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The Efficiency of Cauterization of Clitoris on Serum LH Levels in Cows*

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The aim of the present study was to investigate the effect of cauterization of clitoris on serum Luteinizing Hormone (LH) levels in cows. In this study, 10 cows ranging from 2,5–6 years old were used. Animals were synchronised with double injections of $PGF_2\alpha$ at 11 day intervals. After the first sign of spontaneous estrous, blood samples were collected at 2 hours intervals during 24 hours to monitor LH concentration. When the second spontaneous estrous occurred, clitoris of cows was cauterized with electrocoter under epidural anesthesia and blood samples were collected same as the first spontaneous cycle.

LH concentrations were found 2.18±0.30 ng/ml, 2.03±0.31 ng/ml in control and clitoral cauterization group, respectively. Preovulatory LH peak concentrations were measured 11.56±1.99 ng/ml in control group and 10.55±1.63 ng/ml in cauterization group. The time from the beginning of estrous to the LH peak was found 16±2.61 h in control group and 12±0.97 h in cauterization group. Average duration of LH surge was observed 8.4±0.75 h and 10.4±0.4 h in control and cauterization group, respectively. No significant difference was observed between control and experimental group.

As a conclusion, it was detected that clitoral cauterization applied at the beginning of estrous in cows did not affect serum LH values.

Keywords: *Cow, LH, clitoris, cauterization.*

İneklerde Klitoris Koterizasyonunun LH Salınımı Üzerine Etkisinin Araştırılması

Sunulan çalışma, ineklerde klitoris koterizasyonunun Luteinleştirici Hormon (LH) salınımı üzerine etkisinin araştırılması amacıyla yapıldı. Çalışmanın materyalini, yaşları 2,5–6 arasında değişen toplam 10 inek oluşturdu. Materyal olarak kullanılan hayvanların östrüsleri 11 gün arayla çift doz PGF2α enjeksiyonu ile senkronize edildi. Senkronizasyon sonrası şekillenen ilk tabii siklus kontrol siklusunu oluşturdu. İlk östrüs belirtisi görüldüğü andan itibaren 24 saat boyunca, 2 saat aralıklarla, LH ölçümü amacıyla kan numuneleri alındı. Kendiliğinden şekillenen ikinci siklusta ilk östrüs belirtisinin görülmesiyle birlikte, hayvanın klitorisi epidural anestezi altında koterize edilerek, kontrol siklusunda olduğu gibi, kan numunelerinin toplanmasına devam edildi.

Kontrol ve uygulama grubunda ortalama LH değerlerinin sırasıyla 2,18±0,30 ng/ml ve 2,03±0,31 ng/ml olduğu belirlendi. Ortalama LH pik değeri kontrol grubunda 11.56±1.99 ng/ml, uygulama grubunda ise 10.55±1.63 ng/ml; östrus başlangıcı-LH pik zamanı arasında geçen süre kontrol grubunda 16±2.61 saat, uygulama grubunda 12±0.97 saat olarak belirlendi. Pik süresi kontrol grubunda 8.4±0.75 saat, uygulama grubunda 10.4±0.4 saat olarak tespit edildi. Yapılan uygulamanın kontrol ve uygulama grubunda LH parametrelerini etkilemediği görüldü.

Sonuç olarak, ineklerde östrüs başlangıcında uygulanan klitoris koterizasyonunun, ortalama LH değerlerini etkilemediği tespit edildi.

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Anahtar kelimeler: *İnek, LH, klitoris, koterizasyon.*

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Introduction

Clitoris which has the same embriyonic origin like male penis resides ventral commissure of the vestibule. It is composed of erectile tissue covered by stratified squamous epithelium, and it is well-supplied with sensor nevre endings. Various neural pathways exists between the reproductive system and hypothalamic-pituitary axis (1, 2).

