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RESEARCH ARTICLE

Effect of Sildenafil (Viagra®) on Penile Erection and Semen Volume and Characteristics in Kangal Dogs

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Summary

In this study, the effects of sildenafil administration on penile erection and semen volume and characteristics in Kangal dogs were studied. A total of 5 Kangal dogs, aging 3-6 years, and with a known fertility were used. In each application, sildenafil was administered orally at a dosage of 50 mg, 60 minutes prior to semen collection. Semen was collected 12 times at 3-day intervals by digital manipulation. After collected, semen was examined for volume (first, second, and third fractions), ejaculation time, and spermatological characteristics. Dogs were rested for 3 weeks after the last semen collection. Following the rest period, semen collection was experienced 12 times at 3-day intervals by digital manipulation without sildenafil application. Sildenafil administration enhanced the volume of the second and third fractions, compared to the non-sildenafil group (1.1 ml versus 0.7 ml and 11 ml versus 6 ml, respectively, $P<0.05$), and increased the total ejaculation time (11.8 minutes versus 7 minutes, $P<0.05$). On the other hand, there were no significant differences between the sildenafil and non-sildenafil groups for spermatological characteristics ($P>0.05$). As a conclusion, sildenafil could be used in dogs, having problems in response to digital manipulation, for penile erection and successful semen collection.

Keywords: *Dog, Penile erection, Semen characteristics, Sildenafil*

Sildenafil (Viagra®)'in Kangal Köpeklerinde Penis Ereksiyonu, Sperma Hacmi ve Özellikleri Üzerine Etkisi

Özet

Bu çalışmada, Kangal köpeklerinde penis ereksiyonu, sperma hacmi ve özellikleri üzerine sildenafilin etkisi çalışıldı. Yaşları 3 ila 6 arasında değişen toplam 5 baş fertilitesi bilinen Kangal ırkı köpek kullanıldı. Her bir uygulamada sperma alma işleminin 60 dakika öncesinde köpeklere 50 mg dozunda oral yolla sildenafil uygulandı. Sperma toplama işlemi parmak maniplasyon yöntemiyle 3 gün arayla 12 kez denendi. Spermanın alınma işleminden sonra, sperma hacim (birinci, ikinci ve üçüncü fraksiyon yönüyle), ejakülasyon zamanı ve spermatolojik özellikler bakımından değerlendirildi. Köpekler 3 hafta dinlendirildikten sonra, sildenafil uygulaması yapılmaksızın sperma toplama işlemi parmak maniplasyon yöntemiyle 3 gün arayla 12 kez denendi. Sildenafil uygulaması, sildenafil uygulanmayan gruba göre spermanın ikinci (sırasıyla, 1.1 ve 0.7 ml) ve üçüncü fraksiyon (sırasıyla 11 ve 6 ml) hacmine önemli oranda katkıda bulundu ve total ejakülasyon zamanını artırdı (sırasıyla, 11.8 ve 7 dakika, $P<0.05$). Fakat, sildenafil uygulanan ve uygulanmayan gruplar arasında spermatolojik özellikler yönünden önemli farklılıklar gözlenmedi ($P>0.05$). Sonuç olarak, sidenafil penis ereksiyonu ve başarıyla spermanın toplanmasında parmak maniplasyon işleminde problem yaşayan köpeklerde kullanılabilir.

Anahtar sözcükler: *Köpek, Penis ereksiyonu, Sperma özellikleri, Sildenafil*

INTRODUCTION

Penile erection is initiated by neuronal impulses in parasympathetic pelvic nerves that cause arteriolar vasodilatation and relaxation of smooth muscle elements in the corpus cavernosum, the erectile tissue of the penis [1]. Relaxation is mediated by nitric oxide, which is synthesized during sexual stimulation in

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the nerve terminals of parasympathetic nonadrenergic noncholinergic neurons in the penis as well as by the endothelial cells of the blood vessels of the corpora cavernosa [2,3].

