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Research Article

Effects of Exogenous Oxytocin on Serologic and Seminal Steroids and Semen Characteristics in Rams

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Abstract: The objective of this study was to investigate the effect of IV injection of oxytocin (OT) on serum and seminal plasma levels of oestradiol (E2), testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), and semen characteristics in rams. Twelve 3-year-old Awassi adult rams were randomly divided into 2 equal groups (control and treatment). Physiological saline (0.5 ml) was injected IV into each ram in the control group, whereas OT was administered IV at a single dose of 5 IU to each ram in the treatment group. Blood samples were collected from the jugular vein and semen samples were taken by an electroejaculator from all rams sequentially at 10, 60, and 120 min after the IV injection of physiological saline or OT. Administration of OT had no effect on blood or seminal plasma E2 or DHEA-S levels, or on serum T at any collection time compared to the control group. However, while OT increased significantly the level of seminal plasma T at 10 min, its effect was absent at 60 and 120 min after the injection compared to the control group. OT did not alter the semen viscosity, sperm motility, or abnormal sperm rate at all times in comparison to the control values. Although semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number of the treatment group increased significantly at 10 min compared to the control group, all these parameters decreased markedly at 60 and 120 min in comparison with 10 min after injection in both the control and treatment groups. In conclusion, exogenous OT increases seminal plasma T, semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number in the first ejaculate of the rams 10 min after the IV administration.

Key Words: Oxytocin, oestradiol, testosterone, dehydroepiandrosterone sulphate, semen characteristics, ram

Egzojen Uygulanan Oksitosinin Koçlarda Serolojik ve Seminal Steroidler ile Spermatolojik Özellikler Üzerine Etkileri

Özet: Bu çalışma damar içi oksitosin (OT) enjeksiyonunun koçlarda serum ve seminal plazma östradiol (E2), testosteron (T), dehidroepiandrosteron sülfat (DHEA-S) düzeyleri ve spermatolojik özellikler üzerine etkilerini araştırmak amacıyla yapılmıştır. Çalışmada 3 yaşlarında 12 yetişkin İvesi koçu kullanıldı ve her bir grupta 6 koç olacak şekilde kontrol ve uygulama grubu olmak üzere rasgele iki gruba ayrıldı. Kontrol grubundaki her bir koça 0.5 ml fizyolojik tuzlu su damar içi verilirken uygulama grubundakilere ise 5 IU OT uygulandı. Fizyolojik tuzlu su ya da OT uygulamasından sonraki 10, 60 ve 120. dakikalarda V. jugularisten kan ve elektroejakülatör yardımıyla da sperma örnekleri toplandı. Kontrol grubu ile karşılaştırıldığında, OT uygulaması serum ve seminal plazma E2 ve DHEA-S düzeyleri ile serum T miktarını tüm zamanlarda etkilemedi. Bununla birlikte OT kontrol grubu ile karşılaştırıldığında 10. dakikadaki seminal plazma T düzeyini önemli derecede artırırken 60 ve 120. dakikalarda ise etkisiz kaldı. Kontrol grubuyla karşılaştırıldığında tüm zamanlarda, OT spermanın akışkanlığını, motilitesini ve anormal spermatozoon oranını değiştirmedi. Uygulama grubunun sperma miktarı, kitle hareketi, spermatozoon yoğunluğu, toplam spermatozoon sayısı ve toplam motil spermatozoon sayısı kontrol grubuna göre 10. dakikada önemli derecede artmasına rağmen, tüm bu parametreler 10. dakika ile karşılaştırıldığında 60 ve 120. dakikalarda hem kontrol ve hem de uygulama grubunda önemli derecede azaldı. Sonuç olarak koçlara damar içi OT uygulaması 10. dakikada alınan ilk ejakülattaki seminal plazma T, sperma miktarı, kitle hareketi, spermatozoon yoğunluğu, toplam spermatozoon sayısı ve toplam motil spermatozoon sayısını artırmaktadır.

Anahtar Sözcükler: Oksitosin, östradiol, testosteron, dehidroepiandrosteron sülfat, spermatolojik özellikler, koç

Introduction

Oxytocin (OT) is a naturally occurring hormone in the female and male organism of all mammalian species. Its chemical structure is a nonapeptide. Endogenous OT is produced in the nucleus supraopticus and the nucleus paraventricularis of the hypothalamus. By neurosecretion

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the hormone migrates to the posterior lobe of the hypophysis, where it is stored, and it is released in response to nervous stimuli (1).

