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Research Article

Effects of Rams and Luteal or Follicular Phase Ewes on Preovulatory LH Surge Characteristics in Ewes*

Sedat YILDIZ, Metehan UZUN, Mehmet KAYA
Department of Physiology, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY
Ömer UÇAR
Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY
Metin ÇENESİZ
Department of Physiology, Faculty of Veterinary Medicine, University of Kafkas, Kars - TURKEY

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Abstract: This study aimed to investigate whether the preovulatory LH surge mechanism is involved in sheep-to-sheep interaction for oestrous synchronisation. For that purpose, anoestrous ewes were inserted with intravaginal progestagen sponges for 13 days to induce ovulation. Upon sponge withdrawal, they were introduced either to 4 rams (n = 6, ram group), or to 4 ewes which were in follicular period (n = 6, follicular group), or 4 ewes which were in the luteal period (n = 6, luteal group) or else they were kept alone (n = 6, control group). The ewes were weighed and their body conditions were scored prior to the experiment. There were no significant differences in the body weights and body condition scores of the 4 groups. Blood samples were collected at 2 h intervals from sponge withdrawal (0 h) until the 96^{th} h, and twice daily thereafter for 10 days. LH analyses were carried out on blood samples. According to data from the analyses, no LH surge was observed in the follicular and luteal groups within the 96 h following sponge withdrawal. The periods for observation of an LH surge were 162.7 ± 46.2, 224.0 ± 21.5, 58.7 ± 4.6 and 69.7 ± 6.7 h for the luteal, follicular, ram and control groups, respectively ($P < 0.001$). Although no significant difference was observed between the ram and control groups, the LH surge in these groups occurred significantly earlier than those of the luteal ($P < 0.05$ and $P = 0.075$, respectively) and follicular ($P < 0.001$ for both groups) groups. In conclusion, it appears that female sheep delay the LH surges compared to the ram-introduced or control groups and that female-to-female interaction seems to suppress preovulatory LH surge generation centres.

Key Words: LH surge, pheromones, preovulatory, sheep-to-sheep interaction

Koyunlarda Preovulatör LH Salınımı Üzerine Koçların ve Luteal veya Folliküler Dönemdeki Koyunların Etkisi

Özet: Bu çalışmada, koyunlarda östrus senkronizasyonuyla ilişkili olduğu bildirilen koyun-koyun etkileşiminin, preovulatör LH salınımına etkili olup olmadığını araştırmak amaçlanmıştır. Bu amaçla, anöstrustaki koyunlardan her bir grupta 6 koyun olacak şekilde 4 grup oluşturuldu ve ovulasyonlarını uyarmak için koyunlara 13 gün süreyle intravajinal süngerler takıldı. Süngerlerin çıkarılmasından sonra, grupların bir tanesine başka bir koyun katılmazken (n = 6; kontrol grubu), bir gruba 4 adet koç (n = 6; koç grubu), diğerine folliküler dönemdeki 4 adet koyun (n = 6; folliküler grup) ve son gruba ise luteal dönemdeki yine 4 adet koyun (n = 6; luteal grup) katıldı. Deneme öncesi koyunlar tartıldı ve vücut kondisyon skorları belirlendi. Tüm dört grup arasında canlı ağırlıkları ve vücut kondisyon skorları yönünden anlamlı bir fark gözlenmedi. Kan örnekleri süngerlerin çıkarılmasından sonraki 96. saate kadar 2 saat aralıklarla, bundan sonra 10. güne kadar ise günde iki kez toplandı. LH analizleri, alınan kan örneklerinde enzimimmunoassay yöntemiyle yapıldı. Analizler sonucu elde edilen verilere göre, süngerlerin çıkarılmasını takip eden 96 saat içinde folliküler ve luteal gruplarda LH salınımı gözlenmedi. Luteal, folliküler, koç ve kontrol gruplarında preovulatör LH salınımları sırasıyla 162,7 ± 46,2, 224,0 ± 21,5, 58,7 ± 4,6 ve 69,7 ± 6,7 saatlerde gözlendi ($P < 0,001$). Koç ve kontrol grupları arasında LH salınımı yönünden anlamlı bir fark olmasa da, bu gruplardaki LH salınımı luteal (sırasıyla $P < 0,05$ ve $P = 0,075$) ve folliküler gruplara (her iki grup için $P < 0.001$) göre anlamlı şekilde daha erken oluştu. Sonuç olarak, dişi koyunların preovulatör LH salınımını koç ve kontrol grubuna göre geciktirebileceği ve dişi-dişi etkileşimiyle preovulatör LH salınım merkezlerinin baskılanabileceği anlaşılmıştır.