Sildenafil, an inhibitor of cGMP-specific phosphodiesterase type V (PDE V), is reported to be effective in the treatment of erectile dysfunction in humans [4]. In animal studies, sildenafil facilitates tumescence in the penis of the dog and relaxes the cavernous tissue of the rabbit and human penis [5,6]. Because PDE V is a predominant isoenzyme hydrolyzing cGMP in the corpus cavernosum, it is thought that its inhibition and the subsequent accumulation of cGMP both account for the effect of sildenafil [4]. Sildenafil has a low side-effect profile [7]. Erection and relaxation of the penis develops 30-60 minutes after the use of sildenafil [4]. Injection of sildenafil causes erection in rabbits [8] and enhances sexual activity in rats [9].

Sexual stimulation and semen collection are difficult to achieve during cold winter days, and are sometimes time-consuming and frustrating processes in researches. Therefore, researchers require additional applications such as the administration of sildenafil and pharmacological agents. The aim of this study was to determine the effect of sildenafil on penile erection, volume and semen characteristics in dogs.

MATERIAL and METHODS

A total of 5 Kangal dogs, aging 3-6 years, and with a known fertility were used. The experiment was conducted between January and March. The animals (3-4 year old dogs) were housed at Selçuk University, Faculty of Veterinary Medicine, Education Research and Practice Farm, under uniform nutritional conditions. Sildenafil (Prizer, Viagra) was administered orally at a dosage of 50 mg, 60 minutes prior to semen collection. Semen collection was experienced 12 times at 3-day intervals by digital manipulation and the three fractions (first, second and third fractions) were collected separately. Dogs were rested for 3 weeks after the last semen collection. Following the rest period, semen collection was experienced 12 times at 3-day intervals by digital manipulation without sildenafil application. Response to digital manipulation was evaluated in the sildenafil and non-sildenafil groups. Digital manipulation was applied 3 times, consecutively. Immediately after collection, the ejaculates were immersed in a warm water bath at 37°C until their assessment in the laboratory. Semen assessment was performed in approximately 30 min. Semen was examined for volume of fractions, ejaculation time (from the beginning of digital manipulation until the detumescence of the penis) and spermatological characteristics (percentage of motility, viability, acrosome, head and other (mid-piece and tail) abnormalities).

Motility was assessed using a phase-contrast microscope (x400 magnification), with a warm stage maintained at 37ºC. A wet mount was made using a 5µl drop of semen placed directly on a microscope slide and covered by a cover slip. Sperm motility estimations were performed in 3 different microscopic fields for each semen sample and the mean of the 3 successive estimations was recorded as the percentage of motility [10].

The viability of spermatozoa in samples was assessed by means of the nigrosin-eosin stain method [11]. The final composition of the stain was: Eosin-Y 1.67 g, nigrosin 10 g, and sodium citrate 2.9 g, dissolved in 100 ml distilled water. Sperm suspension smears were prepared by mixing a drop of sperm sample with two drops of stain on a warm slide and spreading the stain with a second slide; viability was assessed by counting 400 cells under phase-contrast at x1000 magnification. Sperm displaying partial or complete purple staining were considered nonviable; only sperm showing strict exclusion of stain were counted as viable.

For the assessment of sperm abnormalities, at least three drops of each sample were added to 1 ml of Hancock solution. One drop of this mixture was put on a slide and covered with a cover slip. The percentage of sperm abnormalities (acrosome, head, mid-piece and tail) were determined by counting a total of 400 spermatozoa under phase-contrast microscope (magnification x1000 and oil immersion) [12].

The study was replicated 12 times. Responses to digital manipulation were compared using the chi-square test, and total ejaculation times were compared by the t-test. Spermatological characteristics were compared using the Mann-Whitney U test between the sildenafil and non-sildenafil groups. Differences with values of $P<0.05$ were considered to be statistically significant.

RESULTS

Differences for semen volume and total ejaculation time between the sildenafil and non-sildenafil treatments are illustrated in *Table 1*.

Table 1. *Semen volume (first, second and third fractions), and total ejaculation time in the groups with sildenafil and non-sildenafil administered*

Tablo 1. *Sildenafil uygulanan ve uygulanmayan gruplarda sperma hacmi (ön, ana ve son fraksiyon) ve ejakülasyon süreleri*

Groups	n	First Fraction (ml)	Second Fraction (ml)	Third Fraction (ml)	Total ejaculation time (min)
Sildenafil	55	0.8±0.20	1.1±0.09 [a]	11.0±0.98 [a]	11.85±0.72 [a]
Non-sildenafil	42	0.7±0.09	0.7±0.08 [b]	6.0±0.80 [b]	7.0±0.84 [b]
P		-	*	*	*

Different letters within the same column are significantly different (P<0.05)*
- : *No significant difference (P>0.05)*

In *Table 1*, there was a significant difference (P<0.05) for the semen volume of the second and third fractions and total ejaculation time between the two groups. In addition, significant differences were detected (P<0.05) for the erection numbers between the sildenafil and non-sildenafil groups, as shown in *Table 2*.

