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Short term effects of dexamethasone on hyaluronidase activity and sperm characteristics in rams

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Abstract

The aim of this study was to determine the effects of dexamethasone on sperm characteristics and hyaluronidase activity of serum and semen. In this investigation, 14 healthy Akkaraman rams, at the age of 2 years and weighing between 50–60 kg, were used. The rams were randomly divided into two groups. After the last administration of dexamethasone intramuscularly at a dose of 0.25 mg/kg, semen and blood samples were taken at different times. The results showed that the serum hyaluronidase activity was increased significantly ($p < 0.001$) in the treatment group when compared with the control group except for the 1st hour. There was a significant difference ($p < 0.001$, 0.01, 0.05) in the hyaluronidase activity of semen between the treatment group and the control group. Furthermore, there was a significant difference ($p < 0.01$) in sperm concentration between both groups at all the times except the 96th hour. There were statistically significant ($p < 0.05$) differences in semen volume between the treatment and control groups. There were also significant differences ($p < 0.05$) in sperm motility between the treatment and control groups except for the 72 and 96th hours.

These findings indicate that dexamethasone increases hyaluronidase activity of serum and semen, but it decreases sperm concentration, semen volume and sperm motility in rams. Therefore the use of these drugs in breeding rams during breeding season is not suitable.

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1. Introduction

Dexamethasone, a synthetic adrenocortical steroid, practically white, odorless, crystalline powder (Kovarik et al., 1998), is used widely in treating endocrine disorders, rheumatic disorders, collagen diseases, dermatologic diseases, allergic states, ophthalmic diseases, respiratory diseases, haematologic disorders, neoplastic disease, edematous states, gastrointestinal disease, cerebral edema and diagnostic testing of adrenocortical hyperfunction (Kovarik et al., 1998; Ram et al., 1993). In general, the side effects of dexamethasone on male fertility could be not determined until the males were examined andrologically. The transient inhibition of ram fertility by dexamethasone is possible, if one of the following functions interferes: spermatogenesis, sperm maturation within the epididymis, sperm transport, sperm metabolism and motility, semen liquefaction, capacitation, acrosomal reaction, or ovum penetration. It seems that in many species, dexamethasone treatment directly affects testicular function (Abbatichio et al., 1981).

Acrosin, hyaluronidase, esterases and acid hydrolases are acrosomal enzymes (Garner and Hafez, 2000). Hyaluronidase is an acrosomal enzyme, which participates in the dissolution of the cumulus oophorus matrix containing hyaluronic acid and is essential for the fertilization process (Savion et al., 1986). Hyaluronidase has been found in a wide variety of mammalian tissues (Csoka et al., 1997). It has been reported that (Tsantarliotou et al., 2002; Berger and Clegg, 1985) total acrosin activity in spermatozoa was reduced between 7–28 days after dexamethasone administration. Dexamethasone also induced a reduction in mean value and basal level of blood testosterone and inhibited its episodic secretion between 1 and 4 days after administration (Berger and Clegg, 1985). The reduction of acrosin activity appeared relatively soon after dexamethasone administration in Chios rams (Tsantarliotou et al., 2002).

The influence of dexamethasone on sperm characteristics and hyaluronidase activity of semen and serum were investigated in the present study.

2. Materials and methods

2.1. Animals

In this study, 14 Akkaraman healthy rams, at 2 years of age and between 50–60 kg live weights were used. The rams were randomly divided into two groups as control ($n = 7$) and treatment ($n = 7$). The rams were fed on grass supplemented with alfalfa hay and drinking water was provided ad libitum.

2.2. Administration of dexamethasone and sample collection

Semen and serum samples were taken from both control and treatment group rams before the administration of dexamethasone. Then dexamethasone [Dexamethasone 21-phosphate disodium, (Dekort 8 mg/2 ml, DEVA Co., Istanbul)] was injected intramuscularly at a dose of 0.25 mg/kg (this dose is recommended by the manufacturer) body weight to the treatment group, once daily for 2 days. After the last administration of the drug, blood and semen

samples were taken from control and treatment groups after 1, 2, 4, 24, 48, 72 and 96th hours. The blood samples were collected by jugular vein puncture into 10 ml vacutainer tubes and semen samples were collected by artificial vagina from all rams. The blood samples were stored at +4 °C until separation of serum for 2 h.

