The determination of plasma progesterone, oestradiol-17β and luteinizing hormone levels during oestrous period of Tuj ewe-lambs following oestrus induction

M. KAYA*, S. YILDIZ and B. GUVEN

Department of Physiology, Faculty of Veterinary Medicine, University of Kafkas, 36280/KARS, TURKEY

* Corresponding author : Dr Mehmet KAYA, Phone : +90 474 2426802, e-mail : kayamet@hotmail.com

SUMMARY

The aim of the present study was to induce oestrus in the Tuj ewe-lambs that are not able to reach puberty at the first breeding season of their life and to determine plasma luteinizing hormone (LH), oestradiol-17β (E2) and progesterone (P4) concentrations during the oestrous period. A total of 19 Tuj ewe-lambs, 9 months aged and weighing about 37 kg, received an intravaginal sponge containing fluoroestrogene acetate (FGA) for 14 days and 400 IU PMSG was injected intramuscularly on the day of sponge removal. During a 104 h period, blood samples were collected from the jugular vein at 2, 6 and 24 h intervals for the determination of plasma LH, E2 and P4 concentrations, respectively. Oestrous behavior was assessed utilizing teaser rams. Oestrous behaviour and preovulatory E2 and LH peaks were determined in 16 of the 19 lambs. Preovulatory E2 and LH surges were observed 29.8 and 43.9 h and the peak values were 36.4 pg/ml and 60.9 ng/ml, respectively. Plasma P4 concentrations generally did not change throughout the study period and found to be lower than 1 ng/ml. A significant positive correlation was observed between the time of occurrence of LH peaks and the onset of oestrous behaviour. In conclusion, the results show that (i) oestrus could successfully be induced, (ii) preovulatory E2 and LH surges could be determined and (iii) oestrous behaviour could be observed in 9 months old Tuj ewe-lambs. Thus, pregnancy might be possible in these lambs early in their life.

Keywords : Tuj lamb - progesterone - luteinizing hormone - oestradiol-17β - œstrus.

RÉSUMÉ

Détermination de la concentration plasmatique en progestérone, oestradiol-17β et LH durant l’œstrus induit chez les agnelles de race Tuj. Par M. KAYA, S. YILDIZ et B. GUVEN.

Cette étude avait pour but d’induire l’œstrus chez des agnelles de race Tuj qui ne peuvent pas atteindre la puberté à la première saison de reproduction et d’évaluer la cinétique des concentrations plasmatiques de LH, d’oestradiol-17β (E2) et de progestérone (P4) pendant l’œstrus induit. Dix-neuf agnelles âgées de 9 mois, pesant environ 37 Kg, ont été traitées par la pose d’une éponge vaginale imprégnée de fluoroestrogène (FGA) pendant 14 jours, suivie de l’administration intramusculaire de 400 UI de PMSG, le jour du retrait de l’éponge. Des prélèvements de sang ont été réalisés par ponction de la veine jugulaire pendant 104 h à des intervalles respectifs de 2, 6 et 24 h après l’administration de PMSG pour évaluer respectivement les concentrations plasmatiques de LH, E2 et P4. La détection de l’œstrus a été réalisée par présentation d’un bélier. Les manifestations de l’œstrus, et l’occurrence des pics préovulatoires de E2 (36.4 ng/ml) et de LH (60.9 ng/ml) ont été observés chez 16 agnelles, respectivement 30 et 44 h après le retrait des éponges. La concentration plasmatique de progestérone est restée stable et inférieure à 1 ng/ml. Une corrélation positive significative a été mise en évidence entre l’occurrence des pics préovulatoires de LH et le début des manifestations d’œstrus.

En conclusion, ces résultats démontrent qu’un traitement hormonal permet d’induire l’œstrus chez les agnelles Tuj dès la première saison de lutte après leur naissance.


Introduction

Tuj sheep, a well adopted breed for the geography and climate of the highlands, originated in the Caucasus and are found throughout the northeast of Turkey. Although the mature sheep are able to reproduce normally at these conditions, ewe-lambs are normally not able to reach puberty during the first year of their life. Shortage in feed due to long and cold winters together with the seasonality of reproduction in this breed results in postponement of reproductive activity to the next breeding season. Hence a whole year of production is lost for the farmers of the region.

Puberty in sheep mainly depends on critical body mass (approximately 70-75 % mature body weight) that is necessary to achieve pregnancy [2] and on the environmental cues including daylight [16]. Both the body mass and daylight can be manipulated to achieve higher reproductive performance but under extensive farming conditions their manipulation are impractical.

