Journal of the Hellenic Veterinary Medical Society

Vol 70, No 1 (2019)

A Comparative Study of Seminal Plasma and Blood Serum Macro and Trace Elements in the the Breeding (October) and the Non-Breeding (April) Seasons in Merino Ram

B. BULBUL, P. P. AKALIN, N. BAŞPINAR, M. N. BUCAK, M. KIRBAŞ, C. ÖZTÜRK, S. GÜNGÖR, K. AKBULUT

doi: 10.12681/jhvms.20341

To cite this article:

BULBUL, B., AKALIN, P. P., BAŞPINAR, N., BUCAK, M. N., KIRBAŞ, M., ÖZTÜRK, C., GÜNGÖR, S., & AKBULUT, K. (2019). A Comparative Study of Seminal Plasma and Blood Serum Macro and Trace Elements in the the Breeding (October) and the Non-Breeding (April) Seasons in Merino Ram. *Journal of the Hellenic Veterinary Medical Society*, *70*(1), 1365–1372. https://doi.org/10.12681/jhvms.20341

J HELLENIC VET MED SOC 2019, 70(1): 1365-1372
ΠΕΚΕ 2019, 70(1): 1365-1372

Research article
Ερευνητικό άρθρο

A Comparative Study of Seminal Plasma and Blood Serum Macro and Trace Elements in the the Breeding (October) and the Non-Breeding (April) Seasons in Merino Ram

B. Bulbul[1], PP. Akalın[2], N. Başpınar[3], MN. Bucak[4], M. Kırbaş[1], C. Öztürk[5], Ş. Güngör [6], K. Akbulut[1]

[1]*Bahri Dağdaş International Agricultural Research Institude, Konya/Turkey,*

[2]*Hatay Mustafa Kemal University, Veterinary Faculty, Department of Biochemistry, Hatay/Turkey,*

[3]*Selcuk University, Veterinary Faculty, Department of Biochemistry, Konya/Turkey,*

[4]*Selcuk University Veterinary Faculty, Department of Reproduction and Artificial Insemination, Konya/Turkey,*

[5]*Aksaray University Veterinary Faculty, Department of Reproduction and Artificial Insemination, Aksaray/Turkey,*

[6] *Mehmet Akif Ersoy University, Department of Reproduction and Artificial Insemination, Burdur, Turkey*

ABSTRACT. In this study, it was aimed to investigate the concentrations of macro and trace elements in seminal plasma and blood serum in the breeding (October) and the non-breeding (April) seasons in Merino Ram. Nineteen Merino Rams, aged 18-24 months, were involved in the study. Blood (once) and ejaculate samples (6 replicates) were taken in the breeding (October) and the non-breeding (April) seasons. Blood serum, seminal plasma and diet Calcium, Sodium, Potassium, Magnesium, Phosphorus, Sulfur, Zinc, Selenium, Chrome, Manganese, Nickel, Molybdenum and Boron concentrations were determined by ICP-AES. In blood serum, Sodium and Selenium concentrations were higher ($p<0.05$ and 0.001, respectively) in the the breeding season than in non-breeding season, whereas Potassium, Chromium and Boron concentrations were lower ($p<0.05$, 0.001 and 0.001, respectively) in the breeding season than in the non-breeding season. In seminal plasma Calcium, Sodium, Zinc and Manganese concentrations were higher ($p<0.05$, 0.001, 0.01 and 0.05, respectively) in the breeding season than in the non-breeding season, whereas Phosphorus, Chrome, Molybdenum and Boron concentrations were lower ($p<0.001$, 0.001, 0.05 and 0.001, respectively) in the breeding season than in the non-breeding season. No difference was detected regarding the other elements. The higher levels of Cr and B in the non-breeding season compared to the breeding season both in serum and seminal plasma, regardless of diet intake, suggest that these elements may play a crutial role on male fertility in Merino Ram.

Keywords: Merino ram, seminal plasma, elements, breeding season, non-breeding season.

