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Dear Scientist,

The second International Congress on Advances in Veterinary Sciences & Technics (icavst) was organized in Kiev, Ukraina. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of veterinary area and also to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

We would like to send our special thanks to Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers for their special efforts. and finally the most importantly I would like to thank to all the participants individually who came from far away to join this conference.

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Abbreviation

FVM: Faculty of Veterinary Medicine

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FULLTEXTS

The effect of melatonin on insulin, glucose, hemoglobin A1c and C-reactive protein levels in rats with cerulein-induced acute pancreatitis

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Abstract:

It was investigated the protective effects of melatonin on insulin, glucose, hemoglobin A1c and C-reactive protein levels in rats with cerulein-induced acute pancreatitis. The animals in the control group do not have any application. The animals in the melatonin group were administrated 2 intraperitoneal injections of melatonin (50 mg/kg) at 2-hour intervals. Acute pancreatitis was induced by intraperitoneal administration at first dose of 50 µg/kg and then second dose of 25 µg/kg cerulein at 2-hour intervals in the animals in the acute pancreatitis group. Acute pancreatitis was induced in the same way in animals in melatonin-treated acute pancreatitis group and the rats was received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection. At the end of the study, insulin, glucose, hemoglobin A1c (HbA1c) and C-reactive protein (CRP) levels were determined in blood samples. Plasma insulin and glucose levels in acute pancreatitis group significantly increased compared to the control group ($p<0.05$). In melatonin-treated acute pancreatitis group, plasma insulin levels were significantly higher ($p<0.05$) and plasma glucose levels were significantly lower when compared to acute pancreatitis group ($p<0.05$). There was no significant difference in HbA1c level among groups. Whereas CRP level was significantly higher in acute pancreatitis group than control group ($p<0.05$), pretreatment of melatonin in rats with acute pancreatitis significantly decreased CRP level compared to the pancreatitis group ($p<0.05$). In conclusion, melatonin administration may alleviate the abnormalities in insulin, glucose and CRP levels caused by acute pancreatitis.

Key words: Cerulein, acute pancreatitis, melatonin, HbA1c, CRP

Serulein ile Akut Pankreatit Oluşturulan Ratlarda Melatoninin İnsülin, Glikoz, Hemoglobin A1c ve C-reaktif protein Düzeyleri Üzerine Etkileri

ÖZET

Bu çalışmada serulein ile akut pankreatit oluşturulan ratlarda melatoninin insülin, glikoz, hemoglobin A1c ve C-reaktif protein düzeyleri üzerine etkileri araştırılmıştır. Kontrol grubundaki hayvanlara herhangi bir uygulama yapılmamıştır. Melatonin grubundaki hayvanlara 2 saat aralıkla 50 mg/kg melatonin 2 kez intraperitoneal olarak uygulandı. Akut pankreatit oluşturulan gruptaki hayvanlarda 2 saat aralıkla ilk dozda 50 µg/kg, ikinci dozda 25 µg/kg serulein intraperitoneal uygulanarak akut pankreatit oluşturuldu. Akut pankreatitli ratlara melatonin ön uygulaması yapılan gruptaki hayvanlarda ise aynı yolla akut pankreatit oluşturuldu ve her serulein enjeksiyonundan 30 dakika önce hayvanlara 50 mg/kg melatonin intraperitoneal olarak verildi. Çalışmanın sonunda, kan örneklerinde insülin, glikoz, hemoglobin A1c (HbA1c) ve C-reaktif protein (CRP) düzeyleri belirlendi. Akut pankreatit grubunda plazma insülin ve glukoz düzeyleri kontrol grubuna göre önemli oranda artış gösterdi ($p<0.05$). Akut pankreatit öncesi melatonin uygulanan grupta plazma insülin seviyesi akut pankreatit grubuna göre daha yüksek ($p<0.05$), plazma glikoz seviyesi ise anlamlı olarak daha düşük bulundu ($p<0.05$). HbA1c düzeyinde gruplar arasında önemli farklılık bulunmadı. CRP düzeyi akut pankreatitli grupta kontrol grubuna göre anlamlı olarak yüksek bulunurken ($p<0.05$), akut pankreatit oluşturulan ratlara melatonin ön uygulaması CRP düzeyini pankreatitli gruba göre anlamlı olarak düşürdü ($p<0.05$). Sonuç olarak, melatonin uygulaması akut pankreatitin neden olduğu insülin, glikoz ve CRP seviyelerindeki anormallikleri hafifletebilir.

Anahtar Kelimeler: Serulein, akut pankreatit, melatonin, HbA1c, CRP

INTRODUCTION

The incidence of acute pancreatitis increases in humans and the incidence has been doubled since the 1960s. This disease, which is characterized by significant systemic inflammatory response, leads to various degrees of organ dysfunction. The mortality rate in patients with multi-organ failure is as high as 50% (Baddeley et al., 2011; Stevenson and Carter, 2013). In terms of veterinary medicine, the processes and pathophysiology in pancreatitis, which are more common in

cats and dogs, are similar to those in humans (Xenoulis et al., 2008; Richard et al., 2014). The main causes of acute pancreatitis include alcohol, hypertriglyceridemia, hypercalcemia, autoimmune pancreatitis, pancreatic canal obstruction, dysfunction of oddi sphincter, ascariasis, hereditary pancreatitis, cystic fibrosis, viral infections (HIV, adenovirus, etc.), ischemia, embolism and vasculitis (Baddeley et al., 2011; Stevenson and Carter, 2013).

In recent years, ascorbic acid, N-acetyl-cysteine, green tea extracts and antioxidant

combinations of organic selenium, β -carotene, vitamin C and vitamin E have been used to decrease the degree of acute pancreatitis. Lycopene, resveratrol and glycyrrhizin have also been used in various studies as natural compounds with antioxidant effects (Eşrefoğlu et al., 2006; Babu et al., 2009; Yu and Kim, 2014). In general, these studies suggest that oxidative stress plays an important role in the stimulation of inflammatory signaling pathways and in the pathogenesis of pancreatitis, whereas antioxidant therapy may be valuable for the prevention or treatment of this disease in terms of reducing ROS levels. However, it is emphasized that the duration and dose of antioxidant treatment should be carefully determined in order to prevent possible side effects and negative consequences (Yu and Kim, 2014). One of the substances used for this purpose is melatonin, also known as the hormone of darkness. Melatonin is produced from L-tryptophan by a four-step process in the pineal gland as an indole (L-tryptophan \rightarrow 5-hydroxytryptophan \rightarrow 5-hydroxytryptamine = serotonin \rightarrow N-acetylserotonin \rightarrow melatonin). For the synthesis of melatonin, N-acetyltransferase is considered to be a rate-limiting enzyme (Stefulj et al., 2001). The synthesis of melatonin in the pineal gland and its release into the bloodstream follow the circadian rhythm. Plasma concentration is at maximum levels during the darkness, while it is at the lowest levels during the day (Reiter, 1991; Jaworek et al., 2005).

It was aimed to determine the protective effects of melatonin on insulin, glucose, hemoglobin A1c and C-reactive protein levels in rats with cerulein-induced acute pancreatitis.

MATERIAL AND METHOD

In the study, 32 adult, male Wistar Albino rats were used. Properly living conditions (heat, humidity and light) for rats were provided during the study period. The animals were divided into four groups. All animals were fasted before at the beginning of study, while it allowed to drink water. The study protocol was approved by The Ethical Committee of Selcuk University *Experimental Medicine Research and Application Center* (Report no. 2017-16).

Group I (C) (n=6): Group I animals was not applied.

Group II (M) (n=6): Group II animals was intraperitoneally administered 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) per rat twice for two hours intervals.

Group III (AP) (n=10): Animals of group III was received two intraperitoneal injections of cerulein (Sigma-Aldrich, St. Louis, MO, USA) (50 μ g/kg and 25 μ g/kg bw, respectively) at two hours intervals.

Group IV (APM) (n=10): Animals of group IV was received two intraperitoneal injections of cerulein (50 μ g/kg and 25 μ g/kg bw, respectively) at two hours intervals and the rats was received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection.

After 12 hours from the last cerulein injection, blood samples were taken from all animals. In these blood samples, insulin, glucose, HbA1c and CRP levels were determined. Insulin, glucose, HbA1c and CRP levels were determined by using Abbott kits in Abbott-C8200 autoanalyzer.

The data obtained from the study were analyzed by one-way ANOVA (SPSS 19).

Differences among the groups were determined by Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

RESULTS

The effect of melatonin on insulin, glucose, HbA1c and CRP levels in experimentally induced acute pancreatitis were summarized in Table 1.

Table 1 The effect of melatonin on insulin, glucose, HbA1c, and CRP levels in experimentally induced acute pancreatitis (Mean \pm SE).

	Insulin (U/ml)	Glucose (mg/dl)	HbA1c (%)	CRP (mg/L)
C	9.49 $\pm 0.98^c$	86.67 $\pm 8.53^c$	3.42 ± 0.38	3.20 $\pm 0.51^c$
M	10.35 $\pm 0.53^c$	93.17 $\pm 7.19^c$	3.23 ± 0.47	3.12 $\pm 0.40^c$
AP	13.29 $\pm 0.79^b$	177.20 $\pm 7.58^a$	3.48 ± 0.47	16.35 $\pm 1.20^a$
APM	17.91 $\pm 0.76^a$	124.70 $\pm 10.33^b$	3.36 ± 0.36	12.08 $\pm 0.82^b$

^{a-c} The difference between mean values with different superscripts in the same column is significant at the $p < 0.05$ level.

DISCUSSION

Cerulein-induced acute pancreatitis causes an increase in acute phase proteins. CRP, an acute phase protein, was significantly higher in the acute pancreatitis group compared to the control group ($p < 0.05$, Table 1), whereas melatonin pretreatment to the rats with acute pancreatitis significantly decreased the CRP

level compared with the pancreatitis group ($p < 0.05$, Table 1). This increase of CRP level in the pancreatitis group can be evaluated with the increase in proinflammatory cytokines as a result of inflammation due to cerulein. In some studies, it has been reported that increases in proinflammatory cytokines (such as TNF- α and IL-6) leading to the production of more inflammatory mediators and acute phase proteins by provoke events such as inflammation processes and insulin resistance (Fontana et al., 2007; Agil et al., 2013). Significant changes in CRP levels with melatonin pretreatment are based on the reduction of the production of proinflammatory cytokines by various antiinflammatory mechanisms against inflammation and modulate release and production of cytokine in various cells and tissues, such as in liver hepatocytes (Pepys and Hirschfield, 2003; Dominguez-Rodriguez et al., 2004; Dominguez-Rodriguez et al., 2006).

In the study, HbA1c level occurred as a result of protein glycation depend on hyperglycemia was not different from control group in experimental acute pancreatitis group. With melatonin pretreatment in rats with acute pancreatitis, determined changes in HbA1c level was not significant compared to the pancreatitis group. The absence of any difference in HbA1c level in this study can be attributed to be based on acute pancreatitis

basis of the study and short term of fluctuations determined in glucose level. It is reported that glycated hemoglobin may be considered as a marker when exposed to prolonged hyperglycemia or plasma glucose levels.