Normally, in Tuj ewe-lambs, body mass increases from birth in March until October but it is not sufficient to evoke reproductive potency. By the time when body mass is sufficient, then breeding season is missed. In other words, they are able to reach sufficient body weight around December, i.e. during the anoestrus season. We postulated that an oestrus activity can be induced in December and that normal hormonal and oestrous behaviour responses can be obtained after hormonal treatment.

There are various methods for oestrus induction but among them progesterone-impregnated sponges and PMSG applications are reported to be the most successful during breeding [8] or non-breeding season [28] in lambs. Successes of these treatments are dependant on the production of E2 peak, LH surge [14] and oestrous behaviour [22].
The aim of the current study was, therefore, to induce oestrus in prepubertal Tuj ewe-lambs in December and to evaluate the plasma concentrations of LH, progesterone and oestradiol-17β during the induced cycle. In addition, oestrous behaviour of the lambs was screened utilizing teaser rams and the relationships among all parameters were also assessed.

Materials and methods

ANIMALS

This study was conducted in the College Farm of Kafkas University at Kars in Turkey (latitude 40° 36' N, longitude 43° 06' E), with an elevation of 1750 m above sea level. Nineteen healthy female Tuj lambs (9 months old) (BW=36.9±0.1 kg) and 4 teaser rams (3-4 yr old) were used. The lambs were fed hay plus barley daily and had free access to water and mineral licking blocks during the study period.

INDUCTIONS OF OESTRUS

Each lamb was inserted with 40 mg of fluorogestone acetate containing intravaginal sponges (FGA, Syncro-Part, Sanofi-Dogu Ilaç AS, Istanbul, Turkey) for 14 days. On the day of sponge withdrawal, 400 IU pregnant mare serum gonadotropin (PMSG, Syncro-Part, Sanofi Dogu Ilaç AS, Istanbul, Turkey) were intramuscularly administered to all lambs. The time of PMSG administration at sponge removal is referred to time 0. Oestrous behavior was assessed between 08:00-24:00 h by utilizing teaser rams.

SAMPLE COLLECTION

Blood samples (5 ml) were collected from the jugular vein at 2, 6 and 24 h intervals for 104 hours for the determination of plasma LH, E2 and P4 concentrations, respectively. Samples were immediately centrifuged at +4°C, 3000 x g for 15 min and plasma stored at -20°C until analyses.

HORMONE ASSAYS

Plasma samples were analyzed using a double-antibody EIA technique for determination of P4 [25], LH [21], and E2 [12]. All assays were carried out in 96 well microtitre plates (Nunc-Immunoplate, Cat. No. 439454, Brand Products, Denmark) and standards, samples and controls were evaluated in duplicate. The levels of quantification of the assays were 0.11 ng/ml for P4, 0.11 ng/ml for LH and 10.13 pg/ml for E2. Intra- and interassay coefficients of variations were 11% and 12% for P4, 11% and 13% for LH and 4% and 5% for E2, respectively.

STATISTICAL ANALYSIS

Mean, standard deviation (SD) and standard error of the mean (SEM) were calculated by using the MINITAB statistical package (Version 11.2, MINITAB Inc., State College, PA, USA). Correlation and regression analysis were also carried out using the same program. Data are presented as mean ± SEM.

The baseline concentration value for LH and oestradiol was calculated from the data obtained 24 h before the surges. The onset of the LH and E2 surges was determined according the method described by CARATY and SKINNER [7]. The area under the LH and E2 plasma concentration time curve was calculated.

Results

OESTROUS BEHAVIOUR

Oestrous behavior was observed in 16 out of 19 lambs at 54.4±2.4 h after PMSG administration. The success rate of oestrus induction was therefore calculated to be 84%. However, at sponge removal, it was noticed that in two lambs among the 3 that did not exhibit oestrus, sponges were not present in the vagina at the time of removal.

PROGESTERONE, LH AND OESTRADIOL CONCENTRATIONS

Plasma P4 levels remained below 1 ng/ml (0.50±0.04 ng/ml) and did not change throughout the 104 h following sponge removal in 16 lambs. The first values of P4 levels of the 3 remaining lambs were slightly higher than 1 ng/ml at the time of PMSG administration.

The preovulatory surges of E2 and LH were observed only in the 16 lambs that exhibited oestrus behaviour. In these lambs, basal levels of E2 and LH were lower than 1 ng/ml and 20 pg/ml, respectively. Table I gives mean basal and peak values of plasma E2 and LH concentrations. Table II gives mean values of the time lag between PMSG administration and the onset, the time to maximum concentration and the end of E2 and LH peaks. It was observed that the surge of oestradiol is followed by a surge of LH and that oestrous behaviour followed these two surges (Figure 1).

