Kafkas Univ Vet Fak Derg
x (x): xxx-xxx, 2019
DOI: 10.9775/kvfd.2018.21521

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Article in Press

The Effects of Oxytocin and $PGF_2\alpha$ Injections on Semen Quality and Libido in Buck

Çiğdem ÇEBİ ŞEN [1,a] Koray TEKİN [2,b] Beste ÇİL [2,c] Ergun AKÇAY [2,d]

[1] The University of Harran, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-63300 Şanliurfa - TURKEY
[2] The University of Ankara, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, TR-06110 Ankara - TURKEY

[a] ORCID: 0000-0001-6876-2069; [b] ORCID: 0000-0002-3862-2337; [c] ORCID: 0000-0003-2822-1625; [d] ORCID: 0000-0002-7491-5671

Article ID: KVFD-2018-21521 Received: 10.12.2018 Accepted: 10.05.2019 Published Online: 16.05.2019

How to Cite This Article

Çebi Şen Ç, Tekin K, Çil B, Akçay E: The effects of oxytocin and $PGF_2\alpha$ injections on semen quality and libido in buck. *Kafkas Univ Vet Fak Derg,* 2019 *(Article in Press).* DOI: 10.9775/kvfd.2018.21521

Abstract

The aim of the present study was to evaluate the effects of exogenous oxytocin and $PGF_2\alpha$ on seminal quality and libido sexualis in bucks. To investigate the role of these hormones on male fertility, semen samples from 20 Norduz bucks (3-4 years of age) were collected with an artificial vagina twice a week with five replications in breeding season. Bucks were randomly assigned to five groups, control group was administered with 2 mL of sodium chloride, 0.9% (w/v)) i.m., whilst the experimental groups were administered with oxytocin 10 IU, i.v. (Group 1, n = 5), oxytocin 20 IU, i.v. (Group 2, n = 5), $PgF_2\alpha$ 5 mg, i.m. (Group 3, n = 5) or $PgF_2\alpha$ 10 mg, i.m. (Group 4, n = 5) 20 min before each collection. There was no statistical difference between the treatment groups in terms of motility (P>0.05). However, semen volume, concentration, abnormal spermatozoa rate, intact membrane rate and libido results were statistically significant among the different groups (P<0.05). As a result, administration of 20 IU oxytocin twenty minutes prior to semen collection in bucks did not improve overall semen quality, however, libido, semen volume, and sperm concentrations were increased. In contrast to oxytocin, $PGF_2\alpha$ administration has led to a slight decrease in libido and has shown moderate effects on semen quality.

Keywords: *Buck, Libido, Oxytocin, PGF2α, Semen quality*

Tekelerde Oksitosin ve $PGF_2\alpha$ Enjeksiyonlarının Sperma Kalitesi ve Libido Üzerine Etkisi

Öz

Bu çalışmanın amacı, ekzojen yoldan uygulanan oksitosin ve $PGF_2\alpha$'nın seminal plasma ve libido seksüalis üzerine olan etkilerini değerlendirmekti. Bu hormonların sezon içi erkek reprodüksiyonu üzerindeki rolünü araştırmak için, 20 Norduz tekesinden (3-4 yaş) haftada iki kez suni vajina ile alınan sperma numuneleri, beş replikasyon ile gerçekleştirildi. Tekeler rastgele beş gruba ayrılarak, sperm alma işleminden 20 dk önce, kontrol grubu 2 mL sodyum klorür, %0.9 (w/v)) ile i.m., deney grupları ise oksitosin 10 IU, i.v. (Grup 1, n = 5), oksitosin 20 IU, i.v. (Grup 2, n = 5), $PgF_2\alpha$ 5 mg, i.m. (Grup 3, n = 5) ve $PgF_2\alpha$ 10 mg, i.m. (Grup 4, n = 5) olarak uygulandı. Deney grupları arasında motilite açısından istatistiksel fark tespit edilmedi (P>0.05). Ancak sperma hacmi, konsantrasyon, anormal spermatozoa oranı, intakt membran oranı ve libido sonuçları gruplar arasında istatistiksel olarak anlamlı bulundu (P<0.05). Sonuç olarak, sperma alımından yirmi dakika önce 20 IU oksitosin enjeksiyonu sperma kalitesini değiştirmezken, libido, sperma hacmi ve sperm konsantrasyonunu artırdı. Oksitosinin aksine, $PGF_2\alpha$ uygulaması libidoda hafif bir azalmaya neden olurken ve sperma kalitesinde orta derecede etkiler gösterdi.