Plasma insulin level and glucose level in this group due to the formation of acute pancreatitis with cerulein significantly increased compared to the control group ($p < 0.05$, Table 1). With pretreatment of melatonin to rats with acute pancreatitis, plasma insulin level was significantly higher than the pancreatitis group ($p < 0.05$, Table 1) and plasma glucose level were significantly lower than the pancreatitis group ($p < 0.05$, Table 1). The increase in insulin level due to induction of acute pancreatitis with cerulein is interpreted as a strong stimulus of insulin secretion of cerulein. It is stated that cerulein is the most potent stimulant of insulin secretion than cholecystokinin-pancreozymin, a gastrointestinal hormone (Bertaccini et al., 1969; Bertaccini et al., 1970). The findings of our study regarding insulin are consistent with the above reports related to cerulein. Increasing insulin secretion of cerulein is explained by a markedly stimulation of blood flow in the pancreatic vascular area (Dorigotti and Glasser, 1968; Bertaccini et al., 1970). Although the significant increase in plasma glucose level in the pancreatitis group compose a paradox with the increase in

insulin, there are reports that cerulein causes hyperglycemia (Bertaccini et al., 1970). Although this mechanism is not obvious, it is suggested that cerulein may directly or indirectly effect glucose metabolism. It is stated that this increase in glucose level may be related to the release of adrenal catecholamines due to pancreatitis or may be caused by an increase of glucagon concentration in the pancreatico-duodenal venous blood (Bertaccini et al., 1970). The significant increase in plasma insulin levels with pretreatment of melatonin to rats with acute pancreatitis may be attributed to the induction of insulin secretion from β -cells of Langerhans islets in pancreas by melatonin (Shima et al., 1997; Agil et al., 2012). The decrease in plasma glucose level, which is consistent with the increase in insulin levels in the melatonin-treated pancreatitis group, is considered important for the consistency of the results. The antihyperglycemic effect of melatonin is described as stimulating insulin secretion, increasing insulin sensitivity, or both (Sartori et al., 2009; Sanchez-Barcelo et al., 2010). Another pathway related to the antihyperglycemic effect of melatonin is considered as the reduction of glucagon secretion (Nagai et al., 1996a; Nagai et al., 1996b; Shima et al., 1997). The positive effect of melatonin on hyperglycemia and insulin secretion is explained as to provide the positive contribution to regeneration or proliferation of β -cells and may be related to

the improvement in the function of these cells (Kanter et al., 2006; Peschke, 2008; Agil et al., 2012).

In conclusion, melatonin administration may alleviate the abnormalities in insulin, glucose and CRP levels caused by acute pancreatitis.

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The protective effect of melatonin on some antioxidant enzymes in rats with cerulein-induced acute pancreatitis

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Abstract:

The purpose of our study was to evaluate the effect of melatonin on some antioxidant enzymes in rats with cerulein-induced acute pancreatitis. In the study, 32 adult male Wistar Albino rats were used. While the animals in the control group do not have any application, the animals in the melatonin group was administrated 2 intraperitoneal injections of melatonin (50 mg/kg) at 2-hour intervals. Acute pancreatitis was induced by intraperitoneal administration at first dose of 50 µg/kg and then second dose of 25 µg/kg cerulein at 2-hour intervals in the animals in the acute pancreatitis group. Acute pancreatitis was induced in the same way in animals in melatonin-treated acute pancreatitis group and the rats was received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection. At the end of the study, malondialdehyde (MDA), glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels were determined in blood samples taken from all animals. GPx and SOD levels were significantly lower in rats with acute pancreatitis compared to the control group ($p<0.05$), whereas the levels of GPx and SOD were significantly higher in melatonin-treated acute pancreatitis group than acute pancreatitis group ($p<0.05$). There was no significant difference in MDA level among groups. These results suggest that melatonin may have protective effects against acute pancreatitis.

Keywords: Cerulein, acute pancreatitis, melatonin, antioxidant, rats

Serulein ile Akut Pankreatit Oluşturulan Ratlarda Melatoninin Bazı Antioksidan Enzimler Üzerine Koruyucu Etkileri

ÖZET

Çalışmanın amacı serulein ile akut pankreatit oluşturulan sıçanlarda bazı antioksidan enzimler üzerine melatoninin etkisini değerlendirmektir. Çalışmada 32 yetişkin erkek Wistar Albino rat kullanıldı. Kontrol grubundaki hayvanlara herhangi bir uygulama yapılmazken, melatonin grubundaki hayvanlara 2 saat aralıkla 50 mg/kg melatonin 2 kez intraperitoneal olarak verildi. Akut pankreatit oluşturulan gruptaki hayvanlarda 2 saat aralıkla ilk dozda 50 µg/kg, ikinci dozda 25 µg/kg serulein intraperitoneal uygulanarak akut pankreatit oluşturuldu. Akut pankreatitli ratlara melatonin ön uygulaması yapılan gruptaki hayvanlarda ise aynı yolla akut pankreatit oluşturuldu ve her serulein enjeksiyonundan 30 dakika önce hayvanlara 50 mg/kg melatonin intraperitoneal olarak verildi. Çalışmanın sonunda, hayvanlardan alınan kan örneklerinde malondialdehit (MDA), glutatyon peroksidaz (GPx) ve süperoksit dismutaz (SOD) düzeyleri belirlendi. Çalışmada GPx ve SOD düzeyleri akut pankreatitli grupta kontrol grubuna göre anlamlı olarak azalırken ($p<0.05$), akut pankreatitli ratlara melatonin ön uygulaması yapılan grupta GPx ve SOD düzeyleri akut pankreatit grubuna göre anlamlı olarak yüksek bulundu ($p<0.05$). MDA düzeyinde ise gruplar arasında önemli farklılık bulunmadı. Bu sonuçlar melatoninin akut pankreatite karşı koruyucu etkileri olabileceğini göstermektedir.

Anahtar Kelimeler: Serulein, akut pankreatit, melatonin, antioksidan, rat

INTRODUCTION

Acute pancreatitis is an inflammatory condition with a clinical course that varies from mild to severe. Acute pancreatitis is generally a mild and self-limiting disease but severe necrotizing forms associated with a important mortality rate are not rare (Baddeley et al., 2011; Alhan et al., 2004). Although the molecular mechanism has not been exactly elucidated, some studies have reported that reactive oxygen species play an

important role in the early stages of this disease, regardless of the underlying etiology of acute pancreatitis (Dabrowski et al., 1999; Carrasco et al., 2013). It is reported that oxidative agents lead to the release of large amounts of cytokines and chemokines by stimulating phagocytic cells and that they cause tissue damage by direct damage to extracellular matrix and cell membranes. Also, it is stated that they cause breakdown of nucleic acids by destroying lysosomal membranes (Wong and Goeddel, 1988; Ryter

et al., 2007; Zhao et al., 2007). In addition to the notifications supporting the role of oxidative damage in pancreatitis, it is presented important findings about the possibility that oxygen-derived free radical scavengers might be useful in this disease. The ability to correct by free radical scavengers the pancreatic damage caused by the increase in free radical production and loss of antioxidant defense system in pancreatitis are among these findings. It has been suggested that the use of antioxidant enzymes leads to morphological and histological improvements in experimental acute pancreatitis (Rau et al., 2000; Sevillano et al., 2003; Hardman et al., 2005; Rahman et al., 2009). During acute pancreatitis, overproduction of reactive oxygen species (ROS) and decrement of the intrinsic antioxidant defense system capacity cause ROS accumulation, peroxidation of lipid membranes and deterioration of cell integrity in the pancreas (Jungermann et al., 1995; Dabrowski et al., 1999; Jung et al., 2010). Lipid peroxidation occurring in this process is reported to cause increment of membrane permeability and ultimately cell death (Halliwell, 1991; Sies, 1991; Qi et al. 1999).

Melatonin, a pineal secretory product, synthesized from L-tryptophan, has received increased interest due to its antioxidant and immunomodulatory properties (Jaworek et al., 2003). Melatonin, which is well tolerable

without toxic effect, has been suggested to be a powerful free radical scavenger and inhibitor of tissue damage (Teixeira et al., 2003; Peyrot and Ducrocq, 2008; Hardeland et al., 2009). Melatonin, which is generally used in pain, inflammation and anti-aging researches, is also being investigated as a protective against endogenous and exogenous oxidants and as a neuroprotector against brain damage (Barlas et al., 2004; Kireev et al., 2008; Ersahin et al., 2009; Jung et al., 2010). Because melatonin is a lipophilic substance and can easily penetrate into the cell, this indole stabilizes lipid membranes against peroxidation and protects the intracellular structures against harmful effects (Costa et al., 1995; Jaworek et al., 2005). ROS is consistently formed in certain amounts in the body and is eliminated by antioxidant defense systems within normal limits (McCord, 1985; Qi et al., 1999). Endogenous and exogenous oxidants may increase in cases of injury and infection that stress the organism (Jung et al., 2010). One of them is also pancreatitis. It is stated that ROS plays role as important mediators in early and late stages of acute pancreatitis. In the early stages of the disease, the exact site and source of free radical is not clear. In the later stages, it is reported that leukocytes are activated and it increased free radical production in the pancreas (Rau et al., 2000). These radicals can directly affect lipids and

proteins in the cells and can impair the cell membrane and function. Indirectly, free radicals affect arachidonic acid metabolism in two ways (Yamamoto et al., 1985; Farber et al., 1990). First, these radicals decrease tissue circulation by inducing thromboxane synthesis and by vasoconstriction and platelet aggregating. Second, free radicals stimulate the production of leukotriene B₄ by enhancing the activation of leukocytes and the release of lysosomal enzymes. Thus, free radicals mediate further progression of damage in the pancreas. (Tate et al., 1984; Hotter et al., 1997; Alhan et al., 2004).

The purpose of our study was to evaluate the effect of melatonin on some antioxidant enzymes in rats with cerulein-induced acute pancreatitis.

MATERIAL AND METHOD

In the study, 32 adult, male Wistar Albino rats were used. Properly living conditions (heat, humidity and light) for rats were provided during the study period. The animals were divided into four groups. All animals were fasted before at the beginning of study, while it allowed to drink water. The study protocol was approved by The Ethical Committee of Selcuk University *Experimental Medicine Research and Application Center* (Report no. 2017-16).

Group I animals (C, n=6) was no applied. Group II animals (M, n=6) was intraperitoneally administered 50 mg/kg melatonin (Sigma-Aldrich, St. Louis, MO, USA) per rat twice for two hours intervals. Animals of group III (AP, n=10) was received two intraperitoneal injections of cerulein (Sigma-Aldrich, St. Louis, MO, USA) (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals. Animals of group IV (APM, n=10) was received two intraperitoneal injections of cerulein (50 µg/kg and 25 µg/kg bw, respectively) at two hours intervals and the rats was received an intraperitoneal injection of 50 mg/kg melatonin 30 min before each cerulein injection. After 12 hours from the last cerulein injection, blood samples were taken from all animals. In these blood samples, GPx, MDA and SOD levels were determined. GPx, MDA and SOD levels were determined with ELISA (Biotek ELx800, Biotek Instrumentations, Inc, Winooski, VT, USA) using sandwich enzyme-linked immunosorbent method via commercial kits (Elabscience).

The data obtained from the study were analyzed by one-way ANOVA (SPSS 19). Differences among the groups were determined by Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

RESULTS

The effect of melatonin on GPx, MDA and SOD levels in experimentally induced acute pancreatitis were summarized in Table 1.

Table 1. The effect of melatonin on GPx, MDA and SOD levels in experimentally induced acute pancreatitis (Mean±SE).