OT has specific effects on contraction of smooth muscle of uterus and cells of mammary gland in the females, but the physiological function of OT in the male is unclear. However, it has been reported that OT advances the sperm movement during its transport from ductuli efferentes and epididymis (2) by stimulating smooth muscle cells. This function of OT is supported by the localisation of the OT receptors in the epididymis (3), ductus deferens (4), and the presence of receptors on intratubular regions (5), Leydig, and Sertoli cells (6). OT is also produced locally by Leydig cells (7,8) and made in a particular place of the testis, and in the epididymis and prostate (9). During sexual stimulation and ejaculation, OT is released from the neurohypophysis into the peripheral circulation (10). Furthermore, OT administration facilitates semen collection by electroejaculation (11). It has been reported that OT injection before the semen collection increases sperm concentration in several mammalian species such as rams (12), rabbits (13), bulls (14), and buffalos (15).

Testosterone (T), which is secreted from Leydig cells in the testes, regulates the development, growth, and maintenance of secondary sex characteristics in males (16). Some researchers (5,17) have reported that OT treatment reduces the T concentration whereas others (8,18) have reported that this hormone increases the release of T and some (19,20) have documented that OT injection does not alter the concentration of T.

Dehydroepiandrosterone (DHEA) is formed predominantly in the zona reticularis of the adrenal gland (21) and a little in Leydig cells (22), and is reversibly converted to dehydroepiandrosterone sulphate (DHEA-S), which is the most abundant circulating hormone in mammals (21). Both DHEA and DHEA-S are prohormones without known receptors or specific target tissues. The adrenal androgens have minimal androgenic activity, and they contribute to androgenic function by converting intracellular androgens to bioactive androgens and oestrogens (23). Chiodera and Coiro (24) have reported that OT administration reduces the plasma DHEA-S level.

Oestrogens are necessary not only for female reproduction but also for male reproduction. Oestrogens together with androgens have an essential role in the differentiation and functional activity of the epididymis (25,26). Oestrogens are present in the seminal plasma and a substantial source of them is the secretory activity of prostate. This study was conducted to investigate the effect of OT on semen characteristics and levels of E2, T, and DHEA-S in serum and seminal plasma of rams.

Materials and Methods

Animals and location

Twelve 3-year-old Awassi adult healthy rams were used in the present study. The rams, raised at the Centre of Education, Research and Application at the Faculty of Veterinary Medicine, University of Fırat, were used during the breeding season (September) and were kept under natural climate conditions in Elazığ province, located at the latitude of 38°40'N. The animals were fed on grass supplemented with lucerne hay and fresh drinking water was provided ad libitum. The rams were randomly divided into 2 equal groups: control and treatment.

Sample collection and OT administration

Physiological saline (0.5 ml, 0.9% NaCl) was injected IV into each ram in the control group whereas OT was administered IV at a single dose of 5 IU (Oxytocin 10 IU/ml, Vetaş, İstanbul, Turkey) to each ram in the treatment group. Blood samples were collected from the jugular vein and semen samples were taken by an electroejaculator from all rams sequentially at 10, 60, and 120 min after IV injection of physiological saline or OT. This single dose of OT is recommended for the contractility of smooth muscles in sheep. Blood and semen samples were centrifuged at 3000 ×g for 5 min; the serum and seminal plasma were separated and stored at -20 °C until assayed. Serum and seminal plasma E2, T, DHEA- S, and semen characteristics of all rams were determined.

Measurement of steroids

The serum and seminal plasma E2 levels were measured by Double-Antibody RIA using a DSL – 4400 kit (Diagnostic System Laboratories Inc. Texas, USA) in a gamma counter (LKB-Wallac Multigamma) according to the manufacturer’s instructions. The calibration range and sensitivity of the E2 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.

The serum and seminal plasma T and DHEA-S levels were measured by Coated-Tube RIA using Active™ DSL-4000 for T and Active™ DSL-3500 for DHEA-S in a gamma counter according to the manufacturer's instructions. The calibration ranges of the T and DHEA-S kits were 0.1 to 25 ng/ml and 5 to 800 µg/dl, respectively. The sensitivity of the T and DHEA-S kits were 0.08 ng/ml and 1.7 µg/dl, respectively. The intra-assay variation coefficients of the T and DHEA-S kits were 7.8% to 9.6% and 6.3% to 9.4%, respectively. The inter-assay variation coefficients of the T and DHEA-S kits were 8.4% to 9.1% and 9.6% to 10.0%, respectively.