2.3. Semen evaluation

Semen volume was calculated by using graded tubes. Semen samples were decimally diluted with isotonic sodium citrate solution at 37 °C (3%, w/v dissolved in distilled water) at rate of 1:10. A slide was placed on phase contrast microscope and allowed to warm up to 37 °C, and then a small droplet of diluted semen was placed on the slide and percent motility was evaluated visually at a magnification of 400× (Bearden and Fuquay, 1992). Motility estimations were performed from three different fields in each sample. The mean value averaged from three successive estimations was used as the final motility score. Sperm concentration was determined by using haemocytometer (Ax et al., 2000; Gordon, 1999). The percentage of morphologically abnormal spermatozoa was determined from slides prepared with an Indian ink. A total of 300 sperm cells were counted on each slide under phase contrast microscope at 400× magnification (Evans and Maxwell, 1987).

2.4. Enzyme assays

Hyaluronidase activity was measured using the method described by Tanyıldızı and Türk (2004), and Wilkinson et al. (1996). The serum and semen samples were diluted 1 in 5 with 0.15 mol/l sodium chloride before assay. One millilitre of serum and semen samples was supplemented with 0.1 ml acetate buffer and 0.1 ml hyaluronic acid substrate. This mixture was then incubated for 24 h at 37 °C in a thermostatically controlled incubator. The mixture was centrifuged at 500 g for 5 min. Then, 60 µl potassium tetraborate (0.8 mol/l in water, pH = 10) was added to the supernatant. The reaction was terminated by heating at 100 °C in a heating block for 5 min. The reaction mixtures were cooled in an ice–water bath before adding 2 ml of dimethylaminobenzaldehyde (stock DMAB reagent, 10%, w/v in 12.5%, v/v concentrated hydrochloric acid in glacial acetic acid: stock reagent diluted 1 in 10 with glacial acetic acid before use) and then incubated for 20 min at 37 °C in water bath. The reaction mixtures were centrifuged immediately at 1500 × g for 10 min. Then, the supernatants of blood serum and semen samples were taken and measured at 582 nm within 30 min by spectrophotometry (Joyce et al., 1986). Hyaluronidase activity was expressed as the mean (±S.E.M.) µmolNAG/min/l.

2.5. Statistical analyses

The data were presented as the mean ± S.E.M. A chi-square (χ^2) test was used to determine differences in the sperm motility between the control and treatment groups. Independent Students *t*-test was used to determine differences in serum and semen hyaluronidase activity between the control and treatment groups. To determine the differences between time points, post hoc comparisons were made with Duncan's multiple comparison tests. The Pearson correlation test was applied to determine the relationship between hyaluronidase

activity and semen characteristics. All data were analysed using the SPSS software package program (Windows 10.0).

3. Results

The pre-treatment values of all parameters were given in Table 1. There was no significant ($p > 0.05$) difference in the pre-treatment values between the control and treatment group rams.

The mean \pm S.E.M. values of blood serum and semen hyaluronidase activity in the control and treatment groups were presented in Table 2.

While the average serum hyaluronidase activity was $44.37 \pm 0.45 \mu\text{molNAG}/\text{min}/\text{l}$ in the control group, it was found as $121.58 \pm 13.44 \mu\text{molNAG}/\text{min}/\text{l}$ in the treatment group. The average semen hyaluronidase activity was determined to be 28.78 ± 0.50 and $56.18 \pm 3.80 \mu\text{molNAG}/\text{min}/\text{l}$ in the control and treatment groups, respectively. There was a significant difference ($p < 0.001$) in serum hyaluronidase activity between the control and the treatment group at all times except the 1st hour. There were also significant differences at 1, 2 and 48th hours ($p < 0.001$), at 4, 24 and 72th hours ($p < 0.01$) and at 96th hour ($p < 0.05$) in semen hyaluronidase activity between the control and the treatment group.

