

## Effect of clitoral massage on levels of estradiol, testosterone, dehydroepiandrosterone sulphate and pregnancy rate in cows

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### ABSTRACT

This study was conducted to investigate the effect of clitoral massage on levels of estradiol ( $E_2$ ), testosterone (T), dehydroepiandrosterone sulphate (DHEA-S) and pregnancy rate in cows. For this purpose, 38 Holstein cows ranging from 3-6 years old were used. The cows were divided into two groups, as control (n=19) and application (n=19). The degree of intensity of oestrus expression was scored on a scale of 0 to 5 by considering knowledge of anamnesis, findings of rectal palpation and inspection as a criteria. All animals of both groups were artificially inseminated by recto-vaginal method with frozen-thawed bull semen. Clitoral massage was applied to the cows in the application group for 15-30 sec. after artificial insemination. Blood samples were then taken from jugular vein and centrifuged at 5000 rpm for 5 m., and serum samples were stored deep-frozen at -20 °C. Levels of  $E_2$ , T and DHEA-S were determined by RIA method. All animals were examined by rectal palpation of the uterus for pregnancy on day 75 after insemination. Clitoral massage application had no significant effect on the levels of  $E_2$ , T, DHEA-S and pregnancy rate in cows. There was a significant difference ( $P<0.05$ ) in the degree of the intensity of oestrus between control and application groups. A significant negative correlation ( $P<0.01$ ,  $r = -0.93$ ) was found between levels of  $E_2$  and T.

**Key words:** clitoral massage, estradiol, testosterone, dehydroepiandrosterone sulphate, pregnancy rate, cow

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### Introduction

The clitoris, homologous to the glans penis in the male, is located ventrally and about 1 cm inside the labia. It contains erectile tissue, is well supplied with sensory nerves and is erect during oestrus. While not sufficiently prominent to be used in oestrus detection in most species, the clitoris of the mare is an exception (BEARDEN et al., 2004; HERMAN et al., 1994; PINEDA, 2004).

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Oestrogens, including estradiol ( $E_2$ ), estrone and estriol, have been called the “female sex hormone”. The principal actions of oestrogens are their influence on: (a) the manifestation of mating behaviour during oestrus; (b) cyclic changes in the female tract; (c) duct development in the mammary gland; and (d), development of secondary sex characteristics in females (BEARDEN et al., 2004). Androgens containing testosterone (T), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEA-S) are not only the immediate precursors of oestrogens but also regulate the development, growth and maintenance of secondary sex characteristics (LEVIN, 1998). However, the role of androgens in female genital organs remains controversial and poorly understood.

Clitoral stimulation is provided with the massaging of the clitoris by hand for 15-30 sec. after artificial insemination, this application being termed “clitoral massage”. The massaging of the clitoris in cows enlarges the cervix lumen and allows easier passage (POINTNER, 1986), stimulates the release of oxytocin, and this hormone increases the contractions of the uterus toward the oviduct (ÇOYAN and TEKELİ, 1996). At the same time, clitoral massage causes an increase of uterine contractions without enhancing the release of oxytocin (COOPER et al., 1985; COOPER and FOOTE, 1986). The best positive effects of clitoral massage appear in the second half of the oestrus (ÇOYAN and TEKELİ, 1996). It has been reported (RANDEL et al., 1973) that mechanical stimulation of the reproductive tracts hastens LH surge, and ovulation is hastened by depending on this hormone. However, KIRSCH et al. (1985) suggest that stimulation of the pelvic region during both natural mating and artificial insemination does not enhance the release of LH in gilts. Although the application of clitoral massage for 10 sec. increases pregnancy rates in cows it has almost no effect in yearling heifers (RANDEL et al., 1975; SHORT et al., 1979).

It has been reported that oestrogens and testosterone treatments accelerate vaginal and clitoral blood flow (MIN et al., 2002; PARK et al., 2001) and increase vaginal lubrication (PARK et al., 2001), but do not alter relaxation of the clitoral cavernous smooth muscles (KIM et al., 2002) in rabbits. But there is no evidence as to whether clitoral stimulation affects steroids in cows or other species. Therefore, this study was conducted to investigate the effect of clitoral massage after artificial insemination on the  $E_2$ , T, DHEA-S and pregnancy rate in cows.