Anahtar Sözcükler: LH salınımı, feromon, preovulatör, koyun-koyun etkileşimi

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Introduction

In sheep flocks reproductive activity is greatly affected by pheromonal cues originating from other members of the flock (1-5). This has generally been thought to be through male effects on females and vice versa (1-3,6,7). However, there is some evidence that these interactions might also take place between female sheep (8,9). Yildiz et al. (9) suggest that in the tactile and visual absence of rams, ewes synchronise their oestrous cycles. The way in which the ewes synchronise their oestrous cycles is not clear. However, it is possible that the ewes which are not synchronous with the main group either shorten or lengthen their oestrous cycles in order to adapt to the main cycling group. We therefore hypothesise that they regulate these by manipulating the timing of ovulation and hence LH surge characteristics.

It has been observed that odourless compounds from the armpits of women have a synchronising effect on other women (10,11). Moreover, odourless compounds taken during different phases of the oestrous cycle had differential effects on the recipient women. This effect was characterised by accelerating or delaying the LH surge (10). Whether such an interaction takes place between ewes is not yet known, but the evidence suggests that it might be the case (8,9,12). Such information might be useful in finding a cheap and practical means of establishing oestrous synchronisation in sheep. Therefore, the aim of the current study was to compare the effects of rams and ewes in the luteal and follicular phases on the LH surge characteristics in ewes.

Materials and Methods

Animals and Experimental Design: Mature fat-tailed Tuj ewes were divided into 4 groups, a ram introduced group (ram group; n = 6), a follicular phase ewe introduced group (follicular group; n = 6), a luteal phase ewe introduced group (luteal group; n = 6) and a control group (n = 6). On day 0 all the ewes and rams were weighed and their body conditions scored (1-to-5 scale; 13) and they were put into experimental rooms. The rooms were at least 30 m away from each other and were separated by at least 2 walls. Ventilation of the rooms was through the roof and the rooms were surrounded by walls and a door. These rooms were cleaned before use. Additionally, it was ensured that the experimental animal groups did not come into visual or auditory contact with each other. On the day of their arrival at the rooms (day 0), all the ewes were inserted with progestagen impregnated sponges (30 mg flourogestone acetate, Chronogest, Intervet, UK) and these were withdrawn 13 days later (day 13). Luteal phase ewes were obtained by inserting the sponges in another 4 ewes on day 7 and removing them on day 20. In order to secure continuous follicular phase effects, follicular phase ewes were obtained by inserting sponges in 4 other ewes on days 1 and 2 and by removing them on days 11 and 12, respectively. Thus both luteal and follicular phase ewes were ready for introduction on day 13. On day 13, performance-tested rams (n = 4) were introduced into the ram group room, luteal phase ewes (n = 4) were introduced into the luteal group room, and half of the follicular phase ewes were introduced into the follicular group room (the other half were introduced on day 14). As we were not sure whether female sheep would have any effect on LH surge characteristics, and in order to observe the basal level without any introduction, we did not intend to introduce sexually inactive sheep into the control group. Therefore the control group consisted of only 6 ewes in order to gain some idea of the secretion characteristics of LH upon sponge withdrawal without the occurrence of any stimuli. Additionally, PMSG was not injected in order to record normal progress of ovulation upon sponge withdrawal.

Collection and analysis of blood samples for LH: On day 0, animals were introduced at 08:30 for sampling, and blood samples were collected starting from 08:00 (0 h) at 2 h intervals over the course of 96 h and twice daily thereafter, because it was reported that without PMSG injection all LH surges are observed within 96 h upon sponge withdrawal in sheep (14). Blood samples were placed into tubes coated with EDTA and immediately centrifuged at 3000 g. Plasma was separated and stored at –20 °C until the analyses for LH.