Anahtar sözcükler: *Libido, Oksitosin, PGF2α, Semen kalitesi, Teke*

INTRODUCTION

The use of assisted reproductive techniques in goat breeding provides additional advantages for cryopreservation and artificial insemination [1]. In some cases, certain animals constantly have a high libido and good semen quality for evaluation, freezing or insemination, while others are reluctant for collection and have low quality ejaculates with decreased volume and concentration or other inadequate spermatological characteristics [2-4]. During the

İletişim (Correspondence)
+90 312 3170315/4409
ergakcay@gmail.com

last decades, specific hormones (oxytocin, prostaglandins, testosterone and GnRH) were introduced to increase the sperm output, quality of male-related reproductive deficiencies and to regulate the breeding activity [5-9].

Oxytocin is a peptide structure hormone and has numerous peripheral actions such as lactation, smooth muscle contraction, wound healing, natriuresis, sexual arousal and mostly known as social behaviour hormone which increases trust and reduces fear, monogamous pair and maternal bonding [10,11]. Prostaglandin (PG) is mainly used for synchronisation of the females [12,13]. It is a physiologically active lipid compound which is secreted from several tissues and is derived from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes COX1 and COX2 [14]. The development of mice deficient in COX1 and/or COX2 has shown that COX2–null female mice are infertile. PG enhances progressive motility of spermatozoa by stimulating the contraction of the vaginal smooth muscle [15]. Administration of $PGF_2\alpha$ prior to semen collection has led to an increase in sperm output in buffalos [16], dogs [17], and stallions [18,19]. Oxytocin has been known as a female hormone but the role of oxytocin associated with reproductive physiology in the male animal needs to be elucidated. Increasing the contractility in the male reproductive tract by modulating steroidogenesis is the specific role of this hormone [20]. Hypothalamic nonpeptide oxytocin is one of the most potent mediators of drug-induced penile erections in laboratory animals. Moreover, oxytocin treatment prior to ejaculation has improved the ejaculate quality by increasing the concentration of sperm in the ejaculate of the bull [21], ram [22], rabbit [23] and man [24]. Oxytocin receptors have been determined from testes, epididymis, prostate, penis and the epididymal smooth muscle of several species [19,25]. There is a growing evidence that $PGF_{2\alpha}$ and oxytocin are important factors in determining sperm transport throughout the entire epididymis of bulls, buffalos, rams, rabbits and stallions [17]. In parallel with above mentioned studies, we aim to evaluate the effects of exogenous oxytocin and $PGF_2\alpha$ on seminal quality and libido in Norduz bucks.

MATERIAL and METHODS

Animals and Semen Collection

This study was conducted according to ethical laws and regulation of Ankara university animal experiments local ethics committee. 20 Norduz bucks (3-4 years of age) were barned at Research Farm of Ankara University, Faculty of Veterinary Medicine (40°05'53.5"N 32°37'19.6"E). The bucks were maintained under the constant nutritional regime and with water ad libitum.

At the beginning of the study, twenty min prior to semen collection all animals were administered with 2 ml physiological saline (Sodium Chloride, 0.9% (w/v)) i.m. and ejaculations were collected with artificial vagina from each buck as a control group (Control Group, n = 20). Afterwards, bucks were randomly assigned to four groups and each group was administered with oxytocin (Hormonipra, HIPRA) 10 IU, i.v. (Group-1, n = 5), oxytocin 20 IU, i.v. (Group-2, n = 5), $PGF_2\alpha$ (Gestavet-Prost, HIPRA) 5 mg, i.m. (Group-3, n = 5) or $PGF_2\alpha$ 10 mg, i.m. (Group-4, n = 5) twenty min before each collection, with a total of 5 replications. A total of 120 ejaculates (20 for control and 100 for experimental design) were collected with an artificial vagina, twice a week from 20 mature bucks during the breeding season. After collection, ejaculates were placed in a water bath (33°C) for further evaluation of spermatological parameters [26].

Libido Assessment

The behavioural signs of libido (leg kicking, sniffing, vocalization, flehmen reaction, mounting without thrust and mounting with ejaculation) were recorded as the total score for libido. Reaction time was assessed as the interval between the buck's entrance into the collection room and the initiation of ejaculation. Libido was evaluated at a scale of 0 to 4, with 0 being the total absence of sexual desire and 4 being the highest level of sexual desire giving minimal time to mount the teaser animal [27].