Corresponding Author:
Pınar Peker Akalın, Department of Biochemistry, Faculty of Veterinary Medicine, Mustafa Kemal University, 31040, Hatay, Turkey.
Email address: ppakalin@mku.edu.tr, pinarpekerakalin@gmail.com

Date of initial submission: 22-03-2018
Date of revised submission: 03-07-2018
Date of acceptance: 20-07-2018

INTRODUCTION

Seasonal pattern including climate, humidity and daylight length are the environmental factors that influence the reproductive activity of small ruminants, causing seasonal changes in testicular size, weight, secretion, sperm production and mating activity (Folch et al., 1984; Gündoğan, 2006). Seasonal variation in sperm quality has been reported in rams of many breeds (Gündoğan, 2006; Marti et al., 2007; Mickelsen et al., 2006). The seminal plasma, which is a complex mixture of the secretions originating from the seminiferous tubules, tubuli recti, rete testis, ductuli efferentes and epididymis, serve as a nutrient medium in which maturation of the developing spermatozoa takes place (Mann, 1964). Moreover, it affects sperm morphology, motility, acrosome reaction and fertility (Mann and Lutwak-Mann, 1981). The chemical composition and the functions of seminal plasma vary among species. Cations like sodium (Na+) and potassium (K+) in the seminal plasma establish the osmotic balance (Zamiriand Khodaei, 2005) while calcium (Ca2+) is reported to stimulate steriodogenesis in leydig cells (Henricks, 1991). Trace elements are essential for the function of various enzymes and other proteins. Seminal plasma contains several trace elements that play important roles in the semen function, including sperm metabolism and capacitating, and in the acrosome reaction.

Zinc (Zn), selenium (Se), iodine (I), copper (Cu) and manganese (Mn) are trace elements that are reported to affect parameters of fertility (Leonard-Marek, 2000). Zinc is an important micronutrient for health and is known to play a major role in the semen ejaculation as well as to be a cofactor for the DNA - binding proteins. The total content of Zn in human semen is very high and is found to have critical role in the spermatogenesis (Endre et al., 1999).

Calcium is needed for stimulation of steriodogenesis in Leydig cells including sperm maturation and plays a vital role in the regulation of the motility, capacitation, hyperpolarisation and chemotaxis (Carlson et al., 2003; Suares and Ho, 2003). Magnesium (Mg) is reported to be in high concentrations in the prostate gland and may play a role in sperm motility (Edorh et al., 2003). Sodium is also reported to be present in the human seminal plasma at higher concentrations (Jeyendran and Van Der Ven, 1989). Nickel (Ni) revealed negative effects on the structure and function of testis, seminal vesicle, and prostate gland in mice (Pandey and Srivastava, 2000). Selenium is associated with albumin, glutathione peroxidase (GPx) and selenoprotein (Awadeh et al., 1998) and Se dependent enzymes protect membranes from oxidative damage, also its deficiency affected sperm quality (Leonard-Marek, 2000).

In man, the accurate contents of macro- and trace elements have been reported in detail. Abnormal levels of ionised Ca, Mg, Zn (Pandy et al., 1983) and Cu (Stanwell-Smith et al., 1983) in seminal plasma are correlated with infertility in man. On the other hand, only a few studies have been published on serum and seminal plasma macro and trace element status in animals. The breeding season and polygamy of the ram puts it in a place apart from other species that his nutrient requirements (like macro and trace elements) for semen production will be relatively high over a short breeding season (Kendall et al., 2000).

To our knowledge, there was no reported study on blood serum and seminal plasma macro and trace element status in Merino Rams comparing breeding and non-breeding seasons. Thus, the objective of the study was to investigate the concentrations of blood serum and seminal plasma macro and trace elements in the breeding (October) and the non-breeding (April) seasons in Merino Rams.

MATERIAL AND METHODS

Semen and blood samples from 19 Merino Rams (1,5-2 years of age) were used in the study. The rams, belonging to the Bahri Dağdaş International Agricultural Research Institute, Konya-Turkey (located at 37.857063 north latitude and 32.567036 east longitude), were maintained under uniform feeding, housing and lighting conditions within breeding and non-breeding seasons. Rams were fed with a ration composed of alfafla hay, concentrated feed, corn silage and dried grape, had ad libitum access to fresh water. The study was approved by Bahri Dağdaş International Agricultural Research Institude, Local Animal Research, Ethics Commette (No: 22.07.2013/2).