LH Analyses: A sensitive competitive enzyme immunoassay method developed by Mutayoba et al. (15) for bovine LH and modified by Yildiz et al. (16) for ovine LH measurements was used. Briefly, oLH (NIDDK-oLH-I-4 (AFP-8614B)) was labelled with D-Biotinyl-ε-aminocaproic acid N-Hydroxy-succimidine ester (Biotin-X-NHS, SIGMA, Germany). Affinity purified goat IgG anti-rabbit IgG was attached to the solid phase, and labelled and non-labelled (sample) oLH were competed against the anti-oLH raised in the rabbit (NIDDK-anti-oLH-1 (AFP-

192279)). Dilutions of biotinyl LH and oLH antiserum were 1:5,000 and 1:3,200,000, respectively. Standards used in the current study were between 0.39 and 50 ng oLH/ml. The minimum detection limit for the assay was 0.70 ng oLH/ml. Intra- and interassay coefficients of variations were calculated at 2 levels of quality control samples and as quadruplicates in 2 different locations of the plate. At the 3.65 ng/ml level, intra- and interassay coefficients of variation were 8.9 and 17.4%, and at the 7.19 ng/ml level they were 8.2 and 16.4%, respectively. Preovulatory LH surge was defined as the increases in LH secretion above 10 ng/ml.

Statistical analyses: Data were analysed by ANOVA within the MINITAB statistical program (State College, Pennsylvania, USA). When a statistical significance was observed, Student's t-test was used to determine where the difference had occurred. Data were represented as mean ± SEM.

Results

Body weights of the ewes were 60.3 ± 1.2, 60.3 ± 2.9, 58.5 ± 2.1 and 53.5 ± 2.6 kg, and their body condition scores were 2.8 ± 0.2, 2.7 ± 0.1, 2.8 ± 0.2, and 2.8 ± 0.2 for the luteal, follicular, ram and control groups, respectively. There were no significant differences in body weights or body condition scores of the animals among the groups.

Timing of the preovulatory LH surge is shown in Figure 1. Minimum and maximum times at which LH surges were observed were 38 and 312 h for the luteal ewe introduced group, 144 and 272 h for the follicular ewe introduced group, 42 and 72 h for the ram introduced group and 50 and 96 h for the control group, respectively. Additionally, there was no relationship between body condition score and LH surge timing.

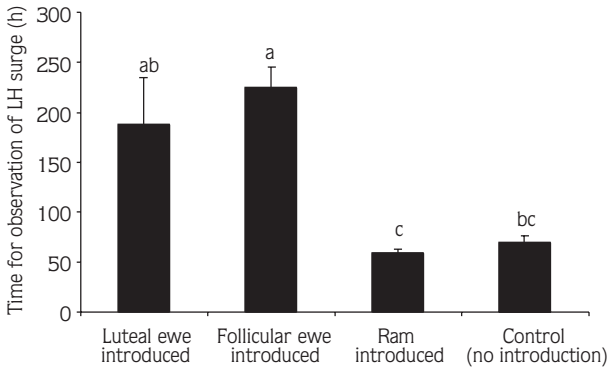

Figure 1. Timing of LH surges following progestagen-impregnated sponge withdrawal in anoestrous ewes kept with luteal or follicular phase ewes, or with rams or kept alone. Data represent mean ± S.E.M. Columns with different letters differ significantly at $P < 0.05$ (ab versus c) or at $P < 0.001$ (a versus c and a versus bc). The luteal group tended to differ from control group ($P = 0.075$).

Since blood samples were taken twice daily after the first 96 h, it was only possible to observe single rises in LH levels that were high enough to be regarded as surges (>10 ng/ml), but this prevented the calculation of the mean, amplitude and duration of surges).

Characteristics of preovulatory LH surges are given in 2 representative ewes for the control and ram groups (Figure 2).

Discussion

This study yielded some data that were beyond our expectations. In the luteal and follicular groups, LH surges were not observed between 0 h (sponge removal) and 96 h. Samples taken twice daily after the first 96 h provided some data for the luteal and follicular ewe-introduced groups. In fact, elevations in LH levels that might be regarded as surges were detected within 10 days of sponge withdrawal in these 2 groups.

The present study shows for the first time that introducing female sheep dramatically affected the onset of LH surge in the experimental sheep group. A female-to-female interaction had previously been suggested in sheep by Zarco et al. (8) and Yildiz et al. (9). In both studies however, the mechanisms of the female-to-female interactions were not clear. The present study suggests that the mechanisms which control the timing of the LH surge are involved. However, our results rather contradict the study by Zarco et al. (8) who reported a positive effect of ewes, following progesterone-impregnated sponge withdrawal, on anoestrous ewes in the nearest adjacent pens. The present study tested whether the automatic process of ovulation is affected in ewes following sponge withdrawal by the presence of other ewes brought to the follicular or luteal phases by manipulating the time of insertion or withdrawal of the sponges. Therefore, the current study and that of Zarco et al. (8) refer to 2 different physiological processes. Introduction of a sexually active female sheep may trigger sexual activity in anoestrous females if they are kept nearby for interaction (8,12). This might be a positive

