH peak during the week of sampling; a further three Young and one Old ewe showed estrus and had ovulated when the ovaries were examined 82 h after the onset of estrus, yet no LH release was detected. Of the 22 Cronolone-treated ewes, two exhibited estrus without an LH release being detected and three had a normal LH release but did not exhibit signs of estrus.

Onset of Estrus and LH Release

In the ewes of Experiment 1, the LH peak began close to the time of onset of behavioural estrus (Table 1). In Experiment 2, the Young and Old ewes at their second estrus after withdrawal of sponges showed a wider range of interval between LH peak and onset of estrus. However, at the *first* estrus after withdrawal of Cronolone-treated sponges (Experiment 3), the LH peak began as early as 22.5 h before the onset of estrus.

TABLE 1

INTERVAL FROM ONSET OF ESTRUS TO START OF PREOVULATORY LH RELEASE IN DIFFERENT GROUPS OF EWES

Experiment	Group	No. of ewes	Interval from onset of estrus to start of LH peak (h)	
			Mean ± SD	Range
1	Untreated (normally cycling ewes)	47	+1.7 ± 2.6	−2.0[a] + 9.0[b]
2	Young	11	−0.3 ± 4.0	−8.0 + 9.5
2	Old	17	+1.7 ± 4.2	−7.0 + 8.0
3	"Cronolone"-treated	19	[c]−2.6 ± 8.1	−22.5 + 9.3

[a] Negative values represent hours before onset of estrus.

[b] Positive values represent hours after onset of estrus.

[c] Significantly different ($P < 0.005$) from the Untreated ewes (Experiment 1).

Interval Between Onset of LH Peak and Ovulation

Ovulation data were collected from 47 ewes in Experiment 1, 11 Young and 17 Old ewes in Experiment 2 and 19 Cronolone-treated ewes in Experiment 3. No ovulation occurred in any group as early as 21 h after the onset of the LH peak. In the ewes of Experiment 2, none had ovulated as late as 22 h after the onset of this peak, however, in the ewes of Experiment 1, seven of 16 ewes had ovulated by 22 or 23 h after the LH peak. All ewes in both experiments had ovulated when examined at 26 or 27 h after the start of the LH peak (Fig. 2).

Figure 3 shows data pooled from all experiments expressed as a percentage of ewes that had ovulated when laparotomized once at different intervals after the start of the LH release. All ewes had ovulated by 26 h after the LH release. At 23 h, 16.6% of ewes had ovulated, whereas by 24 h 66.6% of ewes had ovulated, indicating that a high proportion of ewes ovulated between 23 and 24 h after the start of the preovulatory LH release.

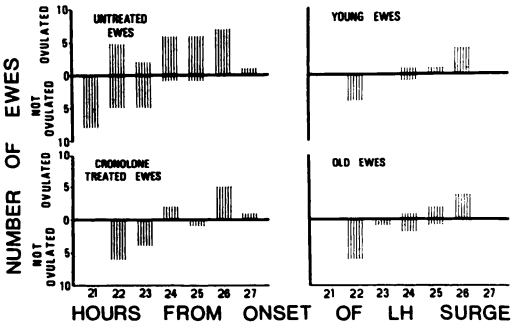

FIG. 2. Number of ewes ovulated or not ovulated at varying times between LH surge and laparotomy.

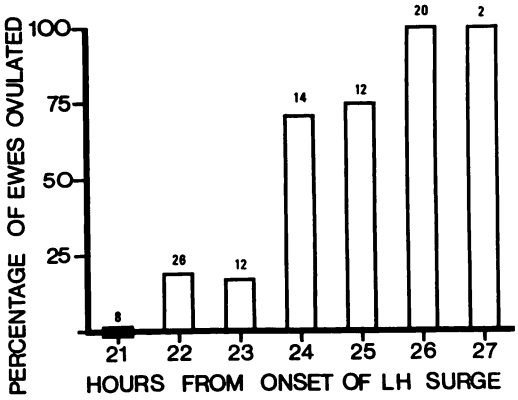

FIG. 3. Percentage of ewes ovulated at varying times between LH surge and laparotomy. Number of ewes in each hour-group shown above blocks.