Ejaculates were collected from the rams using an

artificial vagina, in the breeding (October) and the non-breeding season (April) as 6 replicates (with an interval of 1 day) according to AI standard procedures (n=112 in the breeding and non-breeding seasons each, 2 samples were failed to collect). The ejaculates were cooled at 4 oC immediately after collection and centrifuged at 800 g x 10 min at 4 oC. Seminal plasma was seperated from spermatozoa for analysis in two hours. Blood samples were collected from the jugular vein at the beginning of the sperm collection, once in each season (breeding and non-breeding) . Serum and seminal plasma samples were stored at -70 oC until the analysis of macro and trace element concentrations. Starting 15 days prior to blood and sperm sampling, feed samples (n=8 for each season) were taken with an interval of 1 week.

Serum, seminal plasma and feed macro and trace element concentrations were determined with ICP-AES (Inductively Coupled Plasma Atomic Emission Spektrometer- Varian-Vista Model) using referance material European Referance Materials-LGC (ERM DA120a, Teddington, UK).

The samples of serum and seminal plasma were diluted with deionized water (total volume 1 ml) and 5 ml % 65 HNO3 + 2 ml H2O2 (Merck) was added before digestion in the microwave oven (CEM MarsXpress, Matthews, NC, USA) at 210 oC. Food samples were digested with 7 ml % 65 HNO3 + 3 ml H2O2. The flame conditions were those recommended by the instrument manufacturer for Ca, Na, K, Mg, P, S, Zn, Se, Cr, Mn, Ni, Mo, B (wavelength 393.366, 589.592, 766.490, 279.553, 213.618, 181.971, 213.856, 196.026, 267.716, 257.61, 231.604, 202.03, 249.773 nm and detection limit 0.01, 0.2, 0.5, 0.01, 10, 9, 0.3, 5, 0.5, 0.05, 0.3, 0.8 and 0.07 ppb respectively). All data was obtained using 10 second integration times based on 3 standard deviations and in general compromize conditions were used. Analyzing reference material ERM-DA120a tested the reproducibility of the method. Referance values for Se and Zn were given to be 64.1 and 658 ppb respectively in the procedure. In this study, Se and Zn levels were determined as 52 and 665 ppb, respectively.

STATISTICS

Results were expressed as the mean±S.E.M. t-test was used to determine significance between groups. Correlation between blood and seminal plasma parameters were performed with Pearson correlation. P values below 0.05 were considered to be significantly different.

RESULTS

Blood serum, seminal plasma and diet element concentrations in the breeding and non-breeding seasons were given in Table 1, 2 and 3. In blood serum, Na ($p<0,05$) and Se ($p<0,001$) levels were higher, wheras K ($p<0,05$), Cr and B levels were lower, in the breed-

Table 1: Table 1. Blood Serum Element Concentrations in Merino Rams.
During Breeding and Non-Breeding Season (ppm) (n=19) (mean±S.E.M.)

Parameter	Breeding (October)	Non-Breeding (April)	P
Ca	78.13±1.98	73.82±1.55	-
Na	1354.32±98.57	1094.76±23.57	<0.05
K	199.88±3.29	217.68±3.93	<0.05
Mg	25.74±0.57	24.87±0.57	-
P	171.74±10.31	141.50±3.28	-
S	670.78±10.38	655.73±9.57	-
Zn	0.635±0.279	0.599±0.025	-
Se	0.235±0.019	0.150±0.013	<0.001
Cr	0.041±0.007	0.069±0.005	<0.001
Mn	0.011±0.001	0.010±0.001	-
Ni	0.011±0.001	0.011±0.002	-
Mo	0.023±0.002	0.018±0.001	-
B	0.889±0.008	2.512±0.225	<0.001

Table 2: Seminal Plasma Element Concentrations in Merino Rams.
During Breeding and Non-Breeding Season (ppm) (n=112) (mean±S.E.M.).

Parameter	Breeding (October)	Non-Breeding (April)	P
Ca	86.72±2.43	79.06±2.01	<0.05
Na	1243.02±19.53	1063.82±14.87	<0.001
K	739.48±16.70	729.11±10.92	-
Mg	57.45±1.412	57.39±1.370	-
P	1798.41±41.10	2122.55±39.90	<0.001
S	295.70±7.05	238.67±6.12	-
Zn	3.812±0.363	2.706±0.0690	<0.01
Se	0.406±0.030	0.389±0.025	-
Cr	0.129±0.007	0.164±0.007	<0.001
Mn	0.063±0.008	0.041±0.028	<0.05
Ni	0.043±0.006	0.048±0.0004	-
Mo	0.022±0.002	0.026±0.002	<0.05
B	3.100±0.120	6.463±0.329	<0.001

ing season compared to the non-breeding season. In seminal plasma, Ca ($p<0,05$), Na ($p<0,001$), Zn ($p<0,01$) and Mn($p<0,05$) levels were higher, wheras P ($p<0,001$), Cr ($p<0,001$), Mo ($p<0,05$) and B ($p<0,001$) levels were lower, in the breeding season compared to the non-breeding season. No difference was determined regarding the other elements.