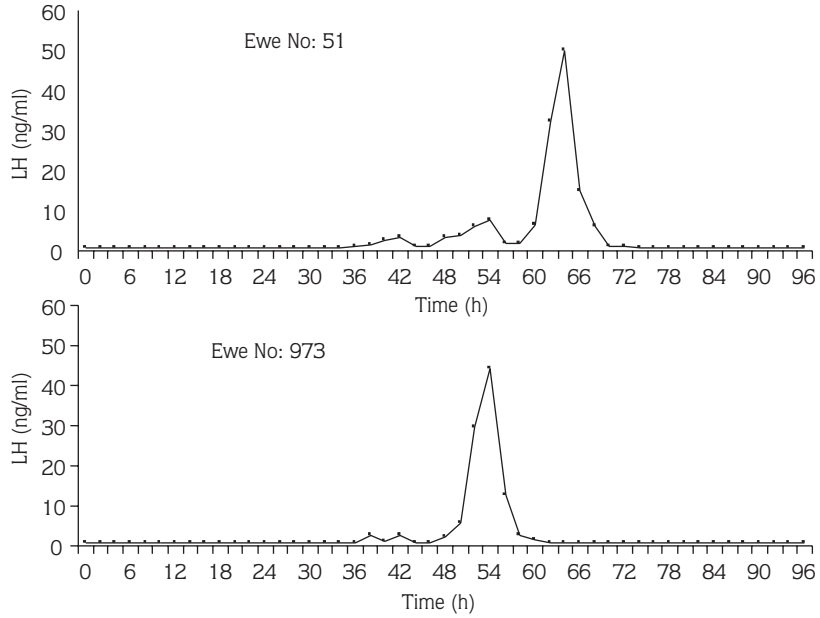

Figure 2. Secretion of LH after progesterone impregnated sponge removal in 2 representative ewes for the control (ewe no: 51) and ram (ewe no: 973) groups.

signal to anoestrous ewes to commence their breeding season. However, when the cyclic activity of the ewe is already triggered, as in the current study by intravaginal sponges, then the course of events might be different. In this case, timing of ovulation might be adjusted by other ewes in the flock. Yildiz et al. (9) reported that if the cycling ewes were kept together for a long time they would synchronise with each others' oestrous cycles. The current study shows that both follicular and luteal ewes negatively affected timing of the LH surge compared to the control and ram- introduced ewes. Additionally, although this was statistically insignificant, follicular ewes affected the other ewes more negatively than the luteal ewes did. Therefore, it might be speculated that a follicular ewe which is close to ovulation or ovulating or has just ovulated forces other ewes to postpone their ovulations. The luteal phase ewes also postpone, but for a shorter time than the follicular ewes, the expected LH surges of other ewes. However, it should be noted that the values obtained for each ewe are not necessarily the same as those observed under practical conditions, but the values show the extent to which ewes can affect others. In fact, within a flock, the LH values in the current study are probably obtained under the pheromenal cues from the ewes in other stages of the oestrous cycle. Indeed, Stern and McClintock (10) have shown that ovulatory and follicular pheromones from donor women differentially affect the cycle length of recipient women. Therefore, it might be speculated that each stage of the oestrus cycle has differential effects in other females. Additionally, female-to-female interaction might be affected by other stimuli, such as the time of the year and nutritional status of the ewe (12,17). In order to be affected by other females in the flock the body condition scores or energy reserves of the ewes should be sufficient. In that respect, Yildiz et al. (16) found that body condition scores were more important for the LH surge characteristics in ewes that received ram introduction at different times following $PGF_{2\alpha}$ synchronisation.

In this study, the timings of LH surge for the control and ram-introduced groups were approximately 10 h later than the values obtained by Yildiz et al. (16), who synchronised oestrous cycles with $PGF_{2\alpha}$ and introduced the rams after the second injection of $PGF_{2\alpha}$. Nevertheless, Yildiz et al. (16) reported no difference in the timing of the LH surge between control and ram-introduced groups, although it was slightly earlier in the latter. The current study and that of Yildiz et al. (16) suggest that the effect of ram introduction on the timing of LH surge in synchronised ewes is insignificant.

In conclusion, it appears that follicular or luteal phase sheep delay the LH surges compared to the ram-introduced or control groups and that female-to-female interaction seems to suppress preovulatory LH surge generation centres. Further studies are, however, needed to determine the interactions during other stages of oestrous cycles by, probably, taking into account the whole behaviour of the flock.

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