### **Materials and methods**

*Animals and location.* This study was conducted in the Elazığ Province of Turkey, located at latitude 38°40'N. In this study, 38 Holstein cows ranging from 3-6 years old were used. The cows were selected from healthy animals which were brought to the Fırat University, Faculty of Veterinary Medicine, Animal Hospital, Unit of Reproduction and Artificial Insemination for insemination. All cows had normal oestrus cycle and were free

Table 1. Criteria for grading intensity of oestrus in cows according to the findings of anamnesis and clinical examination

Intensity of oestrus	Findings of anamnesis	Findings of clinical examination
5 (excellent)	The reduce in the feed consumption The reduce in the milk production Shouting and restlessness Mounting other cows Cervical mucus Permission if other cows mount	Restlessness Moist, red and swollen vulva Abundant, limpid and sticky mucus The increase in the cervix tonus The increase in the uterus tonus Graffian follicle in the ovary
4 (good)	The reduce in the feed consumption The reduce in the milk production Shouting and restlessness Mounting other cows Cervical mucus	Restlessness Light moist, light red, light swollen vulva Abundant, limpid and watery mucus The increase in the cervix tonus The increase in the uterus tonus Graffian follicle in the ovary
3 (normal)	The reduce in the feed consumption The reduce in the milk production Shouting and restlessness Cervical mucus	Light restlessness Light moist, light red, light swollen vulva Little, limpid and watery mucus The increase in the cervix tonus The increase in the uterus tonus Graffian follicle in the ovary
2 (fair)	The reduce in the feed consumption The reduce in the milk production Shouting and restlessness	Light restlessness Light moist, light red, light swollen vulva No cervical mucus Cervix is normal structure The light increase in the uterus tonus Uncertain follicle
1 (poor)	The reduce in the feed consumption Shouting	Calmness Only light moist vulva No cervical mucus Cervix is normal structure The light increase in the uterus tonus No Graffian follicle
0 (no oestrus)	Shouting	Calmness Dry, pale and no swollen vulva No cervical mucus Cervix and uterus are normal structure No Graffian follicle

of reproductive disorders. Lactating cows were at day 60 postpartum, which was learnt by anamnesis from animal owners who had applied similar conditions of maintenance and nutrition to the cows. The animals had been grazed on green pasturage in spring and summer and kept in closed barns in autumn and winter. The animals were fed on barley, bran, sugar beet meal and hay when kept in closed barns. Cows were divided into two groups as control (n=19) and an application group (n=19).

*Determination of oestrus signs, application of clitoral massage, and blood collection.* Signs of oestrus seen by animal owners were taken by anamnesis and recorded before insemination. Additionally, clinical oestrus findings determined by rectal palpation and inspection were also recorded. Degree of intensity of oestrus expression were scored on a scale of 0 to 5 by considering knowledge of anamnesis, findings of rectal palpation and inspection as criteria (Table 1). All animals in control and application groups were then artificially inseminated by recto-vaginal method with frozen-thawed bull semen (BEARDEN et al., 2004). As soon as the control group cows were inseminated, blood samples were taken from jugular vein. Secondly, clitoral massage was applied to cows in the application group for 15-30 sec. after artificial insemination (ÇOYAN and TEKELİ, 1996). Blood samples were taken from the application group 10 m. after clitoral massage. All blood samples were centrifuged at 5000 rpm for 5 m. After centrifugation the serum samples were separated and stored deep-frozen at -20 °C until assay.

*Measurement of steroid hormones and determination of pregnancy.* Serum E<sub>2</sub> level was measured by the Double-Antibody RIA method using DSL – 4400 kit (Diagnostic System Laboratories Inc. Texas, U.S.A.) in gamma counter (LKB-Wallac Multigamma) according to kit manufacturer's instructions. The amount of E<sub>2</sub> was read via RIA data analysis computer program and expressed as pg/mL. The calibration range and sensitivity of E<sub>2</sub> kit were 20 to 6000, and 4.7 pg/mL, respectively. The intra- and inter-assay variation coefficients of the kit were 3.2 to 5.3% and 8.1 to 9.3%, respectively. Serum T and DHEA-S were measured by Coated-Tube RIA using Active™ DSL-4000 for T and Active™ DSL-3500 for DHEA-S in gamma counter, according to kit manufacturer's instructions. Calibration range of T and DHEA-S kits were 0.1 to 25 ng/mL and 5 to 800 µg/dL, respectively. Sensitivity of T and DHEA-S kits were 0.08 ng/mL and 1.7 µg/dL, respectively. The intra-assay variation coefficients of T and DHEA-S kits were 7.8 to 9.6% and 6.3 to 9.4%, respectively. The inter-assay variation coefficients of T and DHEA-S kits were 8.4 to 9.1% and 9.6-10.0%.

