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# Relationship between Vitamin Composition and Spermatological Characteristics in Semen of Different Ram Breeds of Turkey

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**ABSTRACT**

The aim of this study was to evaluate the vitamin levels of ram seminal plasma and the relationship between vitamins and spermatological characteristics. For this aim, nine 3-5 year old rams of Norduz, Karakas and Ile-de-France x Akkaraman (G1) genotypes were provided. Sperm of each ram was collected once per month from June to October of the year 2007 by artificial vagina. In each month, the concentrations of retinol acetate, alpha tocopherol, delta tocopherol, alpha tocopherol acetate and vitamin $D_3$ in seminal plasma were analyzed by using HPLC. Spermatological characteristics viz. sperm volume, pH, mass activity, sperm motility, abnormal spermatozoid, death/alive ratio and sperm concentration were evaluated. As testicular characteristics, scrotal circumference and testicular diameter were measured. Monthly changes and breed differences on the vitamin levels were statistically insignificant, whereas there was a positive correlation was obtained between testis diameter and retinol acetate levels ($p<0.05$) and a positive correlation was also estimated between analyzed vitamins. As a result, the relationships between the testicular, spermatological characteristics, and vitamin compositions should be taken into account in the breeding targets.

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**Authors' Contribution**

**NM, HM and TA conceived and designed the survey. KK collected and statistically analyzed the data. IY analyzed seminal plasma samples via HPLC. TA and KK evaluated spermatological characteristics. NM, KK and MMT contributed to the interpretation of data and drafted the manuscript.**

**Key words**

**Vitamin A, vitamin D, vitamin E, ram, seminal plasma, spermatological characteristics, sperm motility, abnormal spermatozoid, scrotal circumference, tocopherols**

## INTRODUCTION

Reproduction of sheep is very important because it ensures periodical reproduction of several characteristics and thus is a crucial subject in sheep breeding programs to sustain productivity. In sheep breeding, reproductive performance is the key factor that can affect profitability (Jahan *et al.*, 2013). On other hand, the reproductive importance of rams in reproduction activity is generally neglected. In fact, it is not an efficient approach to ignore the effects of rams on modern production principles. On the contrary, the effectiveness of rams on reproduction is more significant in sheep breeding since rams are mated with many ewes in a mating program. The parameters relating to ram testicular characteristics assist breeders to get precious information for both lamb productivity in terms of indirect selection criteria in breeding and sperm production (Karakus *et al.*, 2010). Therefore, rams selected for mating programs should be evaluated on the basis of progressing the characteristics of reproduction before the mating program begins in order that the testicular characteristics which are high heritable were strongly correlated genetically with ovulation proportion of female sheep (Bilgin *et al.*, 2004), and therefore these characteristics of reproduction are reported to be mainly the testis parameters, the sperm characteristics, its biochemical composition, and sexual behavior of animals (Mert *et al.*, 2009). These vital characteristics are affected by several environmental factors such as feeding, mating season, and age, and rams should be equally evaluated based on genotype, age, the season, and the manner of feeding in effective production processes.

It is generally accepted that vitamin- and mineral-supplemented diets that are sufficient to meet the essential requirements in energy, protein, and amino acids improve sperm quality. Energy and protein deficiencies are known to be the most well-known causes of reproductive defects in domesticated animals. The

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nutritional deficiencies have been known to exert a harmful effect on male fertility. Hypovitaminosis A and E are greatly associated with testicular degeneration, sterility, the lack of diet B complex, and the poor intake of feed causes regressive changes in accessory glands (Mann and Lutwak-Mann, 1981). Retinol, an alcohol form of vitamin A, stimulates spermatogenesis. In rat testes, a specific retinol binding protein exists and plays a vital role in the vitamin A expression in the germinal epithelium. Vitamin A and its metabolites play an essential role in spermatogenesis and the reproductive performance of the ram. All physiological functions connected with vitamin A may be adversely affected when it does not take in enough in its feeding regime. Semen output and quality, sexual activity, spermatogenesis and testosterone production are significantly affected by the lack of vitamin A. If rams do not consume enough vitamin A in their feed, their semen output or quality will be poor and unfavorably affect ram fertility (Abdulkareem *et al*., 2005).