Table 1

Pre-treatment values of control and treatment group

Group	Hyaluronidase activity ($\mu\text{molNAG}/\text{min}/\text{l}$)		Sperm concentration ($\dots \times 10^9/\text{ml}$)	Semen volume (ml)	Sperm motility (%)	Sperm abnormality (%)
	Blood serum	Semen				
Control ($n = 7$)	45.12 ± 3.20	30.02 ± 3.18	2.40 ± 0.27	0.93 ± 0.04	70.6 ± 5.4	5.12 ± 0.07
Treatment ($n = 7$)	43.50 ± 2.62	29.73 ± 4.22	2.24 ± 0.48	1.02 ± 0.09	74.1 ± 3.8	6.93 ± 0.90

The data were expressed as the mean \pm S.E.M.

Table 2

The post-treatment values of hyaluronidase activity of blood serum and semen in rams

Hour ($n = 7$)	Blood serum ($\mu\text{molNAG}/\text{min}/\text{l} \pm \text{S.E.M.}$)		Semen ($\mu\text{molNAG}/\text{min}/\text{l} \pm \text{S.E.M.}$)	
	Control ($n = 7$)	Treatment ($n = 7$)	Control ($n = 7$)	Treatment ($n = 7$)
1	43.40 ± 0.51 a	48.32 ± 3.94 b	27.10 ± 1.25 a	72.04 ± 4.38 ad ^{***}
2	43.60 ± 0.47 abc	133.88 ± 6.24 dc ^{***}	29.17 ± 2.51 bc	60.20 ± 6.03 a ^{***}
4	46.50 ± 0.93 c	139.78 ± 6.72 ac ^{***}	28.45 ± 2.31 b	54.27 ± 5.21 c ^{**}
24	44.19 ± 0.32 ab	146.88 ± 6.02 ac ^{***}	30.70 ± 1.82 d	53.21 ± 8.67 c ^{**}
48	45.60 ± 0.44 b	151.94 ± 3.07 a ^{***}	29.75 ± 2.52 cd	62.75 ± 2.41 ac ^{***}
72	43.50 ± 0.49 a	122.19 ± 6.55 d ^{***}	27.15 ± 1.75 a	50.61 ± 3.29 c ^{**}
96	43.80 ± 0.36 a	108.11 ± 4.22 d ^{***}	29.18 ± 2.81 bc	40.22 ± 3.21 c [*]
Mean	44.37 ± 0.45	121.58 ± 13.44	28.78 ± 0.50	56.18 ± 3.80

Differents letters within a column showed significant differences ($p < 0.05$) between serum and semen hyaluronidase activity.

* Significantly different from control group $p < 0.05$.

** Significantly different from control group $p < 0.01$.

*** Significantly different from control group $p < 0.001$.

Table 3
The mean values of sperm concentration in rams

Hour	Sperm concentration ($\dots \times 10^9/\text{ml} \pm \text{S.E.M.}$)	
	Control ($n=7$)	Treatment ($n=7$)
1	2.23 \pm 0.18 ab	1.57 \pm 0.21 b**
2	2.42 \pm 0.15 b	1.44 \pm 0.24 bd**
4	2.19 \pm 0.22 ab	0.82 \pm 0.26 c**
24	2.10 \pm 0.26 a	1.30 \pm 0.27 d**
48	2.23 \pm 0.23 ab	1.46 \pm 0.24 bd**
72	2.34 \pm 0.21 ab	1.67 \pm 0.18 b**
96	2.09 \pm 0.25 a	2.23 \pm 0.51 a
Mean	2.22 \pm 0.04	1.49 \pm 0.15

Differents letters within a column showed significant ($p < 0.05$) differences between sperm concentrations.

** Significantly different from control group $p < 0.01$.

The mean \pm S.E.M. values of sperm concentration in the control and treatment group were presented in Table 3.