Semen Evaluation

Semen volume, total sperm motility, sperm concentration, sperm morphology, membrane integrity and pH were recorded [18].

Sperm Motility

Motility assessment was performed using a phase-contrast light microscopy (x100) (Olympus BH-2, Olympus Optical Co. Ltd., Japan) on a pre-heated stage (37ºC). Five microscopic fields from separate 5 µl aliquots of the same sample were evaluated by two trained technicians. The mean percentage of the three successful evaluations was determined as total motility [18].

Spermatozoa Concentration

Spermatozoa concentration was identified according to the haemocytometry method. Semen was diluted with Hayem solution (1 g NaCl, 5 g Na_2SO_4, 0.5 g $HgCl_2$ and 200 mL bidistilled water) at a ratio of 1:100. Mean spermatozoa count was calculated from three replicate of each sample at a magnification of 400x and recorded as $x10^6$ mL [18].

Sperm Morphology

For morphological assessment of the sperm, a drop of a mixture containing 150 µL semen mixed with 1 mL of Hancock's solution (*Sodium saline solution:* 9.01 g NaCl and 500 mL of double-distilled water. *Buffer solution*: (1) 21.682 g $Na_2HPO_4 \times 2H_2O$ and 500 mL of double-distilled water; (2) 22.254 g KH_2PO_4 and 500 mL of double-distilled water. Amounts of 200 mL of (1) and 80 mL of (2) were mixed to

obtain 280 mL of buffer solution. The final Hancock solution was mixed as follows:150 mL sodium saline solution, 62.5 mL formalin, 150 mL buffer solution and 500 mL double distilled water) was evaluated using a bright field microscopy (x 400) (Olympus CX21FS1, Olympos Optical Co. Ltd., Japan) under immersion oil. At least 200 spermatozoa were counted to determine the percentage of abnormal spermatozoa [18].

The Hypo-Osmotic Swelling Test (HOST)

Spermatozoa membrane integrity was assessed with the hypoosmotic swelling test (HOST), based on swollen and curled tails. 20 µL of semen added into 200 mOsm hypo-osmotic solution (9 g fructose, 4.9 g trisodium citrate and 100 mL distilled water) and the mixture was incubated for 30 min at 37ºC. Subsequent to incubation, 0.1 mL of the mixture was evaluated using a bright-field light microscopy (Olympus CX21FS1, Olympus Optical Co. Ltd., Japan). At least two hundred spermatozoa were evaluated and sperm cells that have coiled or swollen tails were defined as spermatozoa with intact membrane integrity [28].

Statistical Analyses

Semen parameters were statistically analyzed using one-way ANOVA for (GLM procedure of SigmaStat 4.0 Statistical Software) while libido scores were analyzed by Kruskal Wallis Test. Significant differences were considered with $P<0.05$.

RESULTS

According to obtained results, there was not any statistical difference between the dosage groups regarding motility. However, in terms of semen volume, concentration, abnormal spermatozoa rate, intact membrane rate, and libido test, results were statistically significant among the different dose groups ($P<0.05$).

Average semen volume of Group two (1.34 ± 0.16 mL) was found significantly higher than other groups, whereas mean values of $PGF_2\alpha$ groups (Group-3 and Group-4) were found lower than control value ($P<0.05$). When the concentration was taken into account, Group two has statistically higher mean value than $PGF_2\alpha$ groups. For abnormal spermatozoa rate, a significant increase in Group one was observed regarding other groups. A significant decrease of mean intact membrane percentages was evident at Group four comparing to group one *(Table 1)*.

A significant increase in libido evaluation scores for oxytocin groups (3.04±0.64; 3.2±0.5) was observed *(Table 2)*. The bucks, which were administered $PGF_2\alpha$, were reluctant for mating and showed a decrease in libido. Thus, it might have enhanced hyperthermic and psychological stress in animals. Therefore, the duration of ejaculation was longer in $PGF_2\alpha$ groups than the other groups.

DISCUSSION

Numerous pharmacological substances have been proven to improve male reproductive performance in many species [29]. It is a known fact that hormones play the major role in the regulation of male reproductive functions as in sexual arousal, control of sexual behaviour, the onset of erection and ejaculation, and the post-ejaculatory detumescence [30].