Semen evaluation

Semen volume was determined by direct reading of the graduations on the collection tube (from 0.1 to 10 ml). Semen viscosity was established visually with a scale of 0-5 (from watery to creamy). To determine the mass activity, a non-coverslipped drop of non-diluted fresh semen was placed on a warm slide (37 °C) under a light microscope with heated stage at 100× magnification. The condenser diaphragm of the microscope was lowered in order to increase the contrast. The following descriptors were used for mass activity: 5: rapid dark swirls; 4: slower dark swirls and eddies; 3: little slower swirls; 2: no swirls, but prominent individual cell motion; 1: little individual cell motion; and 0: no individual cell motion (1).

Semen samples were diluted with isotonic sodium citrate solution at 37 °C (3%, w/v dissolved in distilled water) at the rate of 1:10 (semen to sodium citrate). A slide was placed on the light microscope and warmed up to 37 °C. A small droplet of diluted semen was placed on the slide and the percent motility was evaluated visually at a magnification of 400×. Motility estimations were performed from 3 different fields in each sample. The mean of the 3 estimations was used as the final motility score. The percentage of morphologically abnormal spermatozoa was determined from the slides prepared with Indian ink. A total of 300 spermatozoa were counted on each slide under the light microscope at 400× magnification. Sperm concentration was measured with a haemocytometer (1).

The total sperm number was calculated by multiplying the semen volume by the number of sperm per millilitre. The total motile sperm number was also calculated by multiplying the total sperm number by the percentage of sperm motility.

Data analyses

The data are presented as mean ± standard error of the mean (SEM). The level of significance was set at $P < 0.05$. Non-parametric Mann Whitney-U test was used to determine the differences between the control and treatment groups. To determine the differences among time divisions, 2-way analysis of variance for repeated measures were used. All data were analysed by SPSS/PC (Version 10.0; SPSS, Chicago, IL, USA).

Results

The serum E2, T, and DHEA-S levels of the rams are given in Table 1. No significant differences were observed between the control and treatment groups, or among time divisions.

The seminal plasma E2, T, and DHEA-S levels are presented in Figures 1-3, respectively. The administration of OT did not affect E2 or DHEA-S levels at any time compared to the control group. However, while OT administration significantly increased ($P < 0.05$) the level of seminal plasma T at 10 min, its effect was absent at 60 and 120 min after the injection compared to the control group.

The values regarding the semen characteristics are given in Table 2. It was observed that OT administration did not alter the semen viscosity, sperm motility, or abnormal sperm rate at any time in comparison with the control group. Although semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number of the treatment group increased significantly ($P < 0.05$) at 10 min after the injection of OT compared to the control group, all these parameters decreased markedly ($P < 0.01$) at 60 and 120 min after the injection in comparison with 10 min in both the control and treatment groups.

Discussion

Synthetic OT is structurally identical to the naturally occurring hormone. OT and its synthetic analogue have been used for a long time in human and veterinary

Table 1. The mean serum oestradiol (E2), testosterone (T), and dehydroepiandrosterone sulphate (DHEA-S) levels of control and treatment groups at all times.

		Time		
		10 min	60 min	120 min
Oestradiol (pg/ml)	Control	24.14 ± 0.86	29.94 ± 0.51	26.52 ± 1.08
	Treatment	21.08 ± 1.07	28.84 ± 0.67	30.42 ± 1.90
Testosterone (ng/ml)	Control	3.78 ± 1.07	4.13 ± 0.96	4.74 ± 0.68
	Treatment	5.37 ± 1.12	3.40 ± 1.04	3.88 ± 0.52
Dehydroepiandrosterone sulphate (µg/dl)	Control	13.55 ± 1.21	11.94 ± 2.85	14.28 ± 2.06
	Treatment	14.15 ± 1.96	13.80 ± 1.50	12.75 ± 0.79

Data are expressed as mean ± SEM.
No significant differences were observed between the control and treatment groups, or among time divisions.