While the average sperm concentration was determined as $2.22 \pm 0.04 \times 10^9/\text{ml}$ in the control group, it was found as $1.49 \pm 0.15 \times 10^9/\text{ml}$ in the treatment group. This difference was statistically significant ($p < 0.01$) at all times except the 96th hour.

There were statistically significant differences ($p < 0.05$) between the control and treatment group in semen volume at all times (Fig. 1). The difference in sperm motility was also significant ($p < 0.05$) except 72 and 96th hours (Fig. 2).

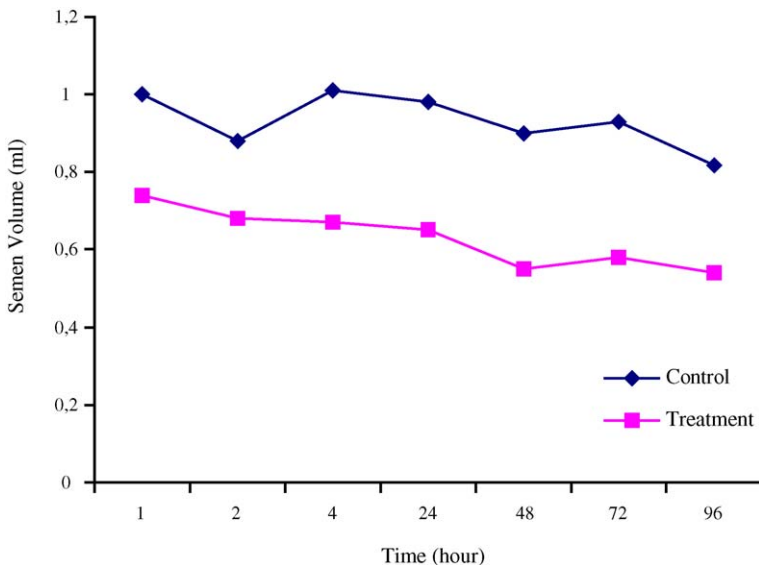


Fig. 1. The mean values of semen volume in control and treatment group in rams. There was a significantly ($p < 0.05$) difference between both groups at all times.

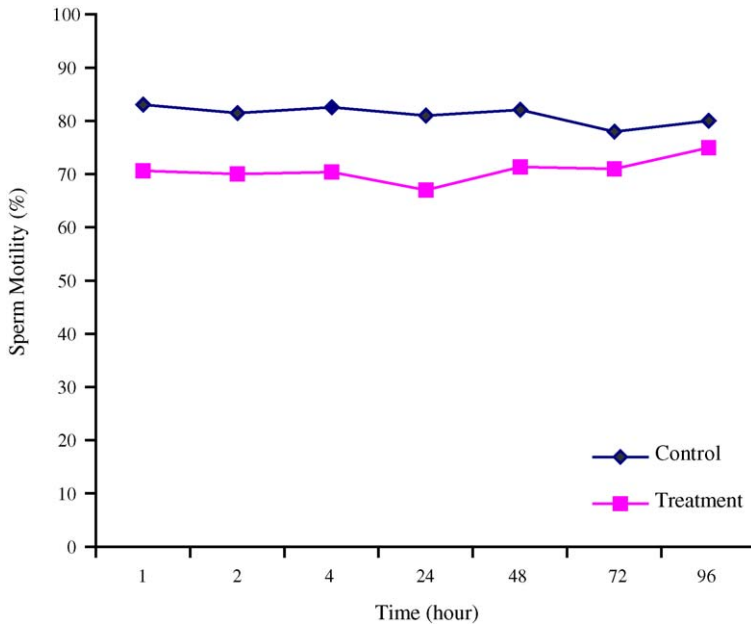


Fig. 2. The mean values of sperm motility in control and treatment group in rams. There was a significantly ($p < 0.05$) difference between both groups at 11 times with the exception of 72 and 96th hours.