Libido is an important factor in male reproduction and oxytocin plays a major physiological role in sexual behaviours. Libido is one of the most important factor in male reproduction and regulated by primarily testosterone, dopamine respectively. However, a study [31] showed that electrical stimulation of the glans penis elicits a specific activation of 40-50% of oxytocinergic neurons in the paranuclear nucleus of the hypothalamus. During ejaculation, oxytocin probably associated with ejaculation through hypothalamus. This is also must be in relation with systemic pulsation of oxytocin on sexual behaviour [32].

Table 1. *Effects of different doses of oxytocin and $PGF_2\alpha$ administration on ejaculate characteristics in bucks*

Treatment	n	Volume (mL)	Motility (%)	Concentration ($x10^9$ sperm/mL)	Abnormal Spermatozoa Rate (%)	Intact Membrane (%)
2 mL PWS (Control)	20	1.14 ± 0.17^{b}	56.50 ± 0.82	3.18 ± 0.006^{ab}	24.55 ± 0.87^{b}	62.00 ± 0.95^{ab}
10 IU Oxytocin (Group 1)	25	1.20 ± 0.18^{b}	56 ± 0.71	3.24 ± 0.06^{ab}	27.76 ± 0.78^{a}	64.52 ± 0.86^{a}
20 IU Oxytocin (Group 2)	25	1.40 ± 0.13^{a}	57 ± 0.82	3.32 ± 0.03^{a}	23.16 ± 0.45^{b}	63.28 ± 0.99^{ab}
5 mg $PGF_2\alpha$ (Group 3)	25	0.96 ± 0.22^{c}	56.4 ± 0.74	3.07 ± 0.05^{b}	25.40 ± 0.63^{ab}	61.40 ± 0.60^{ab}
10 mg $PGF_2\alpha$ (Group 4)	25	0.89 ± 0.15^{c}	54.60 ± 0.64	$3.05\pm0.05_{b}$	23.16 ± 0.47^{b}	60.40 ± 0.65^{b}

^{a,b,c} Different letters within the same column indicate a significant difference ($P<0.05$) One Way ANOVA

Table 2. *Effects of different doses of oxytocin and $PGF_2\alpha$ administration on the libido of bucks*

Treatment	n	Libido Scores
2 mL PWS (Control)	20	2.55 ± 0.51^{b}
10 IU Oxytocin (Group 1)	25	3.04 ± 0.73^{a}
20 IU Oxytocin (Group 2)	25	3.2 ± 0.5^{a}
5 mg $PGF_2\alpha$ (Group 3)	25	2.24 ± 0.44^{bc}
10 mg $PGF_2\alpha$ (Group 4)	25	2.12 ± 0.33^{c}

^{a,b,c} Different letters within the same column indicate a significant difference ($P<0.05$) Kruskal Wallis Test

During the sexual arousal, oxytocin has shown a slight increase while during the ejaculatory phase a considerable increase has been presented in rams, bulls, rabbits and humans as well. Peripherally released oxytocin participates in sexual satiety and assists the sperm transport by contracting the reproductive tract [33].

Although the administration of oxytocin stimulates sexual behaviour and performance in many mammalian species, our data indicate that oxytocin administration 20 min prior to semen collection did not improve the semen quality of bucks, however detectable effects on the ejaculation time and sperm output was observed. In the present study, the role of oxytocin on libido was clearly displayed through an increase in libido test score. In addition to that, the overall duration of semen collection was shortened.

Oxytocin hormone can be effective on mating behaviour and erectile function and it can modulate the androgen regulation. It acts on smooth muscle cells of the epididymis. In addition to that, oxytocin can stimulate the release of endothelin-1 from the caput epididymis [32]. There is a growing evidence that oxytocin is one of the most potent mediators of drug-induced penile erections in laboratory animals, most likely by increasing NO-synthase activity in the paraventricular nucleus of the hypothalamus. Intra-cerebroventricular injection of synthetic oxytocin was followed by yawning and penile erection within 5 min in rats, whereas application of a potent non-peptide oxytocin receptor antagonist, as well as a competitive inhibitor of NOsynthase, reduced penile erections and copulatory behaviour in a dose-dependent manner [34]. The role of central oxytocin in the control of ejaculation has been demonstrated in rabbits and rodents as well. After the oxytocin treatment, the number of intromission before ejaculation was reduced and oxytocin promoted ejaculatory behaviour by shortening ejaculation latency and post-ejaculatory refractory period [35-37]. However, attempts to elicit sexual behaviour in previously nonresponsive male rats were not successful. Consistently with previous research, in the present study, the administration of 10 or 20 IU oxytocin hormone has positively affected the libido and shortened the collection time as well.