A variety of tocopherol isoforms with vitamin E activity is available in nature. Tocopherol esters, acetate and succinate are more stable and water soluble than tocopherol alone and more effective against many diverse oxidative stressors (Almeida and Ball, 2005).

The bioactive form of vitamin D, 1,2 5-dihidroxyvitamin $D_3$, has many important physiological functions such as blood pressure regulation, mammary gland development, mineral ion homeostasis and skeletal integrity, and the promotion of intestinal absorption of Ca and P (Sutton and Mac Donald, 2003). Several studies have previously reported that vitamin D and vitamin D receptors (VDRs) play vital roles in male fertility. However, the detailed studies on the mechanism in testis and spermatogenesis are still scarce. VDR has been shown in the epididymis, spermatogonium and Sertoli cells. These suggest that vitamin D has a major role in the production and transport of sperm (Almeida and Ball, 2005; Sutton and MacDonald, 2003). Kwiecinski *et al.* (1989) demonstrated that vitamin D deficient male rats had reductions in successful mating, low fertility rates when compared with the controls, and incomplete spermatogenesis. Impaired development and degenerative changes in the seminiferous tubules were also noted.

Although the effect of spermatologic characteristics on the fertility of adult Norduz and Karakas rams has been reported by Karakus and Cengiz (2007), the information available on reproduction characteristics of Norduz, Karakas and Ile-de-France x Akkaraman genotypes is still scanty in literature. Hence, this investigation was undertaken to determine the vitamin levels of ram seminal plasma and the relationships between several vitamins, spermatological characteristics and testicular characteristics in Norduz, Karakas and Ile-de-France x Akkaraman genotypes with the objective to increase fertility of these rams.

## MATERIALS AND METHODS

The present study was carried out at the research farm of the Agricultural Faculty of Yuzuncu Yil University during June and October months of the year 2007. Nine 3-5 year old rams, four Norduz, two Karakaş and three Ile-de-France x Akkaraman (G1), were provided. They were housed in a covered shelter with an open-air run, and allowed to walk freely throughout the study. All the rams were fed with the same ration. Sperms were collected from each ram early in the morning once per month for four months using an artificial vagina. The freshly collected sperms were immediately transferred to a laboratory inside the farm and immersed in a water bath at 30ºC.

The volume of semen was recorded immediately after collection using a graduated glass tube. Mass motility of sperms was made on the basis of an arbitrary scale from 0 to 5. Sperm mortality, pH, sperm viscosity (0-5), sperm live/death ratio (%) and sperm concentrations $10^9/ml^{-1}$ were also determined. The evaluations of the parameters of production and quality of sperm were made as described by Tekin (1990). Testicular diameter and scrotal circumference were measured with a metal compass and ribbon meter, respectively.

The extraction procedure was modified from the related literature (Beverly and Sarı, 1983; Miller and Yang, 1985; Pikkarainen and Parviainen 1992; Fechner *et al*., 1998). Seminal plasma samples were homogenized with 250 µl 10 % ascorbic acid and then 250 µl of ethanol was added to each tube and vortexed for one minute. Later, 2 ml of n-hexane was added and vortexed. The samples were then centrifuged at 2,000 rpm for 10 minutes. The hexane-containing upper layer was removed and evaporated to dryness under a nitrogen stream and the residue was dissolved in 250µl of methanol. The retinol acetate, tocopherol and vitamin $D_3$ standards (Sigma Co.) were prepared for analysis. 100µl of seminal plasma extract was injected into HPLC column (C-18, 250x4.6 mm, Ace-Scotland) for the separation and quantitative determination of vitamins. DAD (diode-array detector) was employed at 265 nm wavelength for vitamin $D_3$ 290 nm for tocopherols and 325 nm for retinol acetate. As the mobile phase, methanol-water (98:2) (Merck) was used at 1.5 ml/ min flow rate (Beverly and Sarı, 1983; Pikkarainen and Parviainen 1992; Fechner *et al*., 1998; Reynolds and Judd, 1984).