Several authors (3, 4) have reported that increased biostimulation of the female during natural mating results in higher pregnancy rate. Nikolakopoulos et al. (5) have reported that events around mating including stimulation of the genital tract and uterine distension, often caused an increase in circulating concentrations of oxytocin. It has been reported that increased uterine motility has been observed in cows during exposure to a bull, nuzzling of genitalia, mounting, and copulating, possibly resulting in improved sperm transport (6). Mating can modulate the preovulatory surge of LH by prolonging the

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duration of LH release rather than by increasing plasma concentrations. Mating affects the time of ovulation in spontaneously ovulating species such, as sheep. In sows, natural mating affects ovulation by shortening the interval from onset of estrous to ovulation and by reducing the interval from the first to last ovulation. Naturally mated sows have higher concentrations of plasma LH immediately after mating (2).

Sexual stimulation of cows is an important factor in the efficiency of artificial insemination (AI) (7). A review in the literature has revealed some methods which may increase pregnancy rates in AI. Several researchers (8, 9) have noted that clitoral stimulation at the time of artificial insemination was an effective means of increasing pregnancy rate in cows. It has been shown that mechanical stimulations of the reproductive tract is tend to hastening the LH surge and clitoral stimulation hastens ovulation (10). In beef cattle, clitoral stimulation hastens the onset of ovulation, and stimulation of the cervix reduces the time from the beginning of estrous to the occurence of LH surge (2). It has been also showed that 10 seconds of manual clitoral massage increases conception rates in cows (11, 12). Segura et al. (13) demonstrated that clitoral massage improves pregnancy rates to AI in heifers.

It has been reported that (14, 15) clitoral massage applied at time of AI caused an immediate single uterine contraction in cows, and this response was neurally rather than hormonally mediated. Pointer (16) has demonstrated that after clitoral massage or stimulation, the cervix lumen enlarges and allows an easier passage.

Rodriguez et al. (17) showed that pregnancy rate was higher in animals not receiving clitoral massage than in those receiving it. Zalesky et al. (18) reported the presence of and/or mating a bull did not affect LH secretion or timing of the preovulatory LH surge after $PGF_2\alpha$. Arbeiter et al. (19) observed that a more rapid follicle growth in clitoral massage group and ovulation occured earlier compared with the control group. An experimental study (14) demonstrated that the effect of clitoral stimulation on LH release was negative, in the another study, Kirsch et al. (20) found that stimulation of the pelvic region during either natural mating or AI did not enhance release of LH in the gilt.

It is believed that the cauterization of the clitoris which is a common method used by farmer in Turkey, helps the pregnancy of repeat breeding cows and heifers in some cases after insemination.

Data mentioned above has showed that mating stimulation and clitoral massage affect ovulation time and release of oxytocin and LH from pituitary in the cow. These factors may directly or indirectly affect conception rate (10).

The aim of this study is to investigate the effects of cauterization of clitoris on serum LH levels in cows.

Materials and Methods

Animals and Location: This study was conducted in ELAZIG province of TURKEY, located at latitude $38^0$40'N. In this study, 10 cows (3 Holstein, 4 Brown Swiss and 3 Simmental) ranging from 2,5–6 years old were used. Cows were selected from healthy animals in University of Firat, Faculty of Veterinary Medicine Research and Application farm. All cows had normal oestrus cycle and were free of reproductive disorders. Lactating cows were on day of 60-80 postpartum. The animals were fed on barley, bran, hay and corn silage.

Estrous Synchronization and Blood Collection: The estrous cycles of the cows were synchronized with two doses of $PGF_2\alpha$ analogue (500 µg Cloprostenol, Estrumate, Sanofi Doğu İlaç A.Ş. İstanbul, TURKEY) for 11 days apart. Estrous was detected visually six times daily (0, 4, 8, 12, 16, 20 h), rectal palpation and transrectal ultrasonography.

The first spontanous estrous cycle was control cycle. After the first sign of spontanous estrous, blood samples were taken from vein jugularis. Briefly, a total 5 ml blood sample was taken by jugular veinpuncture at 2 hours intervals during first 24 hours to monitor LH concentration. When the second spontaneos estrous (application cycle) occurred, the clitoris was cauterized with electrocoter under epidural anesthesia. After cauterization, blood samples were collected with the way same in control cycle. Sera were stored – 20 °C until assayed.

Hormone Assays: Serum LH level was detected by Enzyme Immuno Assay (EIA) as detailed by Mutayoba et al. (21). The sensitivity of assay was 0.70 ng/ml. Intra- and inter-assay coefficients of variations for 4.1 ng/ml and 10.0 ng/ml were 8.5, 6.4 and 19.4, 19.8 %, respectively.