Although 10 IU i.v. oxytocin administration did not lead to any statistically significant increase in semen volume or sperm concentration, doubling the dose of oxytocin led to a significant increase in both parameters. Even though parallel studies with our results exist, there is also a contradiction with previous studies. In addition, there is a negative correlation between the volume of semen and DNA fragmentation [38]. The implementation of the exogenous oxytocin hormone just before the ejaculation, bull [21], buffalo [16], ram [22,34], rabbit [23] and rats caused to an increase in the number of sperm in the ejaculate. While a study [39] reported that, administration of 7 IU oxytocin 5 min before ejaculation has been found to increase the number of spermatozoa in rams, Berndtson and Igboeli [21], suggested that, 50 IU iv oxytocin injection has no positive effect on spermatological characteristics in the bull. In another study, after administration of 10 IU of oxytocin in the male do had no effects on ejaculate characteristics [29]. Knight and Lindsay [36] reported that exogenous administration of oxytocin hormone 10 min. prior to semen collection had resulted with an increase of sperm concentration. Moreover, in vitro addition of oxytocin did not improve motility or abnormal spermatozoa rate [40]. This conflict may be due to oxytocin dose and the specific time of hormone administration.

The neuropeptide oxytocin can be found in the mammalian testis and cauda epididymis and it enhances sperm transport by improving seminiferous tubular and testicular capsule contractile activity. It has been shown in vitro that in the absence of oxytocin contractile activity of seminiferous tubules is reduced [41]. However, it can be restored by addition of exogenous oxytocin [42]. The increase of spermatozoa number in the ejaculate after oxytocin administration is assumed that can be related to the forceful contraction of the efferent tubules and testes. Oxytocin increases the seminiferous tubules rhythmic contractions which taken forward the spermatozoa from lumen towards the rete testicles [17,43]. Effect of oxytocin is partly mediated via stimulation of an increase in the synthesis of prostaglandins by the seminal vesicles. It influences the secretion rate of the male accessory glands, which, may account for the increased volume seen in the ejaculate of rams and buffalo following administration. Within the prostate, both directly and via interactions with androgen metabolism, it has been shown to affect gland growth [32].

In the present study, the concentration of spermatozoa number did not increase statistically after with 10 IU oxytocin administration however with 20 IU a significant increase was observed. In other words, this can be related to epididymal contraction dose as well. Motility and semen pH was not affected by the treatment and showed similar results with the control group [29,40]. With 10 IU oxytocin group, there was a slight increase in abnormal spermatozoa rate.

It is a well-known fact that $PGF_2\alpha$ is a component of the seminal fluid in many species. This hormone is related to the increase of contractility in both the male and female genital tracts. It is also believed that prostaglandins are involved in the ejaculation process and may have some effect on libido [44]. Exogenous $PGF_2\alpha$ has been shown to cause masturbation, spontaneous erection and ejaculation in the stallion. It has also been used for ex-copula ejaculation in stallions [18]. In the rabbit corpus cavernosum smooth muscle, endogenous prostaglandins have a local effect on reinforcement of neurally mediated and spontaneous contractions and enhancing the detumescence of the penis [45]. In contrast to our study, in the ejaculate of bulls, rabbits, rams and stallions $PGF_2\alpha$ has been reported to

increase both the volume and spermatozoa concentration [46]. $PGF_2\alpha$ is also believed to cause contractions on the male reproductive system. In the boar, spermatozoa concentration did not affect by $PGF_2\alpha$ administration, although an increase in the volume of the semen has been reported [47]. The mechanism behind this increase has not been fully understood yet. Nevertheless, in the present study, $PGF_2\alpha$ has shown detrimental effects on semen quality, especially with a high dose on intact membrane rate. In addition to that, libido score was decreased following the $PGF_2\alpha$ administration. Among the livestock species, goats are more vulnerable due to their sensitive behavioural pattern. In this study, we also observed that administration of $PGF_2\alpha$ has increased the reaction time before ejaculation, comparing to oxytocin group. Our observation is highly associated with the libido results, which suggest that prostaglandins are involved in the development of hyperthermia and the ACTH response induced by psychological stress.