**Table I.- Vitamin levels (Mean±SEM) in mcg/ml of sperms of rams of Norduz, Karakas and Ile-de-France x Akkaraman ($G_1$) mcg/ml**

| | Number | Retinol acetate | α tocopherol | δ tocopherol | α tocopherol acetate | Vitamin $D_3$ |
|---|---|---|---|---|---|---|
| **Total** | 34 | 0.34±0.03 | 0.77±0.06 | 0.69±0.05 | 1.72±0.12 | 0.18±0.03 |
| **Genotype (G)** | | | | | | |
| Norduz | 15 | 0.33±0.03 | 0.75±0.07 | 0.67±0.06 | 1.64±0.15 | 0.15±0.03 |
| Karakaş | 8 | 0.32±0.04 | 0.82±0.09 | 0.72±0.08 | 1.87±0.20 | 0.25±0.04 |
| Crossbreeds | 11 | 0.36±0.04 | 0.76±0.08 | 0.69±0.07 | 1.77±0.18 | 0.17±0.04 |
| **Month (M)** | | | | | | |
| July | 9 | 0.34±0.04 | 0.73±0.09 | 0.67±0.08 | 1.49±0.20 | $0.16±0.04^{ab}$ |
| August | 9 | 0.35±0.04 | 0.74±0.09 | 0.67±0.08 | 1.79±0.20 | $0.17±0.04^{ab}$ |
| September | 9 | 0.31±0.04 | 0.81±0.09 | 0.81±0.09 | 1.83±0.20 | $0.14±0.04^{b}$ |
| November | 7 | 0.36±0.05 | 0.83±0.10 | 0.83±0.10 | 1.93±0.22 | $0.27±0.05^{a}$ |
| **GxM Interaction** | | ns | Ns | ns | ns | ns |

**[a,b]** Values in column not having a common superscript differ significantly ($p< 0.05$)
ns: non-significant

*Statistical analysis*

Descriptive statistics of the recorded data were reported as Mean±SE. Pearson correlation coefficients were estimated to determine the linear relationships between pairs of the quantitative characteristics assessed in the current survey. All the evaluated characteristics were analyzed by GLM (General Linear Model) for testing hypotheses of fixed effects like genotype (G), month (M) and GxM interaction. Mean separation was performed with Duncan Multiple Comparison Test. All the statistical computations were done by CORR and GLM procedures of SAS statistical package program (SAS, 2009).

## RESULTS

Table I shows descriptive statistics of sperm vitamin levels for breed and month factors under the study. The seminal plasma level differences between breeds evaluated in the current study were non-significant. Monthly changes were also non-significant but vitamin levels from July to November increased (Table I). The significant variation in Vitamin $D_3$ between months was determined ($P<0.05$).

Table II shows the bivariate relationship between trait pairs of spermatological, testicular, vitamin compositions. A positive correlation was found for testicular diameter and retinol acetate level ($P<0.05$). Several significant correlations were obtained between vitamin composition traits ($P<0.01$).

Table II shows the relationships between the sperm characteristics themselves or vitamin values of the rams under investigation. Some negative correlations between sperm mass activity and sperm motility with sperm pH in rams were estimated. When the value of sperm pH increased, the sperm viscosity and sperm motility might decrease because high acidity in semen kills spermatozoa cells. Interestingly, as also perceived in Table II, sperm viscosity was adversely correlated with sperm mass activity and sperm motility, whereas there was a positive correlation between sperm mass activity and sperm motility, as is expected biochemically. When statistically evaluated, Table II showed that there were several positive correlations between sperm mass activity or sperm motility and testicular characteristics such as testicular diameter and scrotal circumference. At the same time, Table II reflected that there were several positive correlations between vitamin parameters, *viz.* α tocopherol and the α tocopherol acetate and vitamin D.