Statistical Analysis: Data was presented as mean ±S.E.M. Nonparametric Wilcoxon Signed Rank Test was applied to determine the differences in the LH between control and application groups. All data was analysed using the SPSS (Version 10.0) software package.

Results

Table I; demonstrates LH concentration (mean), peak LH concentration, time of LH surge, duration of LH surge, onset of LH surge and end of LH surge in control and application cycle in cows. These parameters did not change between two cycles. The preovulatory LH surge occurred at 16±2.61 h and 12±0.97 h after onset of estrous in noncauterized and cauterized cows, respectively. Peak concentrations and duration of preovulatory LH surge were similar for application and control females during the preovulatory LH surge. Onset of LH surge and end of LH surge occurred 9.6±2.56 h and 18±2.53 h in control group and 7.2±3.14 h and 17.6±3.19 h in application group after initial expression of oestrus symptoms (P>0,05).

Table 1. Serum LH peak characteristics. Table I: Serum LH peak characteristics.

Parameters	Control cycle[a] cycle[a]	Application cycle[a]	Significance
Time of LH surge (hour)	16±2.61	12±0.97	-
Peak LH concentration (ng/ml)	11.56±1.99	10.55±1.63	-
Duration of LH surge (hour)	8.4±0.75	10.4±0.4	-
Onset of LH surge (hour)	9.6±2.56	7.2±3.14	-
End of LH surge (hour)	18±2.53	17.6±3.19	-
LH concentration (mean)	2.18±0.30	2.03±0.31	-

-: P> 0,05

Discussion

It has been proposed that a neural pathway from the reproductive tract of ovine females to the pituitary gland exist, and a neural blocking agent, chloropromazine, has blocked ovulation in ewes (22). Randel et al. (11) has proposed that such a neural pathway also exists in cattle. Clitoral stimulation hastens the onset of ovulation, and stimulation of cervix reduces the time from the beginning of estrous to the occurance of LH surge in beef cattle (2).

Naturally mated sows have higher concentrations of plasma LH immediately after mating (2). Tilton et al. (23) have observed that natural mating has a temporary stimulatory effect on plasma LH concentrations approximately 1 h after copulation. In a study made by Zalesky et al. (18) has noted that the presence of and mating with a bull did not affect the release of LH in cows. Kirsch et al. (20) also observed that stimulation of the pelvic region during either natural mating or AI did not enhance release of LH. Cooper and Foote (14) reported that the effect of clitoral stimulation on LH release was negative.

In the present study, it was observed that cauterization of clitoris did not affect LH concentration. LH levels were not significantly different between groups. This finding indicates that cauterization of clitoris did not affect pituitary release of LH. While these results are similar with the results of Zalesky et al. (18) and Kirsch et al. (20), they are inconsistent with the results reported by other authors (2, 6, 10, 11, 24). The levels of serum LH detected in this study are within the range of plasma levels reported by Walters and Schallenberger (25), whereas these findings are different from the finding reported by other researchers (11, 24, 26).

Randel et al. (11), reported that the time from estrous to ovulation was shortened to 4.3 hours in clitoral stimulated group than control group in cows. They claimed that the reason of shortening of this term is hastening of LH surge in the cow. Panth et al. (27) and Singh et al. (9) observed that clitoral stimulation increased pregnancy rate presumably by hastening LH surge. Ziecik et al. (28) observed that mating can modulate the preovulatory surge of LH in gilts by prolonging the duration of LH release rather than by increasing plasma concentrations.

In the present study, cauterization of the clitoris did not affect the rate of LH secretion during preovulatory LH surge. Concentration of LH and duration of the preovulatory LH surge reported in the present study are similar to those reported (25) for cows not exposed to any type of stimuli, and are comparable to those reported by other researchers (9, 11, 18, 27, 28).

In conclusion, clitoral cauterization applied under epidural anaesthesia in healthy cows did not affect the mean LH concentration, LH peak concentration, the time from the beginning of estrous to onset of LH surge, to end of LH surge and to LH peak, mean duration of LH surge.

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