	GPx (pg/ml)	MDA (μmol/L)	SOD (ng/ml)
C	93.82±1.09 ^a	4.22±0.72	8.91±0.72 ^a
M	95.78±1.31 ^a	3.91±0.44	9.32±0.64 ^a
AP	52.37±0.67 ^c	5.13±0.60	5.18±0.70 ^b
APM	69.14±1.04 ^b	4.80±0.72	7.64±0.62 ^a
^{a-c} The difference between mean values with different superscripts in the same column is significant at the $p<0.05$ level.			

DISCUSSION

In the study, GPx and SOD levels were significantly decreased in acute pancreatitis group compared to the control group ($p<0.05$, Table 1), while the increase in MDA level which is lipid peroxidation product was not significant. With melatonin pretreatment to rats with acute pancreatitis, GPx ve SOD levels was significantly higher than the pancreatitis group ($p<0.05$, Table 1), while an important change in MDA level was not detected. During inflammation, it is known that reactive oxygen species in various tissues have dramatically increased, antioxidant capacity is weakened in the inflammatory tissues and this contributes negatively to tissue damage (Dabrowski et al., 1999; Shi et al., 2005; Afonso et al., 2007; Jaworek et al., 2007). Melatonin can neutralize free radicals and reactive nitrogen species occurred by depending on inflammation. It is reported that it has a protective effect on many tissues against the

toxic effects of free radicals (Suzen, 2006). Melatonin, which can easily enter from the cell membranes due to its high lipophilic character, prevents membrane peroxidation as well as it protects intracellular structures from damage (Costa et al., 1995; Jaworek et al., 2007). While melatonin has been reported to have the ability to directly neutralize toxic products (Qi et al., 1999; Jaworek et al., 2003; Szabolcs et al., 2006; Jaworek et al., 2007; Jaworek et al., 2010), it is stated that it can activate antioxidant enzymes such as GPx, CAT, GSH and SOD and indirectly protect pancreatic tissue from oxidative damage by inactivation of reactive intermediates (Jaworek et al., 2003; Muñoz-Casares et al., 2006; Cöl et al., 2009; Jaworek et al., 2010). Melatonin has been reported to increase Nrf2 expression as well as molecular inhibition of proinflammatory pathways (Jung et al., 2010). It is noted that Nrf2 activates signal pathways that play a critical role in the induction of

antioxidant enzymes such as SOD, CAT and GPx (Zhu et al., 2005). In different experimental models of acute pancreatitis, melatonin administration has been shown to reduce the harmful effects of acute inflammation and to alleviate the severity of pancreatitis (Qi et al., 1999; Muñoz-Casares et al., 2006; Szabolcs et al., 2006; Sidhu et al., 2010; Jaworek et al., 2012). In this study, findings regarding to the antioxidant parameters with melatonin application are seen in accordance with the findings obtained in the above mentioned studies.

These results suggest that melatonin may have protective effects against acute pancreatitis.

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Some results of reproductive traits and milk yield of akkaraman sheep under selection for fertility and milk yield

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Abstract:

The aim of this study was to investigate some reproductive traits and milk yield of Akkaraman sheep breed. Akkaraman is a native sheep breed of Turkey. It is reared in Central Anatolia, in where steppe climate condition prevails. It is a fat - tailed sheep breed and tail weight is ranging from 4 to 6 kg. The colour is white, and sometimes black around the nose and eyes. The study was conducted in Gözlü state farm in Konya province. The data were collected from 2700 ewes during 2015 - 2017 years. The means were obtained as 79 % for lambing rate, 53 % for single birth rate, 47 % for twinning rate, 116.2 % for lamb production rate and 1.47 for litter size. The means of 120 day lactation period were 151.7 and 165.5 kg for milk yield and 1264.4 and 1390.2 g for daily milk yield in 2016 and 2017 years, respectively. Finally, it may be concluded, reproductive traits and the milk yields of Akkaraman ewes were better than literature published before. This shows that Akkaraman sheep reproductive traits and milk yields can be increased with selection studies and Akkaraman seems to be a suitable breed for milk production.

Keywords: Akkaraman, Fertility, Lactation, Milk Yield, Sheep

INTRODUCTION

Sheep are utilized for meat and milk production in most of countries. Sheep milk and its products are popular in Mediterranean and Balkan countries (Ünal et al., 2008). In Turkey, sheep breeds are generally rearing for multipurpose. Akkaraman sheep breed is a native breed of Turkey. It is raised in Central

Anatolia. It is a fat tailed sheep breed. The colour is white, and sometimes black around the nose and eyes. This study is a part of a research project concerned with the improvement of the Akkaraman sheep breed by means of pure breeding and crossbreeding (Stage 2). The aim of this part was to

improvement of milk yield of Akkaraman sheep under semi-intensive conditions.

MATERIALS and METHODS

This study was carried out in Gözlü State Farm in Konya Province. Konya is in central Anatolia, where steppe climate condition prevails. In first year data were collected from 1080 ewes for reproductive traits and 907 ewes for milk yield, and second year data were collected from 1620 ewes for reproductive traits and 1237 ewes for milk yield. Ewes were kept Gözlü State farm conditions during the study. Ewes were fed with 500 g sheep milk pellet, 500 g vetch hay and 400 g wheat stem and were grazed in the nearby grazing areas in farm. Measurements of milk yield were made on the 30th, 60th, 90th and 120th test days. Lambs were separated from ewes the night before the test day and test day milking made in the morning and the evening. Measure sensitiveness was 10 ml. Data were collected with repeated measurements on ewes and analysed using Least Squares Mixed Model Procedures of SPSS (Ünal et al., 2008).

RESULTS and DISCUSSION

Some reproductive traits of Akkaraman sheep were given in Table 1 which collected during

2015-2016 and 2016-2017 breeding seasons. The means of test day milk yields of Akkaraman sheep were given in Table 2; the means of daily milk yield and total (120 days) milk yield were given in Table 3, which were collected during 2016 and 2017 years. Generally test day, daily and total milk yields of ewes with twin births are greater than ewes with single births and statistically important. Similarly, in all test days, daily and total milk yields of ewes at older ages are greater than younger ewes and statistically important. These situations are similar to formerly reports for ewes (Akçapınar, 2000). Total milk yield was greater during 2016 and 2017 than many of former publications (Akçapınar et al., 1982; Başpınar, 1985; Küçük and Akçapınar, 1999; Akçapınar, 2000; Ünal et al., 2002). Daily milk yield was higher during 2016 and 2017 than the former publication (Kocakaya et al., 2015) of this research project concerned with the improvement of the Akkaraman sheep breed by means of pure breeding and crossbreeding (Stage 1). Total milk yield was fewer than the former publication (Kocakaya et al., 2015), it could be because of ewes were gets into dry period earlier in 2016 and 2017

Table 1. Some reproductive traits of Akkaraman sheep

Breeding season	Age	Lambing (%)	Single Birth (%)	Twinning (%)	Lamb Production (%)	Litter Size
2015-2016	2	81	80	20	96.6	1.20
	3	84	55	45	121.9	1.45
	4	84	44	56	131.0	1.56
	5	85	34	66	136.0	1.66
	6	90	44	56	140.9	1.56
	Total	84	51	49	125.3	1.49
2016-2017	2	68	66	34	90.6	1.34
	3	80	63	37	110.1	1.37
	4	76	51	49	112.3	1.49
	5	85	46	54	131.2	1.54
	6	74	50	50	111.2	1.50
	Total	76	55	45	110.1	1.45
Total of two season		79	53	47	116.2	1.47

Table 2. One-way analysis of variance (ANOVA) test of Test-Day Milk Yields in 2016 and 2017 by birth type (g) ($\bar{X} \pm S_{\bar{x}}$)

				2016		
		n	30	60	90	120
P			***	***	***	***
Birth Type	Single	460	1171.8±27.2	1659.3±37.2	1074.8±21.3	756.9±14.8
	Twin	447	1320.0±28.1	1955.8±46.1	1222.7±25.0	846.9±17.6
	Total	907	1244.8±19.7	1805.4±29.9	1147.6±16.6	801.3±11.5
				2017		
		n	30	60	90	120
P			***	***	***	**
Birth Type	Single	680	1113.6±21.8	1585.0±31.0	1273.5±24.2	981.2±20.3
	Twin	557	1252.9±25.1	1783.3±35.7	1409.0±26.8	1080.0±24.5
	Total	1237	1176.4±16.6	1674.3±23.6	1334.5±18.1	1025.2±15.7

:P<0.01; *:P<0.001

Table 3. One-way analysis of variance (ANOVA) test of Daily Milk Yield and Total Milk Yield in 2016 and 2017 by birth type ($\bar{X} \pm S_{\bar{x}}$)

				2016	
		n		Daily Milk Yield (g)	Total Milk Yield (kg)
P				***	***
Birth Type	Single	460		1177.7±23.0	141.3±2.8
	Twin	447		1353.6±27.1	162.4±3.3
	Total	907		1264.4±17.9	151.7±2.2
				2017	
		n		Daily Milk Yield (g)	Total Milk Yield (kg)
P				**	**
Birth Type	Single	680		1322.5±25.2	157.6±2.9
	Twin	557		1472.9±28.2	175.1±3.3
	Total	1237		1390.2±18.9	165.5±2.2

:P<0.01; *:P<0.001

Table 4. One-way analysis of variance (ANOVA) test of Test-Day Milk Yields in 2016 and 2017 by age (a) ($\bar{X} \pm S_{\bar{x}}$)

		2016				
	<i>n</i>	30	60	90	120	
	<i>p</i>	***	***	***	***	
	2	121	947.3±35.3	1558.2±61.7	1034.4±38.7	734.9±27.2
Age	3	272	1131.6±35.6	1553.3±50.6	1024.5±28.4	727.0±19.9
	4	336	1334.2±30.9	1969.9±48.1	1232.3±26.0	853.2±17.9
	5	123	1365.4±54.3	1982.7±96.1	1234.6±55.6	849.7±39.0
	6	55	1643.9±95.7	2194.9±125.5	1294.6±62.5	889.8±46.7
	Total	907	1244.8±19.7	1805.4±29.9	1147.6±16.6	801.3±11.5
		2017				
	<i>n</i>	30	60	90	120	
	<i>p</i>	***	***	***	***	
	2	338	930.4±26.4	1324.3±37.5	1094.3±30.9	866.3±24.4
Age	3	150	1193.4±43.8	1698.5±62.4	1346.8±48.2	1038.7±40.7
	4	257	1234.7±37.2	1757.4±52.9	1406.8±41.6	1085.3±36.2
	5	347	1276.5±29.6	1816.8±42.1	1433.7±33.3	1098.2±32.7
	6	145	1388.8±58.5	1976.7±83.2	1516.1±54.9	1121.4±49.0
	Total	1237	1176.4±16.6	1674.3±23.6	1334.5±18.1	1025.2±15.7

***: $P < 0.001$

Table 5. One-way analysis of variance (ANOVA) test of Daily Milk Yield and Total Milk Yield in 2016 and 2017 by age ($\bar{X} \pm S_{\bar{x}}$)

		2016		
	<i>n</i>	Daily Milk Yield (a)	Total Milk Yield (ka)	
	<i>p</i>	***	***	
	2	121	1113.6±40.4	133.6±4.8
Age	3	272	1118.4±31.0	134.2±3.7
	4	336	1364.6±28.1	163.7±3.4
	5	123	1367.9±59.2	164.2±7.1
	6	55	1474.6±68.6	176.9±8.2
	Total	907	1264.4±17.9	151.7±2.2
		2017		
	<i>n</i>	Daily Milk Yield (a)	Total Milk Yield (ka)	
	<i>p</i>	***	***	
	2	332	1108.8±31.2	132.9±3.7
Age	3	150	1406.1±50.0	167.2±5.9
	4	257	1461.4±42.8	174.3±5.1
	5	347	1498.1±34.1	178.2±4.1
	6	145	1613.7±61.6	189.7±6.9
	Total	1237	1390.2±18.9	165.5±2.2

***: $P < 0.001$

CONCLUSIONS

This study was conducted in Gözlü State Farm in Konya Province where steppe climate condition prevails. Ewes were grazed in the nearby grazing areas in farm and fed with 500 g sheep milk pellet, 500 g vetch hay and 400 g wheat stem. Former studies and books reported Akkaraman has 50-80 kg milk yield in a lactation period. So, in this study lactation period milk yield were greater 2 or 3 times than former reports and daily milk yield higher than Sage 1 report of project. In conclusion, Akkaraman sheep reproductive traits and milk yields can be increased with selection studies and Akkaraman breed could be suitable for milk production.