Table 3: Daily Intake of Elements of Rams During Breeding and Non-Breeding Seasons (ppm/ram/day) (n=8).

Parameter	Breeding (October)	Non-Breeding (April)
Ca	16667.78	12789.92
Na	1809.37	348.69
K	25815.63	26659.09
Mg	3125.45	3888.26
P	4735.54	3348.31
S	3323.08	2555.01
Zn	123.33	29.41
Se	2.065	2.789
Cr	2.239	1.879
Mn	111.70	75.26
Ni	5.409	4.035
Mo	5.227	4.178
B	40.89	53.56

There was no significant correlation ($p>0,05$) between blood serum and seminal plasma elements both in the breeding and the non-breeding seasons (Table 4 and 5).

DISCUSSION

There has been little published work on seminal plasma macro and trace element status of ram. To our knowledge, the concentrations of elements regarding the breeding and the non-breeding seasons in rams and other small ruminants have not been reported.

Seminal plasma and blood serum Zn concentrations were reported to be 488.6±76.4 µg/dl and 75.6±8.3 µg/dl respectively in Merino Ram, by Başpınar et al., (1998). Antaplı, (1990) reported blood serum Zn concentrations to be 32.5-150 µg/dl in Merino sheep and our results are in good agreement with Antaplı (1990) . Zinc is an essential element for production of sex hormones, attachment of head to tail in spermatozoa and its deficiency results in disorders of testes development and spermatogenesis (Saaranen et al., 1987; Cigankova et al., 1998). In this study, serum Zn concentrations were not significantly different (0.635±0.279 and 0.599±0.025 ppm in the breeding and the non breeding, respectively) between the seasons whereas seminal plasma Zn concentrations were higher in the breeding than in the non-breeding season (3.812±0.363 and 2.706±0.0690 ppm, $p<0.001$). The breeding season and polygamy of the ram may contribute to the fact that requirements for semen production will be relatively high in the breeding season and thus the higher Zn concentrations in seminal plasma will be required for the duration of

Table 4: Correlations (r-values) of blood serum and seminal plasma elements in the breeding season in Merino rams.

Breeding Season	Blood Ca	Blood Na	Blood K	Blood Mg	Blood P	Blood S	Blood Zn	Blood Se	Blood Cr	Blood Mn	Blood Ni	Blood Mo	Blood B
SP Ca	0,178												
SP Na		0,300											
SP K			-0,373										
SP Mg				0,097									
SP P					0,170								
SP S						0,421							
SP Zn							0,035						
SP Se								-0,001					
SP Cr									0,234				
SP Mn										-0,079			
SP Ni											-0,172		
SP Mo												-0,141	
SP B													0,263

Table 5. Correlations (r-values) of blood serum and seminal plasma elements in the non-breeding season in Merino rams.

Non-breeding season	Blood Ca	Blood Na	Blood K	Blood Mg	Blood P	Blood S	Blood Zn	Blood Se	Blood Cr	Blood Mn	Blood Ni	Blood Mo	Blood B
SP Ca	0,178												
SP Na		0,234											
SP K			-0,244										
SP Mg				-0,219									
SP P					-0,132								
SP S						0,218							
SP Zn							0,211						
SP Se								-0,197					
SP Cr									-0,292				
SP Mn										0,189			
SP Ni											-0,175		
SP Mo												0,053	
SP B													0,182

spermatogenesis (Kendall et al., 2000). The lower concentrations of Zn in the seminal plasma of infertile men compared to fertile individuals (Şeren et al., 2002) may suggest that higher concentrations of Zn in breeding season points out the importance of Zn in spermatogenezis and male fertility.

