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THE SECRETION OF LUTEINIZING HORMONE IN EWES OF FINNISH LANDRACE DURING ESTRUS

By

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PYÖRÄLÄ, E., P. LÄHTEENMÄKI, H. TIIHONEN and S. ÖSTERBERG: *The secretion of luteinizing hormone in ewes of Finnish Landrace during estrus.* Acta vet. scand. 1979, *20*, 216—223. — Luteinizing hormone immunoreactivity was measured in the venous plasma of four cycling Finnish Landrace sheep during the breeding season in connection with one synchronized estrus and the subsequent one. The ewes were slaughtered after the second estrus to establish the number of ovulations. To determine the LH concentration, a heterologous method of assay was used; this was based on the cross reaction of sheep plasma LH in a human LH radioimmunoassay system.

As a result of the investigation, it was found that the peaks of LH were lower during the time of synchronized estrus and that these peaks occurred earlier than in the subsequent estrus. However, the differences were not statistically significant. On account of the limited material, the effect of the occurrence of the LH peak on the number of ovulations could not be established.

Finnish Landrace ewes; luteinizing hormone; estrus.

In several investigations, it has been established that the luteinizing hormone (LH) peak during the heat in the ewe, usually occurs within 16 h after the first signs of estrus (*Wheatley & Ratford* 1969). There exist, however, great differences between breeds (*Land et al.* 1973). The peak values, measured during estrus, also vary in different investigations (*Goding et al.* 1969).

Thimonier & Pelletier (1971) found a higher number of ovulations in those Ile-de-France ewes, in which the LH peak

occurs later during the time of estrus. The frequency of ovulation in Finnish Landrace ewes is known to be high. The aim of the present investigation was to demonstrate the LH peak in Finnish Landrace ewes during a synchronized estrus (Estrus I) and during a subsequent one (Estrus II). The LH concentration and the time of the LH peak were compared in the two heats investigated. The relationship between the number of ovulations and the occurrence of the LH peak was, as far as possible, estimated in connection with the slaughter after the second estrus.

MATERIAL AND METHODS

The material consisted of four cycling Finnish Landrace ewes from a flock of the Agricultural Research Centre at Tikkurila. The estrus synchronization was performed during the breeding season by insertion of "Multisex"-tampons containing 30 chlormadinon-acetate and 1 mg mestranol. Estrus commenced within three days of the withdrawal of the tampons.

Detection of estrus

This was performed with the aid of a color-marked, vasectomized ram. When heat was expected and throughout the time of heat, the ram joined the ewes every 3 h. Thus, it was possible, by the behavior of the ewes at time of inspection, to determine the time of onset and the duration of heat of each ewe.

Collection of blood samples

During Heat I and Heat II, the blood samples were collected from the jugular vein every 3 h. The investigation comprised 12 blood samples/ewe/heat. The first sample was drawn 6 h and the last 39 h after the onset of heat. The plasma was stored at —20°C until the LH assay.

Radioimmunoassay

The measurements of sheep plasma LH concentrations were carried out by a heterologous radioimmunoassay. This was based on the cross-reaction of sheep plasma LH in a human LH radioimmunoassay system. In this double antibody-solid phase assay system (*Karonen et al.* 1978) the labelled hormone was pre-

pared by iodination (^{125}Iodine) of human pituitary luteinizing hormone†. The antiserum was prepared in rabbit against human pituitary LH**. The standard preparation was LER-907†† which is a partially purified human pituitary extract with both LH and follicle stimulating hormone (FSH) activity. The separation of bound and unbound fractions of the labelled hormone was performed with solid phase second antibody (anti-rabbit-DASP®, Organon, Oss, Holland). All samples and standards were run in duplicate, and the volume of samples was 200 ul.

The sensitivity of each LH assay was determined by calculating the amount of unlabelled hormone which corresponded to B^*/B_0^* outside of the 95 % confidence limits of B_0^*/T^*, where B^* = labelled hormone bound to antibody, B_0^* = amount of labelled hormone bound to antibody in the absence of unlabelled hormone and T^* = total labelled hormone added (*Midgley et al.* 1969). The highest sensivity of five assays was 0.37 ng/tube LER-907, and this was regarded as a practical detection limit of the assay. The intra-assay coefficient of variation was calculated from replicate analyses of 10 plasma samples in the same assay, and a value of 13.4 % was obtained. The inter-assay coefficient of variation was calculated from the concentrations of 10 plasma samples assayed in two repeated assays. The value of 19.8 % was obtained. The standard curves were linearised by a logit plot method (*Rodbard & Lewald* 1970) and LH concentrations are expressed in ng of LER-907 per ml plasma.