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The areas of using boron in health

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Abstract:

Turkey is the biggest manufacturer of boron in the world. This situation increases the importance of this element for our country. Therefore, in recent years, studies have been increasing in the field of health as well as in the industrial field. Boron, which is an essential element for plants, animals and humans, plays a role in physiological and metabolic events in the organisms. In addition, boron has important functions in mineral metabolism, lipid metabolism and energy metabolism, immune and endocrine system and brain also has a positive effect on performance and might be effective in preventing osteoporosis, osteoarthritis and arthritis. Several pharmacokinetic studies have shown that boric acid is rapidly and nearly all (>90) absorbed from the gastrointestinal tract. There is evidence that boron has a protective effect against certain types of cancer. Researches are reporting that taking non-toxic doses of borax can change lipid and carbohydrate metabolism in dogs. Sodium borate is known to reduce liver fat in dairy cows during the early period of lactation. There are many studies on the effects of boron on bone metabolism in rats, broiler, laying hen and pigs. It has been shown that boric acid (3% solution) increases treatment success in wound healing. The effects of boron on antibacterial properties, bone and immune response have attracted the intention of researchers in the field of periodontology. The antibacterial and anti-inflammatory effect of boric acid compounds has been reported in general medicine. One of the important results in human studies is that the addition of boron to the diet leads to an increase in the level of 17- β oestradiol and testosterone. To conclusion, boron is used for preventive and curative treatment in many areas of health as supported by scientific studies.

Keywords: Boron, Health, Treatment

BORON AS PROTECTIVE AND TREATMENT IN CANCER

There is evidence that boron has a protective effect against certain types of cancer (Korkmaz, 2007; Scorei and Popa, 2010; Bekirdere and Korkmaz, 2011). Positive results have been found that boron mine prevents prostate cancer (Müezzinoğlu, 2008;

Bekirdere and Korkmaz, 2011). Boron compounds are used in the treatment of cancer with two different applications. These are Bortezomib, which is the active ingredient of cancer drug containing boron compound and Boron Neutron Capture Therapy (BNCT), which is a treatment method mainly used in the treatment of brain and neck cancer (Boren, 2019).

Boron Neutron Capture Therapy (BNCT)

The ideal approach of the treatment of cancer, one of the most critical health problems today, is the selective destruction of tumour cells without damaging the surrounding normal cells. Radiation therapy is still widely used one of the treatment methods of cancer. Therapies using external radiation are among the classical treatment methods. Researchers who work in the field of radiotherapy are looking for ways to deliver a high dose of radiation to the tumour at the same time preventing the surrounding normal tissue from being damaged by radiation. BNCT is one of the methods used in research and clinical applications where radiation is used to treat cancer cells. A non-radioactive boron compound (carrying ¹⁰B) is given intravenously to the blood and is preferably introduced into the cell by the target tumour cells. Then, after capturing the neutrons from the neutron source ¹⁰B in or immediately adjacent to the tumour cells, they are lysed, and high energy charged particles are released. These particles only damage the tumour cells, and the normal cells around are often not damaged. Although, many boron compounds have been synthesised for use in BNCT for nearly 50 years, no carrier containing an ideal ¹⁰B has yet been synthesised (Kahraman, 2004). BNCT is a two-component radiation therapy method used in the treatment of various tumours, especially brain tumours and melanoma (Yenialaca, 2009).

Bortezomib

Bortezomib is the first boron supplemented cancer drug approved by the US Food and Drug Administration (FDA) and is used for the treatment of multiple myeloma. Bortezomib is also used as a proteasome inhibitor in the treatment of multiple myeloma (Boren, 2019).

THE EFFECT OF BORON ON LIPID METABOLISM

In a study to investigate the effect of sodium borax on serum lipid profile in dogs, 10 dogs were fed daily with 4 grams of borax for 30 days, and their serum lipid profiles were examined. As a result of this study, it was found that taking non-toxic doses of borax can change lipid and carbohydrate metabolism in dogs (Başoğlu *et al.*, 2000). In another study conducted to examine the effect of sodium borate on the prevention of liver fat in dairy cows, 13 of 23 pregnant dairy cows were administered 100 mg/kg, PO, 15 days sodium borate for one month before calving and one month after calving. It was concluded that sodium borate decreased liver fat levels in dairy cows during the early period of lactation (Başoğlu *et al.*, 2002).

In a study aimed to reveal the mechanism of action of boron in the correction of pathological changes in the liver, boron was found to be effective in preventing obesity and fatty liver (Başoğlu *et al.*, 2011).

BORON IN PREVENTION AND TREATMENT OF MOVEMENT SYSTEM DISEASES

Boron is primarily found in the structure of bones and teeth. Boron is an essential element for bone health with its role of balancing the absorption of calcium, magnesium and phosphorus in the body. In fact, daily boron supplementation was found to be effective by increasing the oestrogen effect in the treatment of osteoporosis (Nielsen *et al.*, 1987). The element of boron is essential for the preservation of calcium, magnesium and phosphorus in the body, especially in the bones. It also provides maximum usage and protection of these three

minerals (Calcium, magnesium and phosphorus) in the body (Yeşilbağ, 2008).

Although boron is not considered important for animals for many years, further studies have shown evidence that boron is essential for the formation of bones. Low levels of boron can cause joint problems, brittleness of the bones and easy fracture; it is reported to cause disorders in embryonal development in pregnant animals (Pawa and Ali, 2006; Kozat, 2006). Boron is also known to have beneficial effects on bone strength and mineral composition in rabbits fed a high energy diet (Hakki *et al.*, 2015).

Several studies have shown that the addition of boron to animal diets has positive effects, and the majority of these studies have addressed the effects of boron on bone metabolism. However, studies continue on the biological importance of boron mineral and its impacts on metabolism. In the case of boron insufficiency, bending in the bone marrow and delay in the onset of calcification of the cartilage tissue occurs. In poultry diets with cholecalciferol deficiency, boron insufficiency leads to a decrease in chondrocyte density in the growth plate of the bones. According to this information, it is considered that boron matures in the developmental regions of long bones (Yeşilbağ, 2008).

In rats injected with antigen causing arthritis, one group was given a diet limited to the boron, and the other group was given a diet supplemented with boron supplementation. Less neutrophil concentration, natural killer cell and CD8a+/CD4- cells were found in rats receiving boron-restricted diet than that receiving boron supplementation (Nielsen F., 2000; Gezmen-Karadağ and Türközü, 2014). The researchers mentioned that boron has

beneficial effects on bone formation and repair, especially on trabecular and alveolar bone. Male rats fed a boron-free diet (0.1 mg/kg diet), and rats fed a boron supplemented diet (3mg/kg diet) were found to have reduced trabecular thickness and increased trabecular space by boron deprivation compared to computed microtomography (Micro-CT). Micro-CT findings have shown that boron is useful for bone resilience and microstructure, although this is not necessary for this structure. Bioactive materials are used to replace lost bone tissue or to aid healing of bone tissue. These materials are needed to support weak osteoporotic bones and to fill bone defects. The known beneficial effects of boron on bone formation and repair have led to the development of a boron-containing bioactive glass. Bioactive glass is also used as a tissue scaffold for bone tissue engineering and natural bone regeneration. 45S5 bio-glass was found to increase bone formation when modified by adding boron (Nielsen, 1994; Gorustovich *et al.*, 2006; Sağlam *et al.*, 2013).

BORON IN WOUND HEALING TREATMENT

By using the antimicrobial properties of boron derivatives in wounds formed in diabetes mellitus; a study was investigated the efficacy of Z3 containing boron derivative in diabetic rats induced by streptozotocin model. A micro-vessel proliferation was observed, especially in groups where Z3 / Z3 + Boric acid-containing applications. This was found to contribute positively to the tissue healing process, speed and tissue integrity. It was determined that the expression of Vascular Endothelial Growth Factor (VEGF) increased especially in the groups containing Z3 / Z3 + Boric acid and thus contributed positively to tissue healing process, speed and tissue

integrity by microvascular proliferation. It has also been observed that it contains less apoptotic bodies, especially in the groups containing Z3/Z3+ Boric acid (Boren, 2019). Wound healing activities were investigated using a wound model with NaB containing hydrogel in mice. In this study, microscopic and histopathological examinations revealed that wounds healed faster in gel-treated groups than in control groups. It is stated that NaB/PLU gel application increases the constriction and collagen accumulation in the wound area. The results showed that NaB increased migration capacity and superoxide dismutase activity in primary human fibroblasts. The hydrogel containing sodium pentaboratpentahydrate and Pluronic is known to enhance skin wound healing *in vitro* and *in vivo* environment. Boric acid (3% solution) has also been shown to increase wound healing rate (Doğan *et al.*, 2014).

THE ANTIMICROBIAL EFFECT OF BORON

The first boron-containing biomolecule is an antibiotic called Boromycin obtained from a strain of *Streptomyces antibioticus*. Boromycin has been shown to be effective against gram (+) bacteria, some certain fungi and protozoa; however, it is ineffective against gram (-) bacteria. It has been reported that boric acid esters inhibit the clinical isolates of *C. albicans* and show antifungal effect and that 500 mg of boric acid kills the isolates of *C. albicans* by 50-90% within 48 hours. In some studies, it is stated that 0.4-5% concentration of boric acid inhibits the growth of *Candida*. The boric acid solution of 12% have been reported to be effective on gram (-) and gram (+) bacteria such as *Streptococcus mutans* RSHM 06029, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* (Vancomycin resistant), *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC

700603, *Pseudomonas aeruginosa*. In recent years, boric acid quinolone esters have been described as novel antibacterial compounds. One of these compounds is AN0128, which is widely used in the treatment of atopic dermatitis associated with skin colonisation of *Staphylococcus aureus*. AN0128 containing boric acid has been reported to have an antibacterial effect *in vitro* environment on bacteria associated with periodontal diseases, such as *Prevotella intermedia*, *Porphyromonas gingivalis*, *Eubacteria nodatum* and *Treponema denticola* (Luan, 2008; Sağlam *et al.*, 2013).

Boric acid can be used as an antiseptic for minor burns and cuts. It can also be used as pomade during bandage. Highly diluted solutions of boric acid are used as eyewash solutions. The diluted boric acid solution can also be useful to eliminate bacteria (e.g. bacterial vaginosis). It can also be used as an antibacterial compound in the treatment of acne. It is also used in athletic socks to prevent bacterial growth in athletes' feet. It is also used as a solution in the treatment of otitis externa in humans and animals (Etimaden, 2019).