Kaneko, (2008) reported serum Ca concentrations to be 115-128 mg/L which represent higher levels from our results (78.13±1.98 and 73.82±1.55 ppm). In the present study, there was no difference in serum Ca concentrations between seasons, while seminal plasma Ca concentration was higher ($p<0.05$) in the breeding season than in the non-breeding season. The higher concentration in the breeding season may be attributed to the important role of Ca on sperm metabolism, motility, vitality, capacitation, chemotaxis and acrosome reaction (Thomas and Meizel, 1988; Carlson et al., 2003; Suarez and Ho, 2003).

Magnesium concentrations in blood serum (25.743±0.573 and 24.872±0.574 ppm, in the breeding and the non-breeding seasons, respectively) was in good agreement with (Al-Noaemi, 2007) (24.7±11.8 mg/L) in sheep. No data was found about seminal plasma Mg concentrations in ram. Magnesium is suggested to be in high concentrations in the prostate gland and is released into the semen during ejaculation. It is thought to play a role in spermatogenesis (Edorh et al., 2003). However, there was no significant difference regarding Mg concentrations between the seasons either in serum or in seminal plasma.

In adult mice, oral Mo exposure at different doses affected sperm parameters, including sperm motility, sperm count, and morphology; it increased the parameters at a moderate dose (25 mg/L), where negatively affected at high doses (≥ 100 mg/L) (Zhai et al., 2013). Marques et al., (2011) reported sheep serum Mo concentrations to be 0.31±0.16 µmol/L (0.0126-0.0470 mg/L) which are similar with our results (0.023±0.002 and 0.018±0.001 ppm in the breeding and the non-breeding seasons, respectively). No research was obtained regarding seminal plasma Mo concentrations in rams and small ruminants. In this study we could not determine any significant difference in serum and seminal plasma Mo concentrations between the seasons evaluated.

Zemanova et al., (2007) reported semen Ni concentrations to be 0.30 mg/kg in ram. In this study, the lower concentration of Ni in seminal plasma (0.043±0.006 and 0.048±0.0004 ppm in the breeding and the non-breeding season, respectively) may suggest that much of Ni in semen is contained in spermatoza. Ni deprivation in rat was reported to impair reproductive performance, decrease spermatozoa motility in epididymis (Yokoi et al., 2003). However in this study, Ni concentration did not differ between

the breeding and the non-breeding seasons neither in serum nor seminal plasma.

Blood serum Se concentrations were similar to those reported by Ghany-Hefnawya et al., (2007). Se concentration in the semen has been reported in bull and ram and Se is a component of GPx. Glutathione peroxidase in seminal plasma has been shown to have significant positive correlation with sperm number (Smith et al., 1979). Selenium is reported to be actively incorporated into the developing spermatozoa of bulls (Smith et al., 1979) and rams (Pond et al., 1983). Semen concentration of Se has been associated with sperm quality. However, serum Se did not affect sperm quality and oxidative DNA damage in human sperm (Xu et al., 2001). Pirinçi et al., (1999) reported semen Se concentrations to be 0.39-0.41 ppm in human. In this study, seminal plasma Se concentrations were 0,406 ± 0,030 and 0,389 ± 0,025 ppm in the breeding and the non-breeding season, respectively. It is reported that more than 85% of the Se is in the seminal plasma in man (Bleau et al., 1984). No data was obtained about seminal plasma Se concentrations in ram, but the agreement of seminal plasma and semen concentrations may reflect to the fact that much of the semen Se is in the seminal plasma as appeared in men. In this study, serum Se concentration was higher ($p<0.001$) in the breeding season than in the non-breeding season but, there was no difference in seminal plasma Se levels between the seasons. Considering with the diet Se levels (Higher in the non-breeding season than the breeding season, Table 3.), Se absorption seem to be lower in non-breeding season, because Se concentrations were lower in the non-breeding season compared to breeding season in blood serum, Table 1), in this study. We may suggest that, the absorption of Se seem to be at an optimal range for blood levels. Also, the role of Se in blood, regardless of seminal plasma in breeding season can be discussed. Sperm motility was reported to be maximal at semen Se levels ranging between 50 and 69 ng/ml and motility was decreased above and below this range. This result suggests an optimal range for semen Se (Bleau et al., 1984) ,regardless of serum status. However this relation is not clear.