In conclusion, administration of 20 IU oxytocin twenty min before the semen collection have increased both the semen volume and the concentration of spermatozoa in bucks. Administration of oxytocin has increased the libido although there were not any improvements in semen quality. In contrast to oxytocin, $PGF_2\alpha$ administration has led to a decrease of libido and has detrimental effects on semen quality. We concluded that administration of oxytocin stimulates sexual behaviour and performance in bucks.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Paramio MT, Izquierdo D: Assisted reproduction technologies in goats. *Small Ruminant Res*, 121 (1): 21-26, 2014. DOI: 10.1016/j.smallrumres.2014.01.002

2. Singh S, Bhakat M, Mohanty TK, Kumar A, Gupta AK, Chakravarty AK, Singh P: Sexual behavior and its relationship with semen quality parameters in Sahiwal breeding bulls. *Vet World,* 8 (6): 745-749, 2015. DOI: 10.14202/vetworld.2015.745-749

3. Savic R, Petrovic M: Variability in ejaculation rate and libido of boars during reproductive exploitation. *S Afr J Anim Sci*, 45 (4): 355-361, 2015. DOI: 10.4314/sajas.v45i4.1

4. Tirpan MB, Gürler H, Olğaç, KT, Daşkın A: Effects of boron added bull semen extender on post-thaw spermatological parameters. *Ankara Univ Vet Fak Derg*, 65 (2): 123-128, 2018.

5. Monaco D, Fatnassi M, Padalino B, Aubé L, Khorchani T, Hammadi M, Lacalandra GM: Effects of a GnRH administration on testosterone profile, libido and semen parameters of dromedary camel bulls. *Res Vet Sci*, 102, 212-216. 2015. DOI: 10.1016/j.rvsc.2015.08.011

6. Kumar BSB, Pandita S, Prakash BS, Mallick S, Mohanty TK, Mandal DK, Mili B: Luteinizing hormone, testosterone and total estrogens response to exogenous GnRH in crossbred bulls with differing semen quality. *Livest Sci*, 174, 150-153, 2015. DOI: 10.1016/j.livsci.2015.01.019

7. Patel DP, Chandrapal JC, Hotaling JM: Hormone-based treatments in subfertile males. *Curr Urol Rep*, 17 (8): 56, 2016. DOI: 10.1007/s11934-016-0612-4

8. Mylonas CC, Duncan NJ, Asturiano JF: Hormonal manipulations for the enhancement of sperm production in cultured fish and evaluation of sperm quality. *Aquaculture*, 472, 21-44, 2017. DOI: 10.1016/j.aquaculture.2016.04.021

9. Yıldız S, Güngör Ö, Tuncer PB, Taşdemir U, Erol H, Kacar C, Bucak MN: The effects of deslorelin, GnRH analogue with long acted on sexual activities in Angora bucks. *Kafkas Univ Vet Fak Derg,* 15 (1): 95-101, 2009. DOI: 10.9775/kvfd.2008.83-A

10. Gruson D: Oxytocin testing and reproductive health: Status and clinical applications. *Clin Biochem,* 62, 55-61. 2018. DOI: 10.1016/j.clinbiochem.2018.10.016

11. Veening JG, De Jong TR, Waldinger MD, Korte SM, Olivier B: The role of oxytocin in male and female reproductive behavior. *Eur J Pharmacol,* 753, 209-228, 2015. DOI: 10.1016/j.ejphar.2014.07.045

12. Stelletta C, Tekin K, Tırpan MB, Alemdar H, Çil B, Öztutar Stelletta F, Olgaç KT, İnanç ME, Daşkın A: Vulvar thermal pattern following synchronization of estrus is linked to fertility after timed artificial insemination in goat. *Theriogenology*, 103, 137-142. 2017. DOI: 10.1016/j.theriogenology.2017.07.038

13. Tırpan MB, Tekin K, Çil B, Alemdar H, İnanc ME, Olgaç KT, Stelletta C, Daşkın A: The effects of different PMSG doses on estrus behavior and pregnancy rate in Angora goats. *Animal*, 13 (3): 564-569, 2019. DOI: 10.1017/S1751731118001908

14. Saeed SA, Anwar N, Khan KM, Sarfraz N: Effect of chronic treatment with a cyclooxygenase inhibitor on reproductive parameters in male rat. *J Ayub Med Coll,* 21 (3): 66-71, 2009.