The boron-containing molecule has been tested as potent compounds against *Trypanosoma brucei in vitro*, including two subtypes in human (*T. b. Rhodesiense* and *T. b. Gambiense*). The result shows that this new chemical boron formulation is a suitable way to develop new and effective oral treatments for human African trypanosomiasis infection. Oxaborole was found to cause time-dependent rapid killing of parasites and to be effective in acute and chronic infections against *T. brucei* in mouse models (Nare *et al.*, 2010). Many studies are showing that boron-containing Tavaborole has antifungal effects (Gupta, 2014).

ANTIOXIDANT EFFECT OF BORON

Potassium Tetraborate (PTB) has been reported to be non-genotoxic and to increase the antioxidant capacity of human peripheral blood lymphocytes (Türkez and Geyikoğlu, 2010). Serological and histopathological findings suggest that low-dose boric acid, as a proteasome inhibitor, may reduce the adverse effects of ALCL on the cerebral cortex (Türkez and Geyikoğlu, 2010).

So far, many studies have shown that boron compounds have a protective effect against free radical damage, which is the cause of many diseases, including cancer (Barranco and Eckhert 2006; Türkez and Geyikoğlu, 2010).

THE EFFECT OF BORON ON BRAIN FUNCTIONS

Boron insufficiency affects the electrical activity of the brain in adult rats. This is probably related to the electrical distribution of the brain. It is generally accepted that boron plays a vital role in brain activation. The mineral composition of the brain is affected by boron insufficiency. Calcium concentration in whole brain and cortex layer and phosphorus concentration in the cerebellum were higher in boron deficient group than boron supplementation group in rats with cholecalciferol deficiency. In addition, it was found that copper concentration in the brain increased in boron insufficiency (Yeşildağ, 2008).

Studies on animals and humans have shown that boron insufficiency causes a decrease in the electrical activity of the brain. Boron insufficiency causes short-term memory loss, attention-perception disorders and decreases the speed of motor activity in individuals. It is

thought that the basic principle explaining the occurrence of these problems may have an effect on nerve stimulation transport of boron. Changes in electroencephalography (EEG) readings were found in individuals after low boron and magnesium diets. Accordingly, it has been reported that brain functions can be affected by low boron diet and other nutrients (Penland, 1994; Türkez and Geyikoğlu, 2010).

EFFECT OF BORON ON DENTAL DISEASES

The antibacterial properties of boron and its known effects on bone and immune response have attracted the attention of researchers in the field of periodontology. Boric acid compounds have been reported to have an antibacterial and anti-inflammatory effect in general medicine (İnce *et al.*, 2010; Sağlam *et al.*, 2013). Although boron feeding does not affect tooth strength, microhardness and density, it has been reported that it affects tooth mineral density in rabbits fed high energy diet (Hakkı *et al.*, 2015).

Bone regeneration and periodontal regeneration are the main objectives of periodontal therapy. Bone grafts are used to increase periodontal regeneration in periodontal defects. Bioactive materials are used to replace lost bone tissue or to aid healing of bone tissue. These materials are needed to support weak osteoporotic bones and to fill bone defects. The known beneficial effects of boron on bone formation and repair have led to the development of a boron-containing bioactive glass. Bioactive glass is used as a tissue scaffold for bone tissue engineering and natural bone regeneration. When 45S5 bio-glass was modified by adding boron, it was found to increase bone formation. Pan *et al.* (2010) stated that

strontium borate glass is a new generation biomaterial in bone regeneration because it stimulates new bone formation. In order to anti-inflammatory effect is one of the structurally essential elements of periodontal tissues, proteinases are key enzymes in periodontal tissue destruction. The proteinases involved in the degradation of tissue matrix macromolecules are classified into four main classes; metallo (MMP), serine, cysteine and aspartic proteinases. MMPs and a group of serine proteinases are known to be the most effective proteolytic enzymes in periodontal diseases. Serine proteases (elastase, chymase, cathepsin G) are major proteolytic enzymes released from activated leukocytes that break down structural proteins. Boric acid compounds have been reported to affect the activity of serine proteases and inhibit these enzymes. Sağlam *et al.*, performed a study in patients with chronic periodontitis, and stated that subgingival 0.75% of boric acid application in addition to non-surgical periodontal treatment significantly increased the amount of matrix metalloproteinase-8 (MMP-8) in the gingival gut fluid compared to the control group treated with saline (Shirakata *et al.*, 2002; Gorustovich *et al.*, 2006; Koşay, 2008; Pan and Zhang, 2010; Sağlam *et al.*, 2013).

When neutrophils enter the site of inflammation in periodontal disease, neutrophils produce reactive oxygen types containing hydroxyl radicals (OH⁻) and hydrogen peroxide (H₂O₂). These reactive oxygen types can interact with cell membranes and organelles, causing damage to cells and tissues. It has been reported that boron prevents oxidative damage by increasing the antioxidant agent glutathione and its derivatives or by stimulating other agents that neutralize reactive oxygen species. These effects of boron have attracted

the attention of researchers in the field of periodontology. However, there are few scientific studies in the field of periodontology (Sağlam *et al.*, 2013).

In a study to test the efficacy of boric acid-containing AN0128 in the treatment of periodontal disease, AN0128 was applied topically in rats with experimental periodontitis. Significantly less bone loss and less inflammatory infiltrate have been reported in rats treated with AN0128 boron-containing compounds than rats treated topically with carrier and untreated rats. When the alveolar bone area between the molars in rats was examined histologically, 50% bone formation was observed in the alveolar bone area in AN0128 group, and this result was found to be comparable with tetracycline with 33% bone formation in the alveolar bone area and chlorhexidine with 40% bone formation, respectively. Besides, the AN0128 boron-containing compound was reported to reduce inflammatory infiltrate by 42% compared to the carrier-treated group. Demirer *et al.* (2012) gave 3mg systemic boric acid daily for 11 days orally to rats with experimental periodontitis. The rats were sacrificed after 11 days, and histomorphometrically examined the changes in alveolar bone and tissue samples were histopathologically examined. Results have been found that inflammatory cell infiltration, osteoblast count and alveolar bone loss were less in boric acid-treated rats than in the control group. Consequently, it has been reported that systemic boric acid administration reduces periodontal inflammation and alveolar bone loss in rats with periodontal disease (Luan, 2008; Sağlam *et al.*, 2013).

It has been reported in chronic periodontitis patients, in addition to non-surgical

periodontal treatment, subgingival boric acid irrigation was significantly lower than in the control group with physiological saline solution with clinical parameters such as clinical attachment level, plaque index, gingival index and MMP-8 amount in probing, bleeding and gingival fluid (Sağlam, 2011).

THE EFFECTS OF BORON ON HORMONES

One of the crucial results of human studies is the addition of boron to the diet to increase the level of oestradiol 17- β and testosterone. This change in steroid hormone concentration is particularly important for postmenopausal women. Oestradiol 17- β , which is the active form of oestrogen hormone, have the effect of reducing calcium release from the bones. In addition, testosterone is useful in the version of oestradiol 17- β in women. It can be seen in many studies; the addition of boron to the diet increased the testosterone level up to twice. These studies indicate that boron mineral affects the hydroxyl groups of steroid hormones in humans. Also, certain steroid hormones (oestradiol) have been implicated in the hydroxylation step in these studies (Yeşilbağ, 2008).

Boron also affects the activation and metabolism of thyroid hormone. This effect is thought to be caused by stimulating the thyroid hormone receptors and cell membrane. Boron affects thyroid hormone indirectly by affecting serum alkaline phosphatase activity (Gezmen-Karadağ and Türközü, 2014).

THE EFFECT OF BORON ON IMMUNE SYSTEM

It was determined that boron is effective in the immune system and inflammatory events. Bai and Hunt (1996) showed that the immune response of rats against bacterial antigens is suppressed in boron insufficiency. Borax,

which is a source of boron, has been shown to have anti-arthritic effect in some studies (Yeşilbağ, 2008). Studies have shown that boron affects the immune system by increasing serum antibody concentration in rats (Yeşilbağ, 2008).

THE EFFECT OF BORON ON VISUAL FUNCTION

Boron insufficiency in adult zebrafish causes photophobia. Previous studies have shown that boron insufficiency may cause photoreceptor dystrophy. Boron supplemented zebrafish were compared with zebrafish receiving a poor diet of boron, and it was concluded that boron insufficiency decreased in photoreceptor cells due to a decrease in myoid and segment in zebrafish receiving a poor diet from boron (Naghii and Samman, 1999; Gezmen-Karadağ and Türközü, 2014).

RESULT

As a result, boron is used for preventive and curative treatment in many areas of health as supported by scientific researches. Scientific studies about the boron that precious and attractive need to increase in our country, which holds the largest part of the world's boron reserves. To raise awareness about boron mine and to improve the usage area of this vital mine in the light of science; it is indispensable and important, especially for science, living things, profession and country.

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The evaluation of serum oxidative stress and total antioxidant status during estrous cycle in bitches

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Abstract:

Bitches are monoestrous animals, and differs from other domestic animals due to hormonal profile of estrus cycle. Serum progesterone level increase rapidly during preovulatory LH surge. Oocyte maturation, follicular growth, ovulation, steroidogenesis, and luteolysis are physiologic process of reproduction; these physiologic processes affect reactive oxygen species and antioxidant status of animals. In the present study, we aimed to evaluate serum Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI) in bitches during estrous cycle. A total of 13 bitches were used for this study. Vaginal smear samples were taken for determining estrous cycle, and stained with Giemsa. Blood samples were collected for TAS, TOS and progesterone analyzes. The TAS and TOS analyzes were performed using by Erel's method. The OSI was defined as the ratio of the TOS level to TAS level. TAS levels were converted to μmol . Specifically, OSI (arbitrary unit) = $\text{TOS} (\mu\text{mol H}_2\text{O}_2 \text{ equivalent /L}) / \text{TAS} (\mu\text{mol Trolox equivalent /L}) \times 100$. The serum levels of progesterone were measured by canine ELISA kits, according to the manufacturer's instructions. The bitches were divided into four groups as proestrus (n=2), estrus (n=3), diestrus (n=6), and anestrus (n=2) according to vaginal smear and serum progesterone levels. The lowest concentration of TAS (0.97 ± 0.19 mmol/L), and the highest concentration of TOS (52.47 ± 3.05 $\mu\text{mol/L}$) were detected in estrus phase. Mean level of OSI was 3.55 ± 0.47 AU in proestrus, 5.85 ± 1.1 AU in estrus, 4.86 ± 0.52 AU in diestrus, and 4.04 ± 0.49 AU in anestrus phases. In conclusion, the results of our study revealed the physiologic levels of TAS, TOS and OSI showed alteration during estrous cycle in bitches. Therefore, further studies about oxidative stress levels in relation with reproductive physiology are required with a large number of animals.

Keywords: Bitch, Estrous cycle, Total Antioxidant Status, Total Oxidant Status, Oxidative Stress Index

INTRODUCTION

The female dogs are typically classified as monoestrous. The estrous cycle of bitches is much longer than those of other animals, and consists of proestrus, estrus, diestrus and

anestrus stages (Senger, 2005). Endocrine mechanism of canine cycle differs from other mammalian species; ovulation occurs 2-3 days after Luteinizing Hormone (LH) surge at the end of proestrus or at the beginning of estrus

phase. The bitch becomes receptive to the male in estrus phase during rising progesterone levels. Unlike those of other mammals, estrus stage proceeds under the influence of progesterone hormone in bitches (Alaçam et al., 2009; Concannon, 2011). The changes in the reproductive organs in relation with steroids and gonadotrophins are ever-repeating process. During estrous cycle, preantral follicles grow, granulosa cells in the antral follicles transforms into the luteal cells, ovulation occurs, and corpora lutea develops and then regress in the ovary of bitches. In addition to this, proliferative, regressive, and apoptotic changes occur in the endometrium of uterine tissue and vagina synchronously with ovarian changes (Van Cruchten et al., 2004; Sato et al., 2016).