RESULTS

Figs. 1—4 show the LH levels of the sheep during Estrus I and II. The LH peaks of Estrus I were lower and appeared at an earlier stage. During Estrus II, the LH peaks appeared later in relation to the onset of estrus. In ewe No. 1, the LH secretion level remained high for a longer period, without any distinct peak.

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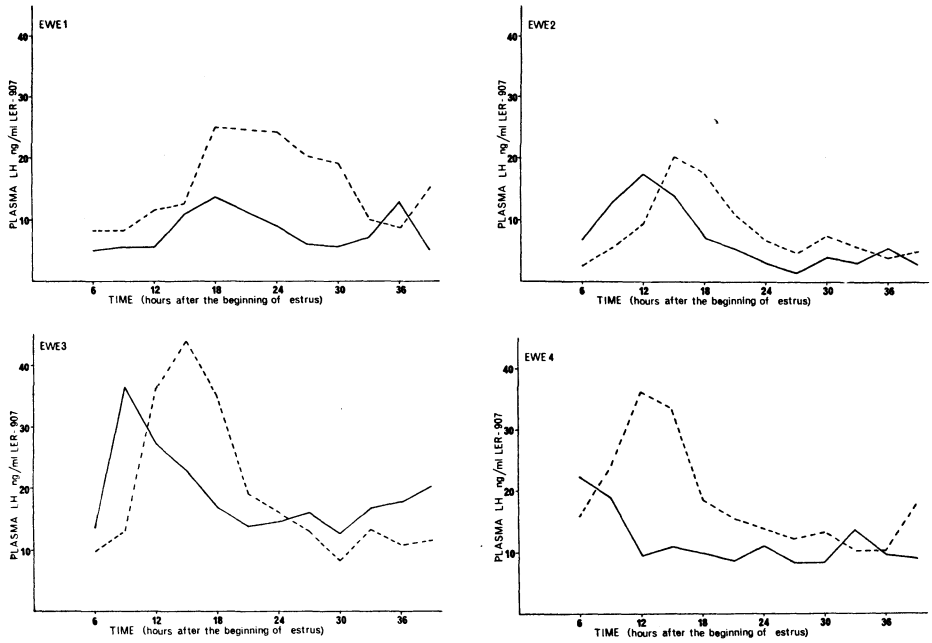

Figures 1—4. The luteinizing hormone (LH) concentrations in jugular venous blood during Estrus I (———) and Estrus II (- - - - -) in ewes Nos. 1, 2, 3 and 4.

Fig. 5 shows the mean values of the LH concentration ± s.e.m. during 6—39 h from the onset of estrus, based on calculations made every 3 h.

The figures also show that the LH level is higher in Estrus II.

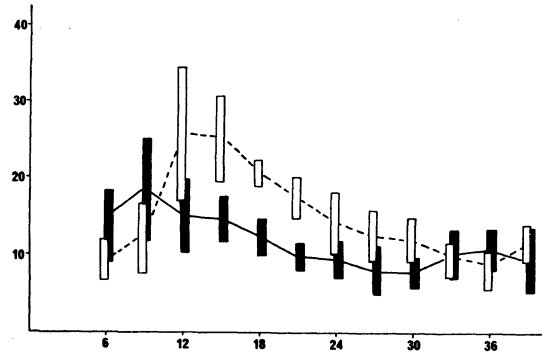

Figure 5. The luteinizing hormone (LH) concentrations (mean ± s.e.m.) in jugular venous blood in four ewes at Estrus I (———) and at Estrus II (- - - - -).

Comparing the LH curve between ewes during both heats, the area of the curve in the synchronized ewes covers but 77 ± 13 % of the area in the subsequent heat. However, the difference is not statistically significant ($P > 0.075$).

The ewes were slaughtered 24 h after the termination of the second heat. Table 1 shows the duration of the heats, the occurrence of the LH peaks and the number of ovulations observed at the time of slaughter.

Table 1. The duration and LH peak values of Estrus I as compared with Estrus II; additionally, the number of ovulations at the time of slaughter is noted.