15. Rossitto M, Ujjan S, Poulat F, Boizet-Bonhoure B: Multiple roles of the prostaglandin D_2 signalling pathway in reproduction. *Reproduction*, 149 (1): R49-R58. 2015. DOI: 10.1530/REP-14-0381

16. Ibrahim MA: Influence of oxytocin and prostaglandin on semen characteristics and process of ejaculation in buffalo bulls. *Acta Vet Hung*, 36, 3-10, 1988.

17. Hess MB: The effects of prostaglandin F2a, oxytocin and gonadotropin releasing hormone on ejaculate characteristics in the dog, *MSc Thesis,* Virginia Polytechnic Institute and State University, 2002.

18. Şen ÇÇ, Akcay E: The effect of oxytocin and prostaglandin hormones added to semen on stallion sperm quality. *Turk J Vet Anim Sci,* 39, 705-708, 2015. DOI: 10.3906/vet-1412-69

19. Palmer CW, Amundson SD, Brito LFC, Waldner CL, Barth AD: Use of oxytocin and cloprostenol to facilitate semen collection by electroejaculation or transrectal massage in bulls, *Anim Reprod Sci*, 80, 213-223, 2004. DOI: 10.1016/j.anireprosci.2003.07.003

20. Nicholson HD, Guldenaar SEF, Boer GJ, Pickering BT: Testicular oxytocin: Effects of intratesticular oxytocin in the rat. *J Endocrinol*, 130 (2): 231-238, 1991. DOI: 10.1677/joe.0.1300231

21. Berndtson WE, Igboeli G: Spermatogenesis, sperm output and seminal quality of Holstein bulls electroejaculated after administration of oxytocin. *J Reprod Fertil,* 82, 467-475, 1988. DOI: 10.1530/jrf.0.0820467

22. Nicholson HD, Parkinson TJ, Lapwood KR: Effects of oxytocin and vasopressin on sperm transport from the cauda epididymis in sheep. *J Reprod Fertil*, 117, 299-305, 1999. DOI: 10.1530/jrf.0.1170299

23. Fjellström D, Kihlström JE, Melin P: The effect of synthetic oxytocin upon seminal characteristics and sexual behaviour in male rabbits. *J Reprod Fertil,* 17, 207-209, 1968. DOI: 10.1530/jrf.0.0170207

24. Filippi S, Vannelli GB, Granchi S, Luconi M, Crescioli, C, Mancina R, Natali A, Brocchi S, Vignozzi L, Bencini E: Identification, localization and functional activity of oxytocin receptors in epididymis. *Mol Cell Endocrinol*, 193, 89-100, 2002. DOI: 10.1016/S0303-7207(02)00101-6

25. Whittington K, Assinder S, Gould M, Nicholson H: Oxytocin, oxytocin associated neurophysin and the oxytocin receptor in human prostate. *Cell Tissue Res*, 318, 375–382, 2004. DOI: 10.1007/s00441-004-0968-5

26. Tırpan MB, Tekin N: Effects of boron (sodium pentaborate), added instead of Tris components, on freezing and post-thaw quality of Angora buck semen. *Ankara Univ Vet Fak Derg*, 62, 295-302, 2015.

27. Kerketta S, Singh M, Patel BHM, Verma M, Prasad JK, Upadhyay D,

Bhushan B: Study on buck's mating behaviour, libido score and semen biology in local goat of Rohilkhand region, India. *Indian J Anim Sci*, 48, 491-495, 2015. DOI: 10.5958/0976-0555.2014.00017.X

28. Revell SG, Mrode RA: An osmotic resistance test for bovine semen. *Anim Reprod Sci*, 36, 77-86, 1994. DOI: 10.1016/0378-4320(94)90055-8

29. Traas AM, Kustritz MVR: Effect of administrating oxytocin or prostaglandin F2α on characteristics of the canine ejaculate. *Can Vet J*, 45, 999-1002, 2004.