## DISCUSSION

Lipid peroxidation has been recognized for many years and well defined as a cause of oxidative stress, which is an important damaging process leading to motility loss and reduced fertilizing ability that occurs spontaneously in mammalian spermatozoa (Jones and Mann, 1977; Jones *et al.*, 1979). Reactive oxygen species (ROS) involve the peroxidation of polyunsaturated fatty acids present in the sperm's plasma membrane. It increases DNA fragmentation and adversely affects the function of spermatozoa. In addition, spermatozoa are

**Table II.- Correlations between sperm and testis characteristics and vitamin levels (mcg/ml) in rams of Norduz, Karakas and Ile-de-France x Akkaraman ($G_1$).**

| | Sperm viscosity (0-5) | Sperm mass activity (0-5) | Sperm motility (%) | Abnormal spermatozoa ratio (%) | Sperm death/alive ratio (%) | Sperm conc. ($10^9$/ml) | Testis diameter (cm) | Scrotal circum-ferences (cm) | Retinol acetate (mcg/ml) | Alpha Tocopherol (mcg/ml) | Delta tocopherol (mcg/ml) | Alpha tocopherol acetate (mcg/ml) | Vitamin $D_3$ (mcg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sperm volume | 0.13 | 0.10 | . | . | 0.10 | 0.33 | 0.13 | 0.28 | . | 0.11 | 0.15 | 0.24 | . |
| pH | -0.24 | -0.57** | -0.71** | 0.26 | -0.15 | -0.10 | . | . | 0.10 | 0.24 | 0.23 | 0.14 | . |
| Sperm viscosity | | 0.37** | 0.51** | . | 0.28 | 0.24 | 0.34 | 0.35 | . | -0.22 | -0.26 | -0.16 | . |
| Sperm mass activity | | | 0.87** | . | . | . | 0.46** | 0.44** | 0.14 | . | . | . | 0.14 |
| Sperm motility | | | | 0.16 | . | 0.11 | 0.37* | 0.35* | . | -0.11 | -0.16 | . | . |
| Abnormal spermatozoa ratio | | | | | 0.24 | 0.15 | 0.23 | 0.19 | 0.17 | . | . | 0.21 | 0.14 |
| Sperm death/alive ratio | | | | | | 0.11 | . | . | -0.16 | -0.22 | -0.33 | . | -0.10 |
| Sperm concentration | | | | | | | 0.25 | 0.23 | . | . | . | . | . |
| Testicular diameter | | | | | | | | | . | . | . | . | . |
| Scrotum circumferences | | | | | | | | | 0.33 | | . | | -0.10. |
| Retinol acetate | | | | | | | | | . | | 0.13 | . | 0.24 |
| Alpha tocopherol | | | | | | | | | | 0.71** | 0.79** | 0.59** | . |
| Delta tocopherol | | | | | | | | | | | 0.69** | 0.85** | . |
| Alpha tocopherol acetate | | | | | | | | | | | | 0.57** | 0.11 |
| Vitamin $D_3$ | | | | | | | | | | | | . | . |

* p< 0.05
* * p< 0.01

capable of generating ROS with many physiologic functions such as controlling acrosome reaction, sperm capacitation and sperm-oocyte fusion (Aitken, 1995; Storey, 1997). The over production of ROS by defective spermatozoa can have negative effects on sperm function. It is generally believed that the extreme production of ROS or weakness of the antioxidant defense mechanism results from oxidative stress. During the process intracellular ATP concentration falls, sperm motility decreases and lipid peroxidation begins in the sperm plasma membrane. As a consequence, increased cell permeability, enzyme inactivation and spermicidal end product formation occurs (Baumber *et al.*, 2000).

Antioxidant vitamins A, E, C and β-carotene have potential health promoting properties. Vitamin E or α-tocopherol plays an essential role in the cell to protect it against oxidative attack. Tocopherol scavenges oxygen radicals and intercepts lipid peroxyl radicals in the chain reactions of lipid peroxidation. A variety of tocopherol isoform exists in vitamin E. Tocoperol esters (α-tocopherol acetate or succinate) are often utilized in biological studies because of their greater stability and water solubility than tocopherol alone (Halliwell and Gutteridge, 1999). These esters are rapidly accumulated within mitochondria and gradually release to subcellular membranes, thus protecting cell membranes from oxidative damage (Halliwell and Gutteridge, 1999; Mickle *et al.*, 1989; Wu *et al.*, 1990).