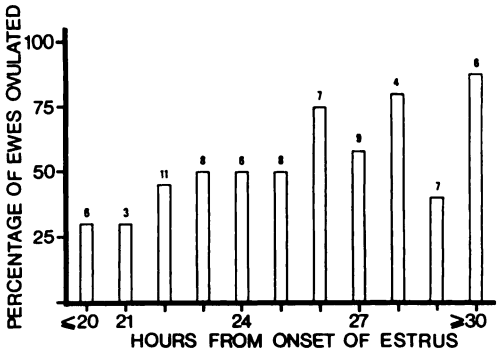

FIG. 4. Percentage of ewes ovulated at varying times between onset of estrus and laparotomy.

Onset of Estrus to Ovulation

In Fig. 4 data from the ewes in Experiments 1 and 2 have been pooled and expressed as a percentage of ewes that had ovulated when laparotomized at different intervals after the onset of estrus. The data

TABLE 2

OVULATION IN RELATION TO START OF LH RELEASE AND ONSET OF ESTRUS

Experiment	Group	Range in interval (h) from: LH surge to ovulation	Range in interval (h) from: Onset of estrus to ovulation[a]
1	Untreated (normally cycling ewes)	21–26	19–35.0
2	Young	22–26	14–35.5
2	Old	23–26	16–34.0
3	"Cronolone"-treated	23–26	0.5–35.3

[a] Calculated from data on interval from estrus to LH surge (Table 1) and from LH surge to ovulation.

from the Cronolone-treated ewes were excluded. There was considerable variability in interval from onset of estrus to ovulation. In Table 2, the range in interval between onset of estrus and ovulation (calculated from data on interval from estrus to LH release and from LH release to ovulation) is compared to the range in interval from LH release to ovulation. There was no obvious correlation between the onset of LH release or first signs of estrus with time of day.

DISCUSSION

These data confirmed our previous finding (Cumming *et al.*, 1970) that the LH peak of ewes at the first estrus after withdrawal of Cronolone sponges can occur well before the onset of estrus. In comparison to the LH peak detected in untreated ewes, the LH peak in some of the Young and Old ewes at the second estrus after sponge withdrawal did occur slightly earlier relative to the onset of estrus (Table 1). However, progestogen treatment caused no deviation from the normal time relationship of LH peak and ovulation (21–26 h) at either the first or second synchronized estrus (Fig. 2).

With hourly blood sampling it is only possible to identify the onset of the LH peak to within ±1 h. It is thus possible that ovulation follows the onset of the LH peak within even narrower time limits than indicated in Fig. 3, probably ovulation occurs within 23–25 h after the onset of the LH peak.

In all animals, including those in their first estrus after Cronolone treatment, the interval between the onset of the LH peak and ovulation remained constant. In an earlier paper, Cumming *et al.* (1970) reported that in Cronolone-treated ewes, the onset of the LH peak occurred much earlier in relation to the onset of estrus than in untreated ewes. The results of the present paper confirm the proposition that ovulation would also occur early in relation to estrus after Cronolone treatment. In fact one of these ewes had ovulated 3.5 h after the onset of estrus. In the ewes which were cycling naturally, ovulation occurred from 19–35 h after the onset of estrus.

The time relationships of estrus to LH peak, LH peak to ovulation and estrus to ovulation were similar in both young maiden and old multiparous ewes. Dzuik (1970) found that optimum fertility results from mating about 12 h before ovulation. Blockey and Cumming (1970) demonstrated that in ewes from the same commercial flock as that which supplied the Young and Old ewes used in this present study, the mean duration of estrus in young maidens (12.6 h) was shorter ($P < 0.01$) than that of older parous ewes (20.5 h). It appears that a large proportion of young ewes would have little chance of being mated at a time that was optimum for fertility as they would no longer be in estrus. This could be a contributing factor to the well-documented generally lower-reproductive rates of young ewes (e.g., Turner and Dolling, 1965).

In conclusion, the major finding of this study is the constancy of interval between the LH peak and ovulation. The data showing that a high proportion of ewes ovulate between 23–25 h after the start of the LH peak agrees well with the findings of Ortavant, Thibault and Winterberger (1949) and Dzuik *et al.* (1964), who found that ovulation occurs approximately 23–24 h after the administration of HCG. Until the roles of FSH and prolactin in the ovulatory complex have been elucidated, it is not possible to conclude that LH is the sole ovulatory hormone in sheep. However, the data presented here and the results of HCG administration strongly suggest that LH is the gonadotrophin that triggers the ovulatory mechanism.

It is obvious that a knowledge of the time of onset of the LH peak permits far more accurate predictions to be made about the time of ovulation. Such knowledge could have considerable use in experimental situations. However, the require-

ment for multiple blood sampling and LH determinations for each animal makes the present procedure too cumbersome and costly for commercial use.

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