Oxidative stress is defined as a result of an increased number of lipid and protein oxidation products, and decreased number of antioxidants. Under normal conditions, pro-oxidants and antioxidants remain in balance in aerobic cells (Agarwal et al., 2005). The oxidative stress balance not only plays a crucial role for healthy body, but it is essential also for reproductive system physiology. It is well known that the reproductive physiology is a continuous process with follicular growth, preovulator luteinizing hormone (LH) surge, oocyte maturation, ovulation, formation of corpus luteum, steroidogenesis, luteolysis, endometrial proliferation and regression,

fertilization, embryonic growth, and pregnancy (Agarwal et al., 2005; Rizzo et al., 2009a; Rizzo et al., 2012). Reactive oxygen species (ROS) are markers of oxidative stress, and ROS are both by-products and influencers of normal reproductive metabolism, especially for steroidogenic cells (Sugino, 2006). However, in some cases ROS production may be increase, and enzymatic or non-enzymatic antioxidants such as superoxide dismutase, catalase, vitamin E, vitamin C, selenium, zinc, and glutathione fail to reduce ROS excess; then, oxidative stress causes damage in cells and tissues. In female reproduction, insufficient antioxidant defense mechanism and/or excessive production of ROS may play an important role on infertility, repeat breeding, inadequacy of oocyte maturation or embryo development, cystic ovarian diseases, endometriosis, preeclampsia, abortions (Rizzo et al., 2007; Rizzo et al., 2009b; Lázár, 2012; Lu et al., 2018). The levels of antioxidant and oxidant components influences oxidative balance in the aerobic cells. Individual metabolites of ROS, which are superoxide, hydrogen peroxide and hydroxyl, may not necessarily reflect the whole condition of oxidative stress in the organism. Therefore, the measurement of TAS, TOS and the calculation of OSI reflect the relation the between ROS formation and antioxidant activity (Erel, 2005). The purpose of the study was to evaluate serum TAS, TOS and OSI in bitches during estrous cycle.

MATERIAL AND METHODS

The present study was performed in 13 healthy bitches from different breeds and of varied ages (between 1-8 years) that were brought to our clinic for routine ovariohysterectomy. Research material was formed obtained blood from these bitches. The stages of estrous cycle were determined using by vaginal cytology and serum progesterone level. This research was maintained in accordance with the directions of Guide for the Care and Use of Animal in Research (AKUHADYEK-24-18).

To determine vaginal cytology, the vulva was cleaned with antiseptic solution, vaginoscopic examination was performed and then vaginal smear samples were collected to detect the estrous cycle. The samples were stained by Giemsa stain and evaluated under the light microscope. The stages of estrous cycle were classified as proestrus (presence of erythrocytes, gradually increase in cornified cells, decrease in polymorphonuclear leukocyte cells), estrus (cornified and anuclear in all cells, no polymorphonuclear leukocyte cells), diestrus (non-cornified cells, large number of polymorphonuclear leukocyte cells, epithelial cells engulfing a polymorphonuclear leukocyte cells and the foam cell), and anestrus (non-cornified cells and rare polymorphonuclear leukocyte cells) (Root Kustritz, 2012).

Blood samples were collected preoperatively from the *vena cephalica antebrachii* into plain tubes (Vacutainer® SST™ II Advance Serum Tubes) and centrifuged at 3000 rpm for 15 min. The obtained serum was stored at -80°C until analysis. The TAS and TOS levels were spectrophotometrically analyzed and OSI was calculated. Serum progesterone levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA; VersaMax™ ELISA Microplate Reader using a commercial dog kit Cusabio® Canine Progesterone, PROG ELISA). The results of progesterone concentration were presented as ng/ml. The progesterone data was confirmed with findings of vaginal cytology in each case.

Total antioxidant-oxidant levels in the serum were analyzed using a novel automated measurement method (Rel Assay kit, Turkey; Erel 2004; 2005). The antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is determined. This assay relies on the ability of antioxidants in the sample to inhibit the formation of 2,2'-azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid (ABTS)]. The values of TAS are expressed as mmol Trolox equivalent /L (Erel 2004). The color intensity is related to the total amount of oxidant molecules present in the sample. The test is based on the oxidation of ferrous ion to ferric ion in the presence of various

oxidative species in acidic medium and the measurement of the ferric ion by xylenol orange. The assay is calibrated with hydrogen peroxide (H₂O₂). The results are expressed in terms of $\mu\text{mol H}_2\text{O}_2$ equivalent /L (Erel, 2005). The OSI was defined as the ratio of the TOS level to TAS level. TAS levels were converted to μmol . Specifically, OSI (arbitrary unit) = $\text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent /L}) / \text{TAS } (\mu\text{mol Trolox equivalent /L}) \times 100$. All results are expressed as the mean \pm standard error of the mean (SEM).

RESULTS

The bitches were divided into four groups as proestrus (n=2), estrus (n=3), diestrus (n=6), and anestrus (n=2) according to vaginal smear and serum progesterone levels. Mean values of progesterone were 1.38 ± 0.47 in proestrus; 4.86 ± 0.50 in estrus; 13.0 ± 5.31 in diestrus; 0.40 ± 0.09 in anestrus (Figure 1). The lowest concentration of TAS (0.97 ± 0.19 mmol/L), and the highest concentration of TOS (52.47 ± 3.05 $\mu\text{mol/L}$) were detected in estrus stage. Mean level of OSI was 3.55 ± 0.47 AU in proestrus, 5.85 ± 1.1 AU in estrus, 4.86 ± 0.52 AU in diestrus, and 4.04 ± 0.49 AU in anestrus phases (Figure 2).

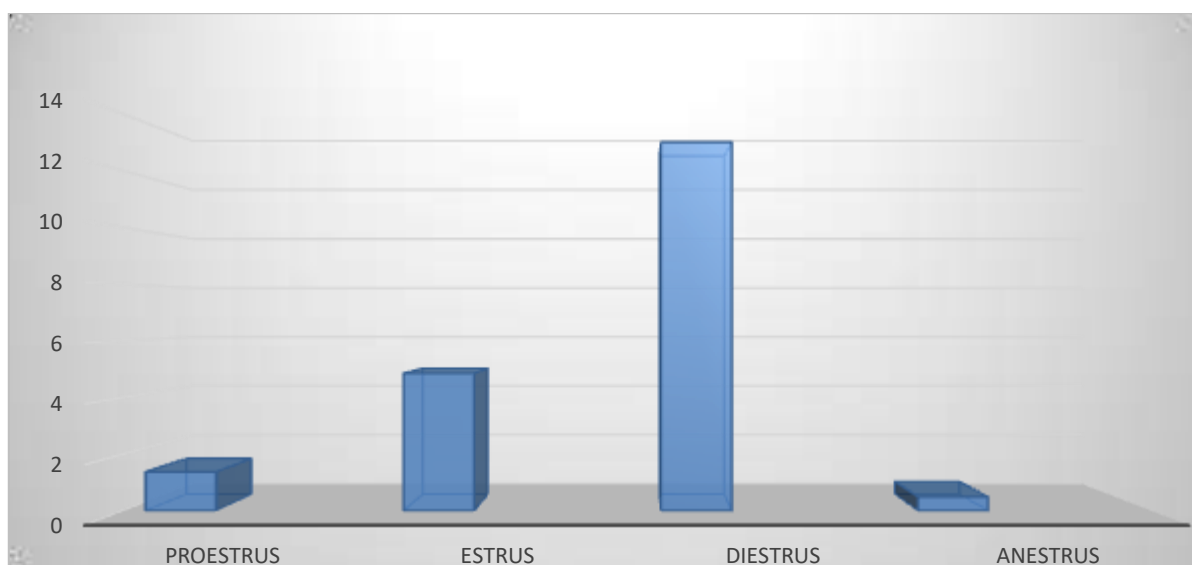


Figure 1. Serum progesterone concentrations (ng/ml) in different phases of the estrus cycle

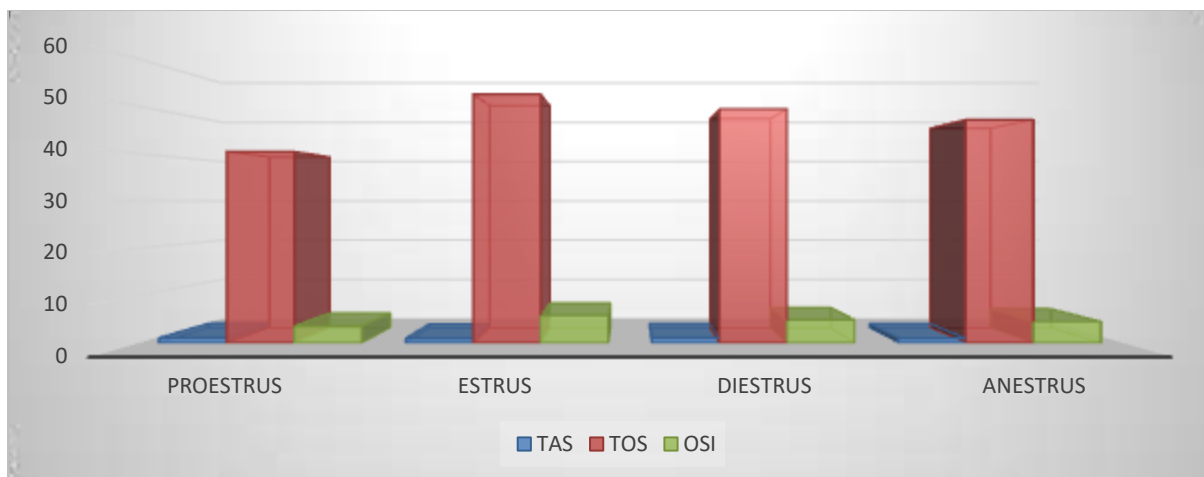


Figure 2. TAS (mmol/L), TOS (μmol/L), and OSI levels in the different phases of the estrus cycle

DISCUSSION

Detection of estrous cycle stage in bitches is an important issue due to the diagnosis of correct breeding timing, pathological conditions such as pyometra, vaginal tumours, disorders ovarian cycle, ovarian remnant syndrome. The most preferring ways to detect cycle stage are vaginal cytology and blood progesterone level analyze (Graves, 2006; Root Kustritz, 2010). In the present study, the stage of estrous cycle was detected using by vaginal cytology and progesterone determination as mentioned above.

Many studies reported that oxidative stress plays key roles in reproductive physiology in human, rodents, and ruminants (Agarwal et al., 2005; Rizzo et al., 2007; Lázár, 2012; Lu et al., 2018). However, there are a few studies about oxidative stress and reproduction in bitches (Rizzo et al., 2009a; Pech et al., 2019). In the present study, we evaluated serum TAS and TOS levels, and OSI in bitches for the first

time. According to our results, the lowest concentration of TAS, and the highest concentration of TOS and OSI were detected in estrus phase. These results indicate that preovulator and ovulatory period cause oxidative stress imbalance due to acute inflammatory process, increased immune and metabolic activities (Rizzo et al., 2009a). The highest concentration of TAS was measured in proestrus phase in the present study. This antioxidant status in bitches confirms the antioxidant effect of high estrogen level and its angiogenetic factor as other female mammals (Agarwal et al., 2005).