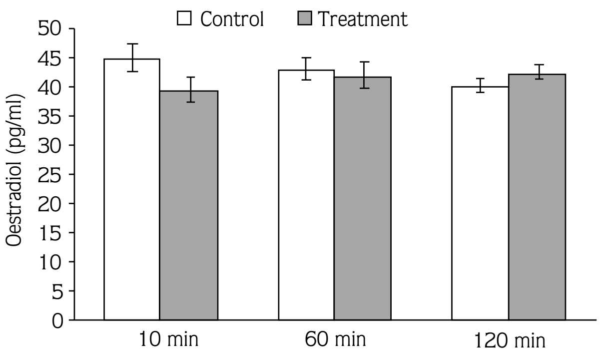

Figure 1. The mean seminal plasma oestradiol (E2) levels of the control and the treatment groups at all times.
No significant differences were observed between the control and the treatment groups, or among time divisions.

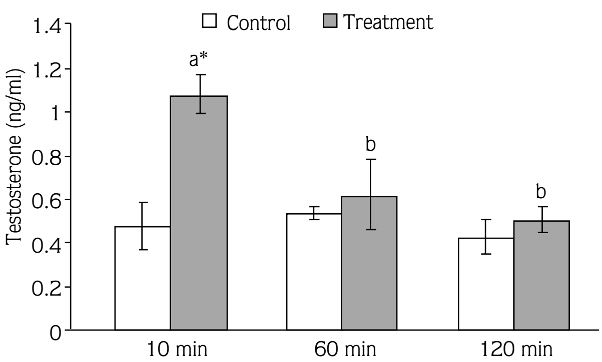

Figure 2. The mean seminal plasma testosterone (T) levels of the control and the treatment groups at all times.
(*): $P < 0.05$, vs. control.
(a, b): Different letters show significant differences ($P < 0.01$) among time divisions.

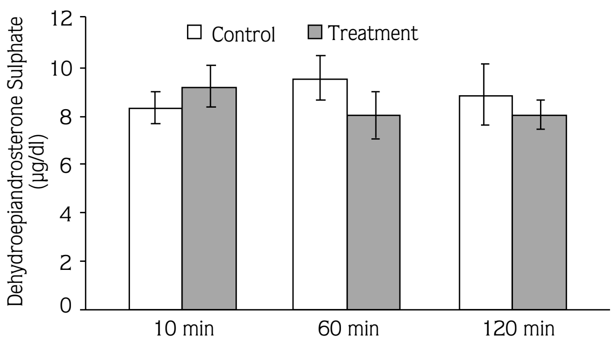

Figure 3. The mean seminal plasma dehydroepiandrosterone sulphate (DHEA-S) levels of the control and the treatment groups at all times.
No significant differences were observed between the control and the treatment groups, or among time divisions.

medicine. Therefore, many studies have been conducted on the relationship between OT and other hormones. However, the data pertaining to the effect of OT on T are controversial, because some researchers (5) have reported that OT treatment reduces the T concentration whereas others (8) have reported that this hormone increases the release of T, and some (19) have also documented that OT does not alter the concentration of T. In the present study it was observed that the administration of OT did not affect the levels of serum T at any time compared to the control group. However, OT administration increased significantly the level of seminal plasma T at 10 min, but its effect was absent at 60 and 120 min compared to the control group. This increase in seminal plasma T of the treatment group at 10 min after the injection may be due to the elevation of testosterone level in prostatic fluid by OT administration. This

Table 2. The mean values pertaining to the semen characteristics of the control and treatment rams at all times.

Semen characteristics		Time		
		10 min	60 min	120 min
Volume (ml)	Control	0.93 ± 0.11^{a}	0.70 ± 0.08^{b}	0.30 ± 0.02^{c}
	Treatment	1.26 ± 0.15^{a*}	0.75 ± 0.19^{b}	0.35 ± 0.05^{c}
Viscosity (0-5)	Control	3.69 ± 0.16	3.82 ± 0.24	3.45 ± 0.14
	Treatment	3.94 ± 0.06	3.50 ± 0.16	3.50 ± 0.12
Mass activity (0-5)	Control	4.00 ± 0.19^{a}	3.08 ± 0.05^{b}	2.99 ± 0.09^{b}
	Treatment	4.50 ± 0.19^{a*}	3.43 ± 0.28^{b}	3.10 ± 0.18^{b}
Sperm motility (%)	Control	80.63 ± 3.33	77.52 ± 3.21	75.16 ± 2.37
	Treatment	86.25 ± 1.83	81.88 ± 1.32	78.13 ± 1.88
Sperm concentration ($\times 10^9$/ml)	Control	2.93 ± 0.22^{a}	2.06 ± 0.16^{b}	1.89 ± 0.10^{b}
	Treatment	3.56 ± 0.16^{a*}	2.36 ± 0.21^{b}	2.08 ± 0.12^{b}
Total sperm number ($\times 10^9$)	Control	2.72 ± 0.42^{a}	1.44 ± 0.28^{b}	0.56 ± 0.09^{c}
	Treatment	4.48 ± 0.61^{a*}	1.77 ± 0.69^{b}	0.73 ± 0.16^{c}
Total motile sperm number ($\times 10^9$)	Control	2.19 ± 0.27^{a}	1.11 ± 0.08^{b}	0.42 ± 0.08^{c}
	Treatment	3.86 ± 0.32^{a*}	1.44 ± 0.15^{b}	0.57 ± 0.05^{c}
Abnormal sperm rate (%)	Control	9.88 ± 1.38	10.54 ± 2.35	12.46 ± 2.04
	Treatment	8.83 ± 1.33	11.63 ± 1.81	11.70 ± 1.30