Ewe No.	Estrus I duration, h	LH peak h after onset of estrus	Estrus II duration, h	LH peak h after onset of estrus	Number of ovulations
1	48	18	72	18	1
2	48	12	51	15	3
3	48	9	57	15	4
4	57	6	75	12	2
Mean ± s	50.3±4.5	11.3±5.1	63.8±11.6	15.0±2.4	

DISCUSSION

In most sheep breeds, the sexual receptivity has a duration of about 30 h. However, in the Finnish Landrace and the Merino, the receptivity lasts longer. The steep rise in estradiol, in connection with the heat, releases the secretion of LH. The peak LH value usually occurs 16 h after the onset of externally detectable heat.

The heats of the investigated ewes were long (Table 1). To detect the end of estrus is more difficult than to ascertain the symptoms of onset, although both are equally important. The duration of the synchronized estrus is usually shorter.

As can be seen in Figs. 1—4, the peak LH values appeared 3—6 h earlier during Estrus I. Only ewe No. 1 was quite exceptional in that its LH secretion was highly atypical throughout the time of the experiment. In peripheral blood, the maximum LH concentrations rose towards the end of the heat. The probable cause of this phenomenon might be that the gestagens, used for

synchronization, disturbed the release mechanism of LH secretion during Estrus I; thus, maintaining a lower level of concentration. The estrogenic compound, mestranol, contained in the tampons, together with the endogenous estradiol, might exercise a synergistic influence, causing a more rapid rise in LH, resulting in peak values.

During the present investigation, a maximum concentration of 43.9 % was observed. Compared with the values arrived at in several other works, with peak values of up to 200 ng/ml (*Goding et al.* 1969), the obtained value is remarkably low. This is probably caused by the method used, a heterologous immunoassay (*Karonen et al.* 1978).

Land (1971) (cit. *Robertson* 1977), on the other hand, suggests, comparing the Finnish Landrace ewes with other breeds, "that a higher level of gonadotropic stimulation is an important factor underlying the various aspects of increased activity in the Finnish Landrace sheep". This suggestion could not be proved in the present work as comparative investigations in other breeds do not exist, using the method applied in this work.

It has been established, when synchronizing the estrus in cattle and sheep, that the fertility rate is low after insemination during the first heat. In the ewe, the impaired fertility is thought to be caused by failure in sperm transport (*Gordon* 1976). It is difficult to judge whether the differences between the two heats (Fig. 5) are connected with impaired fertility during the synchronized estrus or not.

In most sheep breeds, one—two ovulations occur during the period of estrus. In the Finnish Landrace and the Romanov breed, up to five ovulations may take place. According to *Thimonier & Pelletier* (1971) and *Robertson*, the appearance of the LH peak during the latter part of estrus may account for a higher number of ovulations. On account of the small material of the present work, no conclusions can be drawn on this hypothesis. Table 1 shows the number of ovulations in the four investigated ewes. Ewe No. 1, showing an atypical LH curve, had also ovulated, but only one follicle had ruptured. The LH peak during Estrus II occurred 15 ± 2.4 h after detection of estrus. In breeds, with a shorter estrus, ovulation time is, usually, 7.5 h after the onset of estrus. Typical for the Romanov breed is the lateness of the appearance of the LH peak (17.6 h), as recorded by *Land et al.* (1973). The determination of the correct time of artificial

insemination in different breeds might be of importance, as the time of ovulation is closely connected with the occurrence of the LH peak.

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SAMMANFATTNING

LH-sekretionen hos tackor av finsk lantras under brunsten.

Immunoreaktiviteten gentemot LH bestämdes i venöst plasma hos fyra tackor av finsk lantras i samband med cykeln under sexualsäsongen vid en synkroniserad brunst samt den därpåföljande brun-

sten. Tackorna slaktades efter den andra brunsten för att komma underfund med antalet gula kroppar. En heterologisk assay-metod användes för bestämmandet av LH-koncentrationen; metoden var baserad på „kors-reaktionen" av plasma LH av får i ett humant LH-radioimmunoassay system.

Undersökningen visade, att LH-topparna var lägre i samband med synkroniserad brunst samt uppstod tidigare än under den följande brunsten. Skillnaderna var emellertid inte statistiskt signifikanta. På grund av det begränsade materialet kunde effekten av tidpunkten för LH-toppen på antalet ovulationer inte med säkerhet bestämmas.

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