CONCLUSION

The results of our study revealed the physiologic levels of TAS, TOS and OSI showed alteration during estrous cycle in bitches. Therefore, further studies about oxidative stress levels in relation with reproductive physiology are required with a large number of animals.

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The effect of intravaginal sponge treatment on fertility rates in ewe of Sonmez

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Abstract:

The object of this trial was to determine the efficacy of vaginal sponge treatment to synchronize oestrus in Sonmez (ChiosxTahirova) and Merino ewes during the non-breeding season. The experiment was performed under natural conditions in early February in Afyonkarahisar province, located in the west of Turkey. This region is situated at 38° 45' N, 30° 32' W, and at an altitude of 1021 m above sea level. A total of twenty three Sonmez and twenty nine Merino ewes were used. Progesterone impregnated intravaginal sponges were inserted for 12 days. A total of 600 IU of eCG was injected intramuscularly following the removal of sponges. The ewes were mated with fertile rams after twelve hours of removal of vaginal sponges. Rams were removed after 4 days. The pregnancy of ewes was determined by transrectal ultrasonography using a real-time B-Mode ultrasound with linear - array transrectal probe on day 45 following the mating. It was observed that higher ($p < 0.05$) pregnancy rate was recorded in Sonmez ewes % 73.9 (17/23) than those detected in the Merino ewes % 44.82 (13/29). It is suggested that the response of the treatment of intravaginal sponge might be better in Sonmez ewes.

Keywords: Sonmez, Ewe, Progestagen, Pregnancy rate

Sönmez Irkı Koyunlarda Intravaginal Sünger Uygulamasının Fertilitite Oranlarına Etkisi

Özet

Bu çalışmada Sönmez (Sakız X Tahirova melezi) ve Merino koyunlarının sezon dışı senkronizasyon uygulamasında vaginal sünger uygulamasının etkinliği belirlendi. Çalışma standart koşullarda Şubat ayı başında Afyonkarahisar'da bulunan özel bir işletmede yapıldı. Bu bölge 38° 45' N, 30° 32' W ve 1021 m rakımda bulunmaktadır. Çalışmada 23 baş Sönmez ve 29 baş Merinos koyun kullanıldı. Intravaginal sünger uygulaması 12 gün süresince gerçekleştirildi. Süngerlerin çıkartıldığı gün koyunlara 600 IU dozunda eCG intra muskuler olarak uygulandı. Süngerlerin çıkartılmasından 12 saat sonra koç katımı gerçekleştirildi ve çiftleşmeler gözlemlendi. Koçlar koyunlar ile birlikte 4 gün kaldıktan sonra ayrıldı. Koyunlarda gebelik muayenesi çiftleşmeden 45 gün sonra real-time B-mode ultrason lineer prob yardımı ile belirlendi. Gebelik muayenelerini göre Sönmez koyun ırkındaki gebelik oranı % 73.9 (17/23) Merinos koyunlarına göre yüksek elde edildi % 44.82 (13/29, $p < 0.05$). Sonuç olarak Sönmez ırkı koyunlarda intravaginal sünger uygulamasının olumlu olacağı kanısına varıldı.

Anahtar kelimeler: Sönmez, Koyun, Progesteron, Gebelik oranı

GİRİŞ

Türkiye de 2018 yılı TÜİK verilerine göre 31.257.408 baş koyun, 10.419.027 baş keçi mevcuttur. 6 aylıktan küçük kuzu sayısının 1.651.902, 6-12 aylık arası toklu sayısı 4.423.188, 12-24 ay arası şişek sayısının 5.410.157, 2 yaş ve daha yukarı yaşta koyun sayısının ise 19.931.079 olduğu bildirilmektedir (Akdağ ve Akal 2018). Ülkemizde koyun varlığının %93'ü yerli ırklardan meydana gelirken %7'si ise kültür ırkı ve melezlerden meydana gelmektedir (Yavaş, 2008). Ülkemizde 2018 yılı koyun eti üretimi toplam olarak 100.831 ton iken, keçi eti üretimi ise 13.603 ton belirtilmiştir (Akdağ ve Akal 2018). Gelecekteki başarısını ve uzun vadeli sürdürülebilirliğini sağlayacak, günümüz koyun endüstrisi üzerinde etkisi olan en büyük faktörlerden biri üreme performansdır (Dursun ve ark., 2017; Jackson ve ark., 2014).

Doğal aşım yollarıyla yılda bir yavru elde edilirken üremenin denetlenmesi ile bu sayı iki yılda üç yavruya çıkarılabilmektedir. Bu durumda işletme sahibinin karlılığını arttırmakta ve ülkenin gayri safi milli hasılasına katkı sağlayarak aynı zamanda kırmızı et üretiminde sıkıntı yaşadığımız bugünlerde et sektöründe de yol kat etmeyi sağlayacaktır. Damızlık değeri yüksek olan erkek ve dişi hayvan seçilmesi doğacak olan yavruarda ikizlik oranının artmasına, doğum sonrası yavruların mortalite oranında azalmaya, doğacak olan yavruların verim yönünden daha yüksek değerlere sahip olmasını sağlar (Karaca ve ark., 2003).

Progesteron görevi gebeliğin devamını sağlamak ve seksüel siklusun başa dönmesini engellemektir. Progesteronun sentetik türevleri korpusluteum tarafından progesteron üretimini uyarmak için kullanılır. Bu nedenle ortamdan progesteron uzaklaştırılınca korpus luteum luteolize uğrar

ve siklus tekrardan başlar. Progesteronun dışarıdan verilmesi için sayısız yöntem mevcuttur; bunlardan en yaygını yavaş yavaş progesteron salgılayan intravajinal sünger veya yem katkı maddesi olarak verilebilir (Jackson ve ark., 2014).

Üreme mevsiminde ve mevsim dışında olan küçükbaş hayvanlarda östrussenkronizasyonu sıklıkla poliüretan sünger ile yapılmaktadır. Progesteron süngerleri; fluorogestone asetat ve medroksiprogesteron asetatıdır. Süngerlerde fluorogestone acetate seviyesi 20-40 mg arasındadır. Medroksiprogesteron asetat seviyesi ise 40-60 mg arasındadır. Süngerler koyunlara intravajinal olarak yerleştirilir ve olabildiğince serviks yakın yere koyulur. Sünger uygulaması 10-12 gün sonra çıkarılırken tek doz prostaglandin veya PMSG enjeksiyonunun yapılması belirtilmektedir (Jackson ve ark., 2014).

Sunulan çalışmada da Afyonkarahisar bölgesinde bulunan Sönmez (Sakız x Merinos) ırkı koyunlar ile Merinos ırkı koyunlarına intravajinal sünger uygulamasının gebelik oranlarına olan etkisinin ortaya konması amaçlanmıştır.

MATERYAL VE METOT

Çalışma Afyonkarahisar ilinde bulunan özel bir işletmedeki 23 baş Sönmez ile 29 baş Merinos koyun ırkında Şubat ayı içerisinde 38° 45' N, 30° 32' W konumunda deniz seviyesinden 1021 m yükseklikte gerçekleştirildi. Çalışma öncesi hayvanların gebelik kontrolleri yapıldı ve gebe olmadığı tespit edilen hayvanlar çalışmaya dahil edildi. Aynı şekilde koçlarında androlik muayenesi ve daha öncesinde fertilitate başarısı olanlar çiftleştirme amacıyla belirlendi. Belirlenen 4 baş koç senkronizasyona başlamadan önce sürüden ayrıldı. İntravajinal sünger (Esponjavit®, HIPRA Hayvan Sağlığı, Türkiye) uygulaması

başlangıcı 0. Gün olarak kabul edildi ve 12 gün süresince koyunlara progesteron desteği verildi. 12. Gün süngerler çıkartılarak 600 IU dozunda eCG (Gonaser®, HIPRA Hayvan Sağlığı, Türkiye) intra-muskuler olarak uygulandı. Süngerlerin çıkartılmasından 12 saat sonra koçlar çiftleşme için sürüye dahil edildi ve 4 gün süresince çiftleşmeler gözlemlendi. Gebelik muayenesi Sünger çıkartılmasından 45 sonra B-Mode real-time ultrasonun (HT-838, HASVET) lineer probu kullanılarak yapıldı.

İstatistik hesaplamaları

Toplanan verilerin istatistiksel analizi için SPSS paket program sürüm 13 istatistik programı

Tablo 1. Üreme sezon dışı intravaginal yöntemle ile senkronize edilen koyunlarda gebelik oranları

Gruplar	Gebelik oranı %
Sönmez Koyunu	73,9 (17/23)
Merinos Koyunu	44,8 (13/29)
P<0,05	*

*Gruplar arası fark P<0,05 düzeyinde önemlidir.

TARTIŞMA

Sunulan çalışmada Afyonkarahisar bölgesinde sınırlı sayıda bulunan Sönmez ırkı koyunların sezon dışı dönemde progesteron uygulaması ile fertilitite oranları düzeyi Merinos ırkı koyunları ile karşılaştırıldı. Çalışma sonucuna göre Sönmez ırkı koyunların intravaginal sünger uygulamasına cevabı Merinos ırkı koyunlarına göre önemli ölçüde yüksek bulundu. Elde edilen sonuçlardan Merinos ırkı koyunların gebelik oranları ile Yaralı ve Karaca (2004) tarafından Kıvırcık koyunlarında elde edilen gebelik oranları ile benzer, Sönmez koyunun gebelik oranlarından ise düşük bulunmuştur. Ayrıca Kıvırcık koyunlarda (Koyuncu ve ark., 2001), Morkaraman koyunlarda (Baş ve ark., 1986) üreme sezonu

kullanıldı. Kullanılan yöntemle göre gebelik oranlarını karşılaştırmak amacıyla ki-kare testi (χ^2) kullanıldı. Yapılan tüm testlerde p değeri <0,05 anlamlı olarak kabul edildi.

BULGULAR

Çalışma sonucunda yapılan gebelik muayenelerine göre sezon dışı intravaginal sünger uygulaması ile Sönmez ırkı koyunların gebelik oranı % 73.9 (17/23) Merinos koyunlarına göre yüksek elde edildi % 44.82 (13/29, p < 0.05, Tablo 1).

dışında yapılan intravaginal sünger uygulamalarından elde edilen gebelik oranlarından düşük, Sakız x Kıvırcık melezi (Altinel ve ark., 1998) koyunlarından elde edilen gebelik oranlarıyla benzer olarak belirlenmiştir. Oluşan farklılığın uygulama dönemi hormon dozları ve bakım beslenmeye ilgili olduğu kanısına varılmıştır.

Sonuç olarak Sönmez ırkı koyunlarının üreme sezonu dışında emdirilmiş progesteron içeren intravaginal sünger uygulamasının fertilitite başarısına olumlu katkı sağladığı kanısına varılmıştır.