<table>
<thead>
<tr>
<th></th>
<th>Basal concentrations</th>
<th>Peak value of surge</th>
<th>Total area under curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>19.6 ± 0.34</td>
<td>36.4 ± 2.18</td>
<td>232.9 ± 11.9</td>
</tr>
<tr>
<td>LH</td>
<td>0.80 ± 0.08</td>
<td>60.9 ± 4.46</td>
<td>538.5 ± 30.9</td>
</tr>
</tbody>
</table>

Table I. — Mean (±SEM) plasma concentrations and mean area under the plasma concentration time curve for E2 (ng/ml, (ng/ml).h) and LH (ng/ml, (ng/ml).h) obtained in the 16 lambs during the oestrus induced by hormonal treatment.

Revue Méd. Vé., 2005, 156, 12, 628-632
RELATIONSHIPS BETWEEN OESTROUS BEHAVIOUR AND HORMONE SECRETIONS

Pearson correlations are given in Table III for all of the parameters studied. Briefly, the time of occurrence of LH surge was linearly related to the time of occurrence of the oestrous behavior \((r^2=0.88)\). Such a relationship could not be evidenced when considering both the relation between the time of onset of E2 surge and the time of LH surge \((r^2=0.50)\) and the relation between the time of onset of E2 surge and the time of occurrence of the oestrous behavior \((r^2=0.61)\, (\text{Figure 2}).\)

Discussion

The present study shows that oestrus can successfully be induced in prepubertal 9 months old Tuj ewe-lambs by using progestagen-impregnated sponges during non-breeding season. It is known that mechanism leading to ovulation is at highest activity during breeding season and depresses afterwards [20]. This study fulfils the gap in our knowledge about the ovulatory mechanisms in fat-tailed sheep. In fact, it is very difficult to acquire reference values for the fat-tailed sheep in the literature, as the numbers of investigations are quite limited. From that point of view, the current study suggests that the mechanism underlying ovulatory mechanisms are similar to those of tailless breed of sheep and similar cascade of event occur before ovulation and oestrus.

It has been reported that the preovulatory period of the ewe is normally, characterized by progression of hormonal events that includes: \(i\) P4 concentrations decrease, \(ii\) progressive increase in LH pulsatility, \(iii\) sustained E2 increase, and \(iv\) preovulatory LH surge [14]. In our study, the pulsatility of LH secretion was not evaluated but it is generally admitted that the LH preovulatory surge results from an increase in LH pulsatility. Therefore, in the current study, all of these hormonal events were observed in 16 out of 19 lambs and followed by the expression of full behavior of oestrus. In two lambs among the 3 that did not respond to the hormonal treatment, the sponge was not detected in the vagina.

In the current study, plasma P4 levels were below the 1 ng/ml, as described in earlier studies [3, 10, 24, 27]. Plasma P4 levels were only higher than 1 ng/ml at the moment of PMSG administration in 3 lambs. Short periods of elevated serum P4 concentrations (1-4 days) were shown to occur before the first ovulation in peripubertal ewe lambs [5, 9, 15]. The reason for this transient increase in P4 secretion is not fully understood. The source of P4 for these lambs might...
include luteal tissues embedded in the ovarian stroma which cannot be detected macroscopically [5]. Increase in P4 during these times has been implicated to play a role in induction of oestrous behaviour [11] and assurance of normal luteal lifespan [4, 9].

Plasma E2 levels increased before preovulatory LH surge as described by KARSH [14] and PANT [24] and concentrations of E2 were similar to those described in former studies [3, 13, 14, 23, 24]. As for LH, plasma basal level [17, 24] and mean concentration values of preovulatory surge [1, 17, 18, 24] were below the levels obtained for other breeds. The time of occurrence of the preovulatory LH surge after sponge removal is shorter than that described in Finn-Merino cross breed [26], Suffolk [19], and Merino x Finnish Landrace cross breed [6]. The interval between oestrous behavior and preovulatory LH surge was similar to values obtained for Finnish Landrace [1], but higher than that describe for Clun Forest [24] and Galway [1] breeds. This difference may reflect breed differences in hypothalamic or pituitary sensitivity to E2 [18].

On the basis of regression analyses, we have shown that the time of occurrence of oestrous behavior is linearly related to the time of occurrence of the LH peak concentrations.

Collectively, the data presented in this study suggest that fat-tailed Tuji lambs adapted to cold and harsh Caucasian region exhibit full reproductive activity if the mechanism of oestrus is triggered by oestrus induction with progestagen impregnated sponges plus PMSG. However, successful application of this method will necessitate sufficient nutrition and good management techniques.

References