Antioxidant vitamins are vital to improve sperm count and sperm motility. The impacts of vitamin E on seminal plasma characteristics have been studied extensively. Some previous studies showed the positive, whereas others revealed harmful effects on sperm motility (Upreti *et al.*, 1997; Nishimura and Morii, 1992; Brezezinska-Slebodzinska *et al.*, 1995; Beconi *et al.*, 1993; El-Masry *et al.*, 1994; Srivastava *et al.*, 1987). Our results indicated a negative correlation between sperm motility and δ and delta tocopherol levels (Table II).

Increased free radicals during ETS may lead to increased mtDNA deletions. Sperm mtDNA and nDNA are especially sensitive to the damage of ROS because they lack a repair mechanism and the high amount of membrane unsaturated fatty acid content. Excessive production of ROS changes sperm membrane functions and is thought to be an important cause of infertility problems (O'Connell *et al.*, 2002).

Tenlibaeva (1991) fed Karakul rams with a basal diet and vitamin A and E supplemented diets and found that sperm volume per ejaculation or sperm production and percentage fertility of ewes were positively affected by vitamins A and E (Yoshizawa *et al.*, 1997). There are similar studies showing the effects of A, E and D vitamin additions to the diets of ruminants on semen quality.

Most of them reported positive effects on the semen characteristics (Samokhin *et al.*, 1976; Shubin and Shubina, 1977; Sakhin *et al.*, 1979; Kozicki *et al.*, 1978). Marin-Guzman *et al.* (2000) remarked that α tocopherol was accumulated in spermatozoa but not in seminal plasma, and played an important role in spermatogenesis. Vitamin E regulates steps in the development of the germ cells of rats (Cooper *et al.*, 1987).

Abdulkareem *et al.* (2005) informed that vitamin A was important for the survival of sperm in the male genital tract and sperm integrity because in the sperm of rams without adding vitamin A, the number of abnormal spermatozoa was high. In bull and ram sperm abnormal spermatozoa, particularly those with head abnormalities, occur in a very short of time due to the deterioration of sperm DNA transcription. Other semen characteristics are also changed by different mechanisms as a result of Vitamin A deficiencies (Al-Haboby *et al.*, 1997). Abnormal sperm percentage could be a result of direct action of vitamin A on sperm and Sertoli cell functions (Porter *et al.*, 1985; Rajguru *et al.;* 1992). Abdulkareem *et al.* (2005) advised that 100,000 IU/month might be adequate to improve semen quality.

Beyond its classic roles, recent studies have suggested that Vitamin D, 1,25-dihydroxy cholecalsiferol, also played an essential role in female reproduction. Rats could reproduce in the absence of Vitamin D, whereas diminished fertility and reduced litter sizes were noted (Halloran and Deluca, 1979, 1980). Vitamin D deficient male rats had 45 % reduction in successful mating indicating lower fertility ratios with a few abnormal pregnancies and deliveries in females. During this reduced fertility, spermatogenesis fails and Sertoli cells could be destroyed (Walters *et al.*, 1980). Vitamin D binds to the VDR; however, the role of Vitamin D and VDR in male reproduction is not completely known. VDR is found in the smooth muscle of epididymis, spermatogonium, Sertoli cells and head of the sperm indicating an important role in the production and transport of sperm (Kinuta *et al.*, 2000). Some earlier researchers suggest that Sertoli cells are the primary site of vitamin D action. The absence of vitamin D resulted in gonadal insufficiencies, lower sperm count and motility. Histological abnormalities were also demonstrated (Yoshizawa *et al.*, 1997).

## CONCLUSIONS

In conclusion, some negative correlations between sperm mass activity and sperm motility with sperm pH in rams were determined. Sperm viscosity was negatively correlated with sperm mass activity and sperm motility, whereas a positive correlation between sperm mass activity and sperm motility was recorded. Positive correlations between sperm mass activity or sperm motility and testicular characteristics such as testicular diameter and scrotal circumference, and positive correlations between vitamin parameters such as the alphatocopherol and the alphatocopherol acetate and vitamin D were estimated.

*Statement of conflict of interest*

Authors have declared no conflict of interest.

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