All animals were observed for oestrus between 19 and/or 21 days after insemination. Cows which showed no oestrus at this point were examined for pregnancy on day of 75 after artificial insemination by rectal palpation of the uterus.

*Statistical analyses.* Data are presented as mean ± SEM. Nonparametric Mann-Whitney U test was applied to determine the differences in the E<sub>2</sub>, T, DHEA-S and

intensity of oestrus between control and application groups. The chi-square ( $\chi^2$ ) test was used to determine the differences in the pregnancy rate between control and application groups. Spearman rank correlation test was used for relation in the whole parameters. All data were analysed using the SPSS/PC (Version 12.0) and MINITAB® software package program (Version 14.0).

## Results

Steroid hormone levels, degree of the intensity of oestrus, and pregnancy rate in control and application group cows are presented in Table 2. Although application of clitoral massage caused a slight increase in the levels of E<sub>2</sub>, T, DHEA-S and pregnancy rate in the application group compared to the control group, these increases were not statistically significant (P>0.05). There was a significant difference (P<0.05) in the degree of intensity of oestrus between control and application groups. A significant negative correlation (P<0.01, r= -0.93) was found between levels of E<sub>2</sub> and T (Table 3).

Table 2. Levels of steroid hormones, degree of oestrus intensity and pregnancy rate in control and application groups of cows

	Group		
	Control (n=19)	Application (n=19)	Significance
Estradiol (pg/mL)	186.40 ± 13.59	210.70 ± 40.23	-
Testosterone (ng/mL)	0.43 ± 0.04	0.59 ± 0.08	-
Dehydroepiandrosterone sulphate (µg/dL)	7.12 ± 0.94	9.83 ± 0.85	-
Intensity of oestrus (0-5)	3.26 ± 0.19	3.84 ± 0.17	*
Pregnancy rate (%)	63.16 (19/12)	68.42 (19/13)	-

\* P<0.05

Table 3. Correlation coefficients of all parameters pertaining to control and application groups of cows

	Estradiol	Testosterone	DHEA SO <sub>4</sub>	Intensity of oestrus	Pregnancy rate
Estradiol	-----				
Testosterone	-0.93**	-----			
DHEA SO <sub>4</sub>	-0.25	0.32	-----		
Intensity of oestrus	-0.09	-0.18	-0.11	-----	
Pregnancy rate	0.37	0.25	0.16	0.42	-----

\*\* P&lt;0.01

## Discussion

Clitoral massage positively affects the release of certain protein hormones, such as oxytocin and LH (RANDEL et al., 1975). It has been reported that oestrogens and testosterone treatments accelerate vaginal and clitoral blood flow (MIN et al., 2002; PARK et al., 2001) and increase vaginal lubrication (PARK et al., 2001), but do not alter relaxation of the clitoral cavernous smooth muscles (KIM et al., 2002) in rabbits. However, there is no evidence as to whether clitoral stimulation affects steroid hormones in cows and other species. In this study, although the levels of E<sub>2</sub>, T, and DHEA-S were higher in the application group than in the control, the increases were not statistically significant (P>0.05). The intensity of oestrus was found to be higher in the application group than in the control (P<0.05) according to the findings of anamnesis and clinical examination prior to artificial insemination. This situation could be explained by the individual difference in the showing of oestrus behaviours and the non-significant relative increment of the E<sub>2</sub> level.

Oestrogens, representing a group of steroids with a similar physiological activity, are produced by specific cells in the graafian follicle. The thecal cells of the follicle are stimulated by LH to produce androgens which diffuse across the basement membrane, where they are converted to oestrogens by aromatase activity in granulosa cells, which are under the influence of FSH (BEARDEN et al., 2004). Both DHEA and DHEA-S are prohormones without knowing receptors or specific target tissues (WILLEMSE et al., 1994).