Data are expressed as mean ± SEM.
(*): P < 0.05, vs. control
($^{a, b, c}$) Different letters within the same row show significant differences (P < 0.01) among time divisions.

hypothesis is supported by Nicholson and Jenkin (27), who reported that OT treatment increases the testosterone level in prostate tissue. Furthermore, the decrease in the levels of seminal plasma T at 60 and 120 min may be elucidated with the short half-life of OT (approximately 22 min) in small ruminants (28).

DHEA and DHEA-S are distal precursors of oestrogen formation (21). It has been reported (29) that the administration of DHEA or its metabolite causes a significant increase in mean E2 levels. Chiodera and Coiro (24) suggested that IV administration of OT decreases the plasma DHEA-S in humans. OT did not affect the level of serum or seminal plasma DHEA-S at any time compared to the control values in the present study. The difference between our results and those reported by Chiodera and Coiro (24) may be due to the use of different materials and dose. The lack of the effect of OT on DHEA-S may be attributed to the possibility that OT has no significant effect on the adrenal gland, where it is predominantly produced.

Oestrogens and androgens have an essential role in the differentiation and functional activity of the epididymis (25,26). The effect of OT on corpus cavernosum (30) and epididymal contractility (31) is regulated by oestrogens. Berndtson et al. (32) reported that OT inhibits E2 production in bovine granulosa cells without affecting the aromatase activity. There are no available data on the effect of OT on E2 levels in rams. The results of this study showed that OT did not alter either serum or seminal plasma E2 levels. Although the aromatase activity was not measured in the present study, this situation may be explained by the fact that OT has no effect on aromatase activity, which converts androgens to oestrogens.

During sexual stimulation and ejaculation, OT is released from the neurohypophysis into the peripheral circulation (10). Furthermore, OT administration facilitates the semen collection by electroejaculation (11) and causes an increase in the sperm concentration in rams (12), bulls (14), and buffalos (15). Fuchs et al. (33) found that in vitro addition of OT to semen significantly increased the percentage of motile spermatozoa in bulls; whereas Sliwa (34) suggested that intratesticular administration of OT decreased the sperm motility in mice. Walch et al. (35) reported that OT has no detectable effect on ejaculation time and seminal parameters after intranasal application in normal, healthy men. This study showed that OT did not alter the semen viscosity, sperm motility, or abnormal sperm rate at any time in comparison with the control group. Although semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number of the treatment group increased significantly at 10 min compared to the control group, all these parameters decreased markedly at 60 and 120 min in comparison with 10 min in both the control and the treatment groups. The difference between our results and those of previous studies (33-35) might be due to the different materials used and/or the style of OT administration. The increase in the semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number of the treatment group at 10 min may be explained by the acceleration of the sperm movement by OT, which stimulates the smooth muscle cells, during its transport from ductuli efferentes and epididymis (2), which has OT receptors (3,4). The reduction in the semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number at 60 and 120 min compared to 10 min in both the control and treatment groups is probably due to the frequency of semen collections coupled with the decline of circulating OT over the duration of the experiments.

In conclusion, a single dose IV administration of OT enhances seminal plasma T, and semen volume, mass activity, sperm concentration, total sperm number, and total motile sperm number in the first ejaculate (10 min) by hastening the sperm transport from the epididymis. Additionally, it is likely that the collection of semen from rams at short intervals causes more decreases in sperm parameters at 60 and 120 min.

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