Both serum and seminal plasma Cr concentrations were higher ($p<0.001$) in non-breeding season than in breeding season while diet Cr levels were lower in the non-breeding season than in the breeding season (Table 1, 2, and 3). The lower intake of Cr with diet and higher serum and seminal plasma concentrations in non-breeding season suggests different mechanisms may be involved in the absorbtion and distribution of this element. Chromium compounds are reported to induce oxidative stress leading to tissue damage (Stohs et al., 2001). Various studies have shown that hexavalent Cr (+6) caused testicular atrophy, reduced sperm motility and number, increased the number of abnormal sperm in adult rats and mice (Ernst, 1990; Saxena et al., 1990; Ernst and Bonde, 1992). Administration of curcumin (antioxidant) to Cr (+6)-treated rats prevented the Cr(+6)-induced spermatogenic damage, reduced testosterone level, decreased sperm count and generation of free radicals. Testicular tissue, which has high content of polyunsaturated membrane lipids, is a target for metal-induced oxidative stres (Acharya et al., 2004). However, the roles of this element in ram fertility needs further investigation.

Serum B concentrations in sheep were reported to be 1-1.5 ppm (Miyamoto et al., 2000) which is in agreement with our results. To our knowledge, seminal plasma B concentration in ram was not reported. In this study, B concentrations in seminal plasma (3.100±0.120 and 6.463±0.329 ppm, in breeding and non-breeding seasons, respectively) were much higher than in blood serum (0.889±0.008 and 2.512±0.225 ppm in breeding and non-breeding seasons, respectively) indicating the importance of this element for male reproduction. Tarasenko et al., (1972) and Krasovskil et al., (1976) reported that workers exposed to borate dusts and cadmium exhibited reduced sperm count and impaired sexual activity. High dose and long term (38 weeks) administration of boric acid in dogs caused testicular degeneration, including spermatogenic failure and atrophy of the seminiferous epithelium (Weir and Fisher, 1972). Short- and long-term oral exposures to boric acid or borax in laboratory animals have demonstrated that the male reproductive tract is target of boron toxicity. Testicular lesions have been reported in rats administered oral boric acid or borax (Weir and Fisher, 1972; Truhaut et al., 1964; Lee et al., 1978). In this study, both serum (0.889±0.008 and 2.512±0.225 ppm in breeding season and non-breeding season, respective-

ly) and seminal plasma (3.100±0.120 and 6.463±0.329 ppm in breeding season and non-breeding season, respectively) B concentrations were lower ($p<0.001$) in breeding season compared to non-breeding season. The higher levels of diet B concentrations in non-breeding season (53,56 ppm/ram/day) compared to breeding season (40,89 ppm/ram/day) may explain this difference to a certain degree. The dramatically high concentrations of B in non-breeding season may contribute to the lower semen quality. Like Cr, the exact mechanism of absorbtion from intestine, distribution and transfer from the circulating blood into the seminal plasma of B is unclear. The understanding of the role of these elements in non-breeding season in ram needs further investigations.

CONCLUSION

The determination and comparison of macro and trace elements in blood serum and seminal plasma in breeding and non-breeding seasons in ram, in this study, can bring a new perspective, which the breeding season of ram may reveal different levels of elements in semen for spermatogenesis. In this study, the higher levels of Cr and B in non-breeding season compared to breeding season both in serum and seminal plasma, regardless of diet intake suggest that these elements may play a crutial role on male fertility in Merino Ram.

ACKNOWLEDGEMENTS

The authors thank Bahri Dağdaş International Agricultural Research Institude, Konya/Turkey, and Selcuk University, Faculty of Agriculture

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Acharya UR, Mishra M, Mishra I, Tripathy RR (2004) Potential role of vitamins in chromium induced spermatogenesis in Swiss mice. Environ Toxicol Pharmacol 15: 53-59.

Al-Noaemi S (2007) Study the levels of Calcium and Magnesium in the blood of sheep infested with tape worms. Tikrit Journal of Pure Science 14:103-105.

Antaplı M (1990) Koyunların kanında çinko seviyeleri ile karbonik anhidraz aktiviteleri arasındaki ilişkilerin araştırılması. Tr J of Veterinary and Animal Sciences 14:272-281.

Awadeh FT, Abdelrahman RL, Kincaid RL, Finley JW (1998) Effect of selenium supplements on the distribution of selenium amon serum proteins in cattle. J Dairy Sci 81:1089- 94.