Adrenal androgens produce minimal androgenic activity, contributing to androgenicity by converting intracellular to bioactive androgens and oestrogens (LABRIE et al., 1997). In this study a significant negative correlation ( $P < 0.01$ ,  $r = -0.93$ ) was determined between  $E_2$  and T levels. This status shows that almost all androgens, especially T, are converted to oestrogens in the basement membrane of thecal cells in cows.

COOPER and FOOTE (1986) reported that clitoral massage applied for 5 seconds after insemination did not alter the pregnancy rate (control 74.0%, clitoral massage 74.3%) in cows, although it increased in heifers (control 70.6%, clitoral massage 81.9%). Although KARACA et al. (2001) documented that the pregnancy rates of cows with clitoral massage and non-clitoral massage were found to be 74.29% and 67.65%, respectively, these differences were insignificant. SEGURA and RODRIGUEZ (1994) reported that clitoral stimulation increased pregnancy rates (57% versus 45% for treated and control groups, respectively;  $P < 0.02$ ) in Zebu-cross-bred heifers. GLAUBER (1989) also found that conception rates were higher in cows on which clitoral massage was carried out for 10 seconds (65.08%) than in controls (51.43%). LUNSTRA et al. (1983) reported that clitoral stimulation increased the first service pregnancy rate in cows ( $74 \pm 3\%$  versus  $59 \pm 3\%$ ,  $P < 0.05$ ), but not in heifers ( $53 \pm 5\%$  versus  $57 \pm 5\%$ ,  $P > 0.10$ ), and that clitoral stimulation improved pregnancy rates more effectively in 3- to 4-year-old cows, than in both 2-year-old and 5-year-old, or even older, animals. ARBEITER et al. (1985) reported that the pregnancy rate was found to be higher in heifers to which clitoral massage was applied (80%) than that of those to which it was not (40%). In this study, the pregnancy rate found in application and control group cows was 68.42% and 63.16%, respectively. This result is compatible with the findings of some other researchers. COOPER and FOOTE, 1986, and KARACA et al., 2001 reported that clitoral massage had no significant effect on pregnancy rates. Conversely, however, other researchers (GLAUBER, 1989; LUNSTRA et al., 1983; SEGURA and RODRIGUEZ, 1994) reported that clitoral massage had a significant effect on the pregnancy rate in cows. The reason for marked or unmarked increases may lie in different forms of maintenance and nutrition, individual differences between cows, and duration of clitoral stimulation.

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**BOZKURT, T., G. TÜRK, S. GÜR: Utjecaj masaže dražice na razine estradiola, testosterona, dehidroepiandrosteron-sulfata i postotka gravidnosti u krava. *Vet. arhiv* 77, 59-67, 2007.**

**SAŽETAK**

Cilj istraživanja bio je utvrditi utjecaj klitoralne masaže na razine estradiola, testosterona, dehidroepiandrosteron-sulfata i postotak gravidnosti u krava. U tu svrhu pretraženo je 38 krava holštajnske pasmine u dobi od tri do šest godina. Krave su bile podijeljene u kontrolnu i pokusnu skupinu pri čemu je svaka skupina sadržavala po 19 životinja. Stupanj pokazivanja znakova estrusa bio je ocijenjen po skali od 0 do 5 uzimajući u obzir anamnestičke podatke, nalaz rektalne pretrage i promatranje kao kriterije. Sve životinje bile su umjetno osjemenjene smrznutom spermom klasičnom bimanualnom metodom. Masaža dražice provedena je u krava pokusne skupine u tijeku od 15 do 30 sekundi nakon UO. Uzorci krvi uzeti iz v. jugularis, potom centrifugirani na 5000/5 min, a uzorci seruma bili su pohranjeni na -20 °C. Za određivanje razine estradiola, testosterona, dehidroepiandrosteron-sulfata rabljena je RIA. Sve životinje su pregledane rektalno 75 dana nakon UO. Masaža dražice nije značajno utjecala na razine estradiola, testosterona, dehidroepiandrosteron-sulfata kao ni na postotak gravidnosti u krava. Postojala je značajna razlika ( $P < 0,05$ ) u stupnju i jačini estrusa između kontrolne i pokusne skupine. Značajna negativna korelacija ( $P < 0,01$ ,  $r = -0,93$ ) ustanovljena je između razina estradiola i testosterona.

**Ključne riječi:** dražica, masaža, estradiol, testosteron, dehidroepiandrosteron-sulfat, gravidnost, krava

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