30. Veronesi MC, De Amicis I, Panzani S, Kindahl H, Govoni N, Probo M, Carluccio A: $PGF_{2\alpha}$, LH, testosterone, oestrone sulphate, and cortisol plasma concentrations around sexual stimulation in jackass. *Theriogenology*, 75, 1489-1498, 2011. DOI: 10.1016/j.theriogenology.2010.12.010

31. Honda K, Yanagimoto M, Negoro H, Narita K, Murata T, Higuchi T: Excitation of oxytocin cells in the hypothalamic supraoptic nucleus by electrical stimulation of the dorsal penile nerve and tactile stimulation of the penis in the rat. *Brain Res Bull*, 48 (3): 309-313, 1999. DOI: 10.1016/S0361-9230(98)00180-4

32. Thackare H, Nicholson HD, Whittington K: Oxytocin its role in male reproduction and new potential therapeutic uses. *Hum Reprod Update,* 12, 437-448, 2006. DOI: 10.1093/humupd/dmk002

33. Carter CS: Oxytocin and sexual behavior. *Neurosci Biobehav Rev*, 16, 131-144, 1992. DOI: 10.1016/S0149-7634(05)80176-9

34. Walch K, Eder R, Schindler A, Feichtinger W: The effect of single-dose oxytocin application on time to ejaculation and seminal parameters in men. *J Assist Reprod Genet,* 18, 655-659, 2001. DOI: 10.1023/A:1013115301159

35. Lui C, Cui XG, Wang YX, You, ZD, Xu DF: Association between neuropeptide oxytocin and male infertility. *J Assist Reprod Genet,* 27, 525-531, 2010. DOI: 10.1007/s10815-010-9451-2

36. Stoneham MD, Everitt BJ, Hansen S, Lightman SL, Todd K: Oxytocin and sexual behavior in the male rat and rabbit. *J Endocrinol,* 107, 97-106, 1985. DOI: 10.1677/joe.0.1070097

37. Arletti R, Bazzani C, Castelli M, Bertolini A: Oxytocin improves male copulatory performance in rats. *Horm Behav,* 19, 14-20, 1985. DOI: 10.1016/0018-506X(85)90002-9

38. Alcay S, Toker B, Ustuner B, Nur Z, Sagirkaya H, Soylu MK: Investigation of relationships between DNA integrity and fresh semen parameters in rams. *Kafkas Univ Vet Fak Derg*, 20 (5): 793-798. 2014. DOI: 10.9775/kvfd.2014.11144

39. Knight TW, Lindsay DR: Short-and long-term effects of oxytocin on quality and quantity of semen from rams. *J Reprod Fertil,* 21, 523-529, 1970.

40. Çiftçi HB: *In-vitro* effect of oxytocin on the duration of sperm motility and morphology. *J Anim Vet Adv*, 4, 794-797, 2005.

41. Nicholson HD, Warley RTS, Charlton HM, Pickering BT: LH and testosterone cause the development of seminiferous tubule contractile activity and the appearance of testicular oxytocin in hypogonadal mice. *J Endocrinol,*110, 159-167, 1986.

42. Nicholson HD, Warley RTS, Guldenaar SEF, Pickering BT: Ethan-1,2-dimethanesulphonate reduces testicular oxytocin content and seminiferous tubule movements in the rat. *J Endocrinol,* 112, 311-316, 1987.

43. Roser JF: Endocrine and paracrine control of sperm production in stallions. *Anim Reprod Sci,* 68, 139-151, 2001. DOI: 10.1016/S0378-4320(01)00151-8

44. Bygdeman M: Prostaglandins. **In,** Hodgson JE (Ed): Abortion and Sterilization: Medical and Social Aspects. 333-358, Academic Press, London, 1981. DOI: 10.1016/B978-0-12-792030-6.50019-0

45.Hashit ani H, Yanai Y, Shirasawa N, Soji T, Tomita A, Kohri K, Suzuki H: Interaction between spontaneous and neurally mediated regulation of smooth muscle tone in the rabbit corpus cavernosum. *J Physiol,* 569, 723-735, 2005. DOI: 10.1113/jphysiol.2005.099309

46. Veronesi MC, Tosi U, Villani MARTA, Govoni N, Faustini M, Kindahl H, Madej A, Carluccio A: Oxytocin, vasopressin, prostaglandin $F_{2\alpha}$, luteinizing hormone, testosterone, estrone sulfate, and cortisol plasma concentrations after sexual stimulation in stallions. *Theriogenology*, 73, 460-467, 2010. DOI: 10.1016/j.theriogenology.2009.09.028

47. Hemsworth PH, Donnelly J, Findlay JK, Galloway DB: The effects of prostaglandin F2α on sperm output in boars. *Prostaglandins*, 13, 933-941, 1977. DOI: 10.1016/0090-6980(77)90223-4