Başpınar N, Kaya A, Altunok V, Güven B, Kurtoğlu F, Ataman MB (1998) Koçlarda bazi biyokimyasal parametreler ile sperm kalitesi arasindaki ilişkiler, Veteriner Bilimleri Dergisi 14:91-100.

Bleau G, Lemarbre J, Faucher G, Roberts KD, Chapdelaine A (1984) Semen selenium and human fertility. Fertil Steril 42:890-894.

Carlson AE, Westenbroek RE, Quill T, Ren D, Clapham DE, Hille B (2003) CatSper1 required for evoked Ca2+ entry and control of flagellar function in sperm. Proc Natl Acad Sci 100: 14864-14868.

Cigankova V, Mesaros P, Bires J, Ledecky V, Ciganek J, Tom Ajkova E (1998) The morphological structure of the test is in stallions with zinc deficiency. Slov Vet J 23:97-100.

Edorh AP, Tachev K, Hadou T, Gbeassor M, Sanni A, Creppy EE, et al (2003) Magnesium content in seminal fluid as an indicator of chronic prostatitis. Cell Mol Biol 49:419-23.

Endre L, Beck F and Parsad A (1999) The role of zinc in human health. J Trace Elem Exp Med 3:333-375.

Ernst E (1990) Testicular toxicity following short term exposure to tri and hex-avalent chromium: an experimental study in rat. Toxicol Lett 51:269-275.

Ernst E and Bonde JP (1992) Sex hormones and epididymal sperm parameters in rats following subchronic treatment with hexavalent chromium. Hum Exp Toxicol 11:255-8.

Folch J (1984) Influence of age, photoperiodism and temperature on semen production of rams, In: Courot M, eds. The Male In Farm Animal Production, Amsterdam: Martinus Nijhoff: 141-160.

Ghany-Hefnawya Abd El, Lopez-Arellanoa R, Revilla-Vazqueza A, Ramırez-Bribiescab E, Tortora-Perez J (2007) The relationship between fetal and maternal selenium concentrations in sheep and goats. Small Ruminant Research 73:174-180.

Gündoğan M (2006) Some reproductive parameters and seminal plasma constituents in relation to season in Akkaraman and Awassi Rams. Turk J Vet Anim Sci 30:95-100.

Henricks DM (1991) Biochemistry and physiology of the gonadal hormones. In: (eds: Cupps PT . Reproduction in Domestic animals, 4th ed, San Diego: Academic Press). 100, 670.

Jeyendran RS and Van Der Ven HH (1989) Chemical constituents of human seminal plasma: relationship to fertility. Andrologia 21:423-428.

Kaneko JJ (2008) Clinical Biochemistry of Domestic Animals, California, USA: Academic Press.

Kendall NR, McMullen S, Green A, Rodway RG (2000) The effect of a zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. Animal Reproduction Science 62: 277-283.

Krasovskil GN, Varshavskays SP, Borisov AL (1976) Toxic and gonadotropic effects of cadmium and boron relative to standards for these susbstances in drinking water. Envrion Health Perspect 13:69-75.

Lee IP, Sherins RJ, Dixon RL (1978) Evidence for induction of germinal

aplasia in male rats by environmental exposure to boron. Toxicology and applied pharmacology 45: 577-590.

Leonard-Marek S (2000) Why do trace elements hace an influence on fertility? Tierarztliche Praxis Ausgabe Grobtiere 28:60-65.

Mann T (1964) The biochemistry of semen and of the male reproductive tract. London: Methuen & Co LTD.

Mann T and Lutwak-Mann C (1981) Male ReproductiveFunctionand Semen. Berlin: Springer-Verlag: 495.

Marques AVS, Soares PC, Riet-Correa F, Da Mota OI, Silva TLA, Soares FA (2011) Hepaticos de cobre, ferro, molibdenio ezincoemovinos e caprinos no estado de Pernambuco. Pesq Vet Bras 31:398-406.

Marti E, Mara L, Marti JI,. Muino-Blanco T, Cebrian-Perez JA (2007) Seasonal variations in antioxidant enzyme activity in ram seminal plasma. Theriogenology 67:1446-1454.

Mickelsen WD, Paisley LG and Dahmen JJ (2006) Seasonal variations in scrotal circumference, sperm quality, and sexual ability in rams. J American Vet Med Assoc 181:376-80.