Table 2. *Number of erection in the groups with sildenafil and non-sildenafil administered*

Tablo 2. *Sildenafil uygulanan ve uygulanmayan gruplarda ereksiyon sayıları*

Groups	Number of erection	Number of non-erection	Significance
Sildenafil	55	5	P< 0.05
Non-sildenafil	42	18	

Differences for sperm motility, viability and abnormalities of the acrosome, head and other parts (mid-piece and tail) between sildenafil and non-sildenafil treatments are illustrated in *Table 3*. No differences were detected for spermatological characteristics between the sildenafil and non-sildenafil groups (P>0.05).

DISCUSSION

The present results show that sildenafil application increases the semen volume and the number of erections. Recently, it was indicated that sildenafil enhanced penile erection upon electro-stimulation in rats [13]. Taher et al.[14] showed that sildenafil relaxed the penile smooth muscle of monkeys, and also the corpus cavernosum of rabbits.

The total time interval for ejaculation was longer in the sildenafil treated group than in the non-sildenafil group. This situation may have resulted from sildenafil administration, causing longer erection with the resultant effect of enhanced sexual activity and relaxation of the smooth muscle. Consequently, sildenafil could be used in veterinary medicine and experimental studies. However, total ejaculation times were reported to be 10.3 min in the Turkish shepherd dog by Tekin et al.[15] In our study, total ejaculation time was determined to be 11.8 min in the same species. This data confirmed that sildenafil usage enhanced the total ejaculation time and number of erection.

In our study, sildenafil administration enhanced the volume of the first, second and third fractions in dogs. This may be due to the ejaculation time being longer, because longer ejaculation time can enhance the volume of the first, second and third fractions. Additionally, Akcay [16] cited the volume of the third fraction as 9.4 ml in Turkish shepherd dogs. The volume of the third fraction tended to be higher in our study than in the other study. These differences may have resulted from sildenafil administration with the resultant effect of longer ejaculation time and enhanced sexual stimulation.

The present study demonstrated that sildenafil exerts a mating stimulation, which seems to relate to

Table 3. *Mean (±SE) percentages of motility, viability, abnormalities (acrosome, head and other) in the groups with sildenafil and non-sildenafil administered*

Tablo 3. *Sildenafil uygulanan ve uygulanmayan gruplarda ortalama spermatozoa motilitesi, canlı ve anormal (akrozom, baş ve diğer) spermatozoa oranları (%)*

Groups	n	Motility	Viability	Acrosome abnormalities	Head abnormalities	Other abnormalities (mid-piece and tail)
Sildenafil	55	76.3±7.41	11.2±2.97	4.7±1.61	4.4±1.6	11.6±2.93
Non-sildenafil	42	76.2±6.47	11.2±2.84	4.7±1.37	4.6±1.13	12.2±2.23
P	-	-	-	-	-	-

- : *No significant difference (P>0.05)*

both ejaculation and sexual arousal. It has already been shown that copulatory behaviour is modulated by pharmacological agents. Several studies and clinical trials have confirmed the efficacy of sildenafil for treating erectile dysfunction of various causes with a response rate of 70% [17,18]. Additionally, Çoyan and Kaya [19] reported that sildenafil enhanced the performance, number and behaviour of mating. In this study, similar effects were observed in dogs after the use of sildenafil. Sexual desire continued for a while after the sildenafil applications. No significant difference was observed in the percentages of spermatological characteristics in this study. Similar results were obtained by Purvis et al.[20] in humans. Contrarily, Plesssis et al.[21] cited that sildenafil can be used successfully to enhance sperm motility and binding capacity to the oocyte during fertilisation.

In conclusion, sildenafil could be used in dogs having problems in response to digital manipulation for penile erection and successful semen collection. Further investigations are necessary to determine whether decreased libido could successfully be treated with this drug.

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