The mean \pm S.E.M. percentages of abnormal spermatozoa were not affected by treatment with dexamethasone. Abnormal spermatozoa rate was found between 4.67 ± 0.33 and $7.51 \pm 0.33\%$. Additionally, mean \pm S.E.M. percentage for abnormal spermatozoa of the control rams was determined as $6.21 \pm 0.15\%$. There were no significant differences in the abnormal spermatozoa rates between the control and treatment groups. These values were at normal ranges for rams.

No significant correlations were found between hyaluronidase activity and sperm characteristics.

4. Discussion

Hyaluronidase activity, semen volume, sperm motility, concentration, and morphology are important indicators of semen quality and these parameters may provide useful information in determining fertilization potential of animals.

It has been reported (Tsantarliotou et al., 2002) that dexamethasone causes the release of hyaluronidase enzyme from lysosomes in blood. The results of the present study showed that there was a significant increase in the serum hyaluronidase activity of the rams treated with dexamethasone compared to the control group at all times except the 1st hour. This elevation may indicate that dexamethasone increases serum hyaluronidase activity in rams depending on the increase of hyaluronidase released by lysosomes.

As glucocorticoid receptors exist in the epididymis and accessory glands in various species, dexamethasone may have a direct influence on the synthesis and/or release of acrosomal enzyme inhibitors in epididymal fluid or seminal plasma (Savion et al., 1986; Tsantarliotou et al., 2002). Bozkurt et al. (2004) reported that hyaluronidase move from serum to the seminal plasma. In the present study a significant increase was also observed in semen hyaluronidase activity of the rams treated with dexamethasone compared to the control group at all times. The elevation of semen hyaluronidase activity may be explained with the movement of hyaluronidase from serum to seminal plasma.

In the treatment group values for semen volume was less than the control group at all the times. This decrease may be due to the effect of dexamethasone inhibition on luteinizing hormone releasing hormone that caused to decrease of testosterone releasing from Leydig cells (Tsantarliotou et al., 2002). Testosterone has both androgenic and anabolic activities at the target cell (Pineda, 2003). The androgenic activity stimulates function of accessory reproductive organs and epididymis and to be cutted of androgenic activity of testosterone by this drug leads to the decrease in semen volume.

Kumi-Diaka and Townsend (2001), reported that low doses of dexamethasone does not significantly influence sperm motility or sperm morphology; but higher doses dexamethasone significantly interfere with percentage sperm motility in rats. The findings of this study revealed that treatment of dexamethasone caused significant ($p < 0.05$) decreases in the sperm motility in rams at all times except 72 and 96th hours. The reason of these declines may be explained with the decrease of energy substrates in the secretion of accessory glands with the inhibition of testosterone.

Dexamethasone suppresses serum testosterone (Juniewicz et al., 1987) and luteinizing hormone (LH), which influences testicular endocrine function (Matteri et al., 1984; Ssewanyana et al., 1990; Fomicheva, 1985; Juhász et al., 2001), and also indirectly affects sperm maturation within the epididymis, sperm transport, sperm metabolism (Tsantarliotou et al., 2002). There was a significant decrease in sperm concentration between the control and treatment group except the 96th hour. As the period of sampling in the present trial (96th) is limited and duration of the spermatogenic cycle in the ram is 45–51 days (Gordon, 1999) then any effect of dexamethasone on spermatogenesis would not be observed. However, this decrease in the sperm concentration that determined in the present study may be explained with the possible effects of the drug on the smooth muscle contraction and secretions of accessory glands and epididymis itself.

A significant correlation has been reported between seminal hyaluronidase activity and sperm concentration and motility (Tambe et al., 2001; Abdull-Aziz et al., 1995). In the current study no significant correlations were found between all these parameters. The difference to previously reported data by others might have resulted from the method used to measure hyaluronidase activity and sample variation.

5. Conclusion

In conclusion, the findings of this study indicate that dexamethasone increases hyaluronidase activity in serum and semen, but it decreases sperm concentration, semen volume and sperm motility in rams. Our observations indicate that dexamethasone

had significant effects on sperm parameters with the exception of the rate of abnormal spermatozoa.

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