Miyamoto S, Sutoh M, Shiomoto A, Yamazaki S, Nishimura K, Yonezawa C, Matsue H, Hoshi M (2000) Determination of boron in animal materials by reactor neutron induced prompt gamma-ray analysis. J Radioanalytical and Nucl Chem 244: 307-309.

Pandey R and Srivastava SP (2000) Spermatotoxic effects of nickelin mice. Bull Environ Contam Toxicol 64:161-167.

Pandy VK, Parmeshwaran M and Soman SD (1983) Concentrations of morphologically normal, motile spermatozoa: Mg, Ca and Zn in the semen of infertile men. Sci Total Environ 27:49- 52.

Pirinçci I, Tanyildizi S, Ateşşahin A, Bozkurt T (1999) Koçlarda selenyum zehirlenmesinin sperma üzerine olan etkilerinin araştırılması. Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi 13:73-78.

Pond FR, Tripp MJ, Wu ASH, Whanger PD, Schmitz JA (1983) Incorporationof selenium-75 into semen and reproductive tissues of bulls and rams. J Reprod Fertil 69:411-418.

Saaranen M, Suistomaa U, Kantola M, Saarikoski S, Vanha-Perttula T (1987) Lead, magnesium, selenium and zinc in human seminal fluid-comparison with semen parameters and fertility. Hum Reprod 2:475-479.

Saxena DK, Murthy RC, Lal B, Srivastava RS, Chandra SV (1990) Effect of hexavalent chromium on testicular maturation in the rat. Reprod Toxicol 4:223-228.

Şeren G, Kaplan M and Ibar HA (2002) Comparative study of human seminal plasma and blood serum trace elements in fertile and infertile men. Analytical Letters 35:1785-1794.

Smith DG, Senger PL, McCutchan JF, Landa CF (1979) Selenium and glutathioneperoxidase distribution in bovine semen and selenium-75 retention by the tissues of the reproductive tract in the bull. Biol Reprod 20:377-383.

Stanwell-Smith R, Thompson SG, Haines AP, Ward RJ, Cashmore G, Stedronska J, et al. (1983) A comparative study of zinc, copper, cadmium, and lead levels in fertile and infertile men. Fertil Steril 40:670-677.

Stohs JS, Bagchi D, Hassoun E, Bagchi M (2001) Oxidative mechanism in the toxicity of chromium and cadmium ions. J Environ Pathol Toxicol Oncol 20:77-88.

Suarez SS and Ho HC (2003) Hyperactivated motility in sperm. Reprod Domest Anim 38:119-124.

Tarasenko NY, Kasparov AA, Strongina CM (1972) Effect of boric acid on the generative function in males. Giginea Truda Professionalnye Zabolevaniya 11:13-16.

Thomas P and Meizel S (1988) An influx extra calcium is required for initiation of the human sperm acrosome reaction induced by human follicular fluid. Gamete Res 20:397-411.

Truhaut R, Phu-Lich N, Loisillier F (1964) Effects of the repeated ingestion of small doses of boron derivatives on the reproductive functions of the rat. Comptes Rendus de l'Académie des Sciences 258: 5099-5102.

Weir RJ, Fisher RS (1972) Toxicologic studies on borax and boric acid. Toxicology and Applied Pharmacology 23: 351-64.

Xu D, Ong C, Shen H (2001) The associations between concentration of selenium in semen and sperm parameters as well as oxidative DNA damage in human sperm. Chinese Journal of Preventive Medicine 35: 394-396.

Yokoi K, Uthus EO and Nielsen FH (2003) Nickel deficiency diminishes sperm quantity and movement in rats. Biological Trace Element Research 93: 141-153.

Zamiri MJ and Khodaei HR (2005) Seasonal thyroidal activity and reproductive characteristics of Iranian fat-tailed rams. Anim Reprod Sci 88:245-255.

Zemanova J, Lukac N, Massanyi P, J. Trandz J, Burocziova M, Nad P (2007) Nickel seminal concentrations in various animals and correlation to spermatozoa quality. J Vet Med 54:281-286.

Zhai XW, Zhang YL, Qi Q , Bai Y, Chen XL, Jin LJ, Ma XG, Shu RZ, Yang ZJ, Liu FJ (2013) Effects of molybdenum on sperm quality and testis oxidative stress . Systems Biology in Reproductive Medicine, 59: 251–255.