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The Effect of Boron Addition on Some Spermatological Parameters Liquid Storage (+5 °C) Ram Semen

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Abstract:

The study was conducted to determine the effect of Boron on changes of ram sperm motility, abnormal sperm rate and membrane integrity (HOST) during short term storage (+5°C) for 48 hours. Between 3-4 years old three Ramliç rams were used as the material of study during non breeding season. Rams were maintained by Afyon Kocatepe University, Farm of Veterinary Medicine in Afyon, Turkey. Ejaculates were collected from three Ramlic Rams by artificial vagina (AV) and pooled at 37°C for evaluation. The study was replicated six times. Determined principle spermatological properties and having/with normospermie quality will pooled ejaculates were split into four equal aliquots. Each group was separately diluted to final concentration of approximately 120×10^6 (spt/ml) with control (without boron) or boron supplemented extenders (containing 1, 2 and 4 mM, respectively) then cooled to 5 °C within 60 min. Cooled samples were maintained at 5 °C during 0, 24 and 48 h. After 24 and 48 hours of storage, 2 mM Boron treated sperm samples presented higher motility and membrane integrity than control group (P <0.05) in ram semen stored at +5°C. Furthermore, boron addition improved the ability of ram spermatozoa to motility and membrane integrity for short term storage.

Keywords: Sperm, carnosic acid, short term storage, ram

Kısa Süreli Saklanan Koç Spermasına İlave Edilen Borun Bazı Spermatolojik Parametreler Üzerine Etkisi

ÖZET

Sunulan bu çalışma, kısa süreli saklanan (+ 5 ° C) koç spermasına eklenen Boron'un 48 saat boyunca motilite, anormal spermatozoon oranı ve spermatozoon membran bütünlüğü (HOS test) üzerindeki etkilerini belirlemek amacıyla yapıldı. Çalışma materyali olarak üç-dört yaşları arasında sezon dışında üç baş Ramliç koç kullanıldı. Koçlar, Afyon Kocatepe Üniversitesi Veteriner Fakültesi Çiftliğinin bakım ve beslenme şartlarında bulunan koçlardan seçilmiştir. Ejakülatlar, üç Ramliç koçtan suni vajen ile toplandı ve değerlendirme için 37 ° C'de birleştirildi. Çalışma altı kez tekrarlandı. Koçlar normospermi özellik gösterenler arasından seçildi. Her grup ayrı ayrı yaklaşık 120×10^6 (spt/ml) nihai yoğunluğa kadar kontrol (Boron içermeyen) ve Boron ilave edilmiş sulandırıcı (sırasıyla 1, 2 ve 4 mM içeren) ile seyreltildikten sonra 60 dakika içinde 5 ° C'ye soğutuldu. Soğutulan numuneler, 0, 24 ve 48 saat boyunca 5 ° C'de tutuldu. Kısa süreli saklanan sperma örnekleri 24 ve 48 saat saklamanın ardından, 2 mM Boron ile muamele edilmiş grupta, kontrol grubuna göre istatistiki olarak (p<0.05) daha yüksek bir motilite ve membran bütünlüğü göstermiştir. Boron ilavesinin koç spermasının kısa süreli saklanmasında motilite ve membran bütünlüğü kabiliyetini artırdığı sonucuna varıldı.

Anahtar Kelimeler: Sperma, Boron, Kısa süreli saklama, Koç

Introduction

Semen dilution and storage are widely used in artificial insemination (AI) programs. Diluted and cooled ram semen is an alternative to frozen semen when the insemination is done within a short period of time after collection. Compared with fresh semen, cooled ram semen suffers from a decreased in motility and morphological integrity, accompanied by a decline in the survival in the female reproductive tract, reduction of fertility and increased embryonic loss (Maxwell and Watson, 1996). These damages are less pronounced in diluted and chilled semen than in frozen-thawed ram semen (Bucak and Tekin, 2007).

Ramlıç is a type of sheep which combines the high vitality of Dağlıç with the meat and fleece yield characteristics of the Rambouillet breed. The Ramlıç sheep is a significant breed for which research and applications must be carried out in order to develop the freezability of spermatozoa to protect the genes and increase the production of Ramlıç (Kaymakçı and Taşkın, 2008).

Boron is an element commonly found as various compounds in Earth, water, and stone; and 72.1% of the world's total boron reserves are in Turkey. Boron and its compounds have various areas of usage in the industry and farming. In different studies, effects of boron on the reproductive system of different animal have been tried to reveal based on dosage and time, high boron concentration intake results with antioxidant mechanism improvement. Studies have shown that the boron element plays an important role as a macro mineral in the human cell membrane and in the cellular mechanism; it is also noted that it has an anti carcinogenic effect on some types of cancer. Mostly, the effects of the boron element on the reproductive system of rats and mice have

been endeavored to be manifested with various studies based on dosage and duration. The testes which are considered as the most sensitive organ to the boron element have not been studied in terms of spermatological parameters, sperm's freezing ability and the effects on fertilization. It is important to study the effects of using reconstituted boron in the freezing of semen in the reproduction parameters of farmed livestock which have an economic value such as rams because it plays an important role in the cell membrane and cellular mechanism (Treinen and Chapin, 1991, Ku et al, 1991)

The study was conducted to determine the effect of boron on changes of ram sperm motility, abnormal sperm rate and membrane integrity (HOST) during short term storage (+5°C) for 48 hours.

MATERIAL AND METHOD

Between 3-4 years old three Ramlıç rams (Dağlıç x Rambouillet) were used as the material of study during non breeding season. Rams were maintained by Afyon Kocatepe University, Farm of Veterinary Medicine in Afyon, Turkey. Ejaculates were collected from three rams by artificial vagina (AV) and pooled at 37°C for evaluation. The study was replicated six times. Shortly, with the concentration of at least 1×10^9 spermatozoa/mL pooled ejaculates were split into four equal aliquots. Each group was separately diluted to final concentration of approximately 120×10^6 (spt/ml) with control (without boron) or Boron (Sodium pentaborate, $\text{Na}_2\text{O} \cdot 5\text{B}_2\text{O}_3 \cdot 10\text{H}_2\text{O}$) supplemented extenders (containing 1, 2 and 4 mM, respectively) then cooled to 5 °C within 60 min. Cooled samples were maintained at 5 °C during 48 h.

Microscopic Evaluation of Sperm

Motility

Phase-contrast microscopy was performed on a heating plate (200 X) set at 37 °C for the spermatozoa motility assay. Five different areas were examined for each semen sample. The motility averages in the field were recorded as %.

Abnormal Sperm Rate

The abnormal spermatozoa ratio in semen samples was determined by the Giemsa staining method. The prepared slides were counted as 200 spermatozoa for each sample in the immersion lens (1000 X), and the percentage of abnormal spermatozoa ratio was determined as % (10).

Membrane Integrity Test

The hypoosmotic swelling test (HOST) was used as a complementary test to the viability assessment protocol. The assay was performed by mixing 0.1 ml of semen with a 1 ml hypoosmotic solution (9 g fructose, 4.9 g trisodium citrate, distilled water to 1000 ml, 100 mOsm). This mixture was incubated at 37 °C for 30 minutes. After incubation, 0.2 ml of the mixture was spread with a cover slip on a warm slide. A total of 400 spermatozoa were counted with a phase contrast microscope

(1000× magnification, oil immersion). The percentages of spermatozoa with swollen and coiled tails were recorded.

Statistical Analysis

Results were expressed as the mean ± S.E.M. Means were analyzed by one-way analysis of variance, followed by the Duncan's post hoc test to determine significant differences in all the parameters among all groups using the SPSS/PC computer program (Version 13.0; SPSS, Chicago, IL). Differences with values of $p < 0.05$ were considered to be statistically significant.

RESULTS

Before the storage at 5°C, parameters were estimated and saved as primary (Hour 0) (Table 1). The mean primary parameters of the sperm motility, abnormal sperm rate and HOS test were non-significant affected by Boron. Significant differences were observed between the Boron 2 mM and control groups for motility and HOS test levels at 24 h ($p < 0.05$) (Table 2). In terms of higher motility and HOS test values of the 48 h with 2 mM of Boron compared to the control group were statistically significant ($p < 0.05$) (Table 3). No difference was observed between the groups in terms of abnormal sperm rate at all hours.

Table 1. Mean Sperm Motility, Morphology and HOS test obtained at 0. hours in the study (n:6).

Groups	Motility (%)	Abnormal Sperm Rate (%)	Hos Test (%)
Control	80,00±1,29	11,00±1,06	74,5±24,86
1 mM	79,17±0,83	12,00±.,00	74,00±1,86
2 mM	82,50±1,12	12,66±1,28	80,16±1,11
4 mM	80,83±0,83	11,66±0,84	74,5±1,83
P	-	-	-

Table 2. Mean Sperm Motility, Morphology and HOS test obtained at **24.** hours in the study. (n:6).

Groups	Motility (%)	Abnormal Sperm Rate (%)	Hos Test (%)
Control	67,50±2,50 ^b	12,83±0,98	67,00±1,93 ^b
1 mM	71,66±1,05 ^{ab}	14,00±0,93	68,83±0,98 ^{ab}
2 mM	75,00±1,29 ^a	13,83±1,40	74,83±1,30 ^a
4 mM	69,17±0,83 ^{ab}	13,33±1,08	69,5±2,99 ^{ab}
P	*	-	*

a, b : Different superscripts within the same column demonstrate significant differences. (p < 0.05).

Table 3. Mean Sperm Motility, Morphology and HOS test obtained at **48.** hours in the study. (n:6).

Groups	Motility (%)	Abnormal Sperm Rate (%)	Hos Test (%)
Control	58,33±2,10 ^b	14,66±0,95	62,66±2,70 ^b
1 mM	61,66±1,05 ^b	15,50±0,76	60,16±0,60 ^b
2 mM	70,0±1,29 ^a	15,83±1,40	68,83±0,70 ^a
4 mM	60,83±0,83 ^b	15,00±0,93	59,33±1,05 ^b
P	*	-	*

a, b : Different superscripts within the same column demonstrate significant differences (p < 0.05).

DISCUSSION

Liquid storage is used as an alternative of ram semen cryopreservation for short-term storage because of damaging effects of cryopreservation (Gungor et al., 2019). However, spermatozoa metabolism and cold shock cause some negative effects on spermatozoa with the course of time (Maxwell and Salamon 1993).

Motility of spermatozoa is the essential factor for sperm evaluation. In the study of Tirpan and Tekin (2015), boric acid was added and cryopreserved spermatozoa of the

Angora goat and motility values were found similar to our study. In their study, Yeni et al. (2018) added boron to Ramlic ram sperm and obtained the best motility results in 1 mM group. We think this difference is due to their cryopreserved sperm.

Tirpan and Tekin (2015), obtained the lowest rate of abnormal spermatozoon in the group to which they added 0.4 gr Boron, Yeni et al. (2018) the highest values were obtained in the 4mM Boron group. In our study, no significant differences were detected between the control group and the other groups in

terms of spermatozoa abnormal sperm rate results.

Elkomy et al. (2015) reported that rabbits supplemented with different doses of boron in their diets indicated a positive effect in many spermatological rates and conducts, especially in motility values and sperm quality. It was reported that on the molecular level Boron affected the activities of at least 26 enzymes (Hunt et al., 1997) and that a large number of these enzymes were necessary for the energy substrate metabolism. It was reported that boron had several regulating roles on the macro-mineral metabolism (Oliva et al., 2001), the energy metabolism (Banday et al., 2017) and the immune system (Hunt and Idso, 1999) and that after delivery of boron at different levels, the fructose concentration data in seminal plasma decreased significantly compared to the control group while the seminal plasma fructose concentration in the treatment group decreased, spermatozoa concentration per ejaculate increased which could be linked to the consumption of excess fructose to meet the necessary energy for the metabolism.

As a result, it can be pointed out that Boron used in the research may be an alternative substance for the extenders to be positively developed and it has no negative effect along with increasing some parameters in a positive way when short term storage semen parameters are considered. However, further studies must be planned and supported with fertility parameter in order to ground this study more profoundly.

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