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RESEARCH ARTICLE

The effect of litter size and gender on immunoglobulins and oxidative stress in Damascus goats

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Halep Keçilerinde doğan yavru sayısı ve cinsiyetinin immunoglobulinler ve oksidatif stres üzerine etkisi

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Öz

Amaç: Bu çalışmada, keçilerde yavru sayısı (tek-ikiz) ve cinsiyetin immunoglobulin seviyesi ile oksidatif stres üzerine etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Tek ve ikiz yavru doğuran Halep keçilerinden doğum sonrası ilk 20 dakika içinde, kontrol grubu olarak ise gebe olmayan Halep keçilerinden kan örnekleri alındı ve elde edilen kan plazmalarından immunoglobulin G (IgG), immunoglobulin M (IgM), malondialdehid (MDA), nitrik oksit (NO) ve total antioksidan durum (TAS) değerleri ölçüldü.

Bulgular: İkiz gebelerde IgG ile IgM düzeylerinin gebe olmayanlara göre yüksek olduğu belirlenirken (P<0.05), ikiz gebelerin MDA düzeyleri gebe olmayanlardan düşük belirlendi (P<0.05). İkiz doğum yapan keçilerin NO düzeyi ise kontrol grubundan ve tekiz gebelerden daha yüksek olarak belirlendi (P<0.05). Parametreler arasında yapılan korelasyon değerlendirmesinde ise IgG ile IgM arasında pozitif ilişki belirlenirken (r=0.946, P<0.001), diğer parametreler arasında korelasyon belirlenmedi (P<0.05). Erkek yavru doğuran keçilerde IgG, IgM, MDA, TAS ve NO değerleri dişi yavru doğuran keçilere göre daha yüksek belirlenmesine rağmen istatistiki olarak fark görülmedi (P<0.05).

Öneri: Sonuç olarak, ikizliğin immunoglobulin seviyelerinde artışa sebep olduğu, doğan yavru cinsiyetinin ise immunoglobulin seviyeleri ve oksidatif stres üzerinde etkisinin olmadığı belirlendi.

Anahtar kelimeler: Keçi, immunoglobulin G, immunoglobulin M, malondialdehyde, nitrik oksit

Abstract

Aim: The aim of this study is to investigate the effects of litter size (singleton-twin) and gender on the immunoglobulin level and oxidative stress in Damascus goats.

Materials and Methods: Blood samples were taken with in 20 minutes after delivery from the Damascus goats delivering singleton and twin and non-pregnant Damascus goats that were served as control group. The values of immunoglobulin G (IgG), immunoglobulin M (IgM), malondialdehyde (MDA), nitricoxide (NO) and total antioxidant status (TAS) were measured in blood plasma.

Results: IgG and IgM levels in twin pregnant goats were higher than non-pregnant ones (P<0.05); whereas, MDA levels of twin pregnant goats were lower than non-pregnant ones (P<0.05). NO level in goats delivering twin was detected to be higher than pregnant goats delivering single and control group (P<0.05). Positive correlation was determined between IgG and IgM (r=0.946, P<0.001); whereas, no correlation was determined between other parameters (P<0.05). Although IgG, IgM, MDA, TAS and NO values were determined to be higher in goats delivering male kids than goats delivering female kids, no statistical difference was observed (P<0.05).

Conclusion: As a result, twin pregnancy may cause an increase in immunoglobulin levels but the gender of kids did not have any effect on immunoglobulin level and oxidative stress.

Keywords: Goat, immunoglobulin G, immunoglobulin M, malondialdehyde, nitric oxide

Introduction

Maternal immunoglobulins are very crucial defense substances that protect newborn kids from neonatal diseases until their own immune systems develop (Ahmad et al 2000, Galán-Malo et al 2014). Maternal factors such as dystocia, twin pregnancy and number of delivery, as well as litter-related factors such as early access to udder, receiving sufficient colostrum, drying off the kid, and preventing loss of body temperature also play a role in formation of neonatal deaths (Nowak and Poindron 2006, Gapper et al 2007, Hernandez-Castellano et al 2015). Among these factors, colostrum has a distinct importance in terms of transferring its immunoglobulin to the litter. In ruminants, it is extremely critical for preventing death of neonatal offspring to intake sufficient amount of immunoglobulins transferred via colostrum (Vihan 1988, Ahmad et al 2000, Dominguez et al 2001, Tabatabaei et al 2013). Antibody transfer is not possible to occur during the pregnancy period because ruminants have epitheliochorial placenta, immunity is provided by intake of colostrum secreted with the delivery (Dominguez et al 2001, Abdel-Salam et al 2014, Galán-Malo et al 2014). Even though there are numerous studies on serum immunoglobulin levels in normal pregnancy, results are contradictive. There are studies indicating that serum immunoglobulin levels were stabled (Gusdon 1969), increased (Panpatana et al 1974) and decreased (Benster and Wood 1970) during pregnancy in humans. In a study conducted on sheep, any difference was not observed in IgG concentrations of colostrum in multiple births; however, parturition number was reported to be effective on IgG concentration. It was stated in the study that colostrum's IgG and serum IgG concentrations were similar, the rate of death in lambs born of these sheep with low IgG level of colostrum was higher (Tabatabaei et al 2013).

While changes occurring during pregnancy makes mother predisposed to formation of oxidative stress (Mutinati et al 2013), the increase of maternal metabolism leads release of reactive oxygen species (ROS) in excessive amounts during the pregnancy (Rizzo et al 2012). Even though ROS is required for fetal development (Mutinati et al 2013), ROS produced in excessive amount causes oxidative stress (Garrel et al 2010). Total antioxidant status (TAS) can be identified in order to reveal antioxidant capacity (Aytekin et al 2015). Malondialdehyde (MDA) is an indicator of lipid peroxidation. Therefore, it is frequently used for presenting oxidative damage (Morrow 2000, Lykkesfeldt and Svendsen 2007). MDA concentrations are reported to increase in normal and complicated pregnancies (Biondi et al 2005). Increased MDA concentrations generally indicate that oxidative stress has formed during pregnancy (Myatt 2006). However, it was observed in previous studies that antioxidant activity concentrations increased and there was no substantial change in MDA levels during pregnancy (Shilina et al 1999). In a study on Saanen goats, MDA concentrations were determined to decrease considerably during pregnancy and after delivery compared to values obtained before pregnancy. In the same study, it was stated that in comparison with MDA concentrations, antioxidant activity concentrations increased gradually during pregnancy compared to pre-gestational values and there was a significant negative correlation between MDA and antioxidant activity concentrations (Aydin and Köse 2015).

Previous studies have reported that nitric oxide (NO) has important effects on reproductive functions. NO has been suggested to play important roles in endocrine control of reproduction, regulation of ovarian activities, fertilization, conception, and occurrence of delivery (Çelik and Bülbül 2004). NO levels were observed to increase during pregnancy in studies on humans (Yüksel and Yiğit 2015) and goats (Abdel-Ghani et al 2016).

The aim of this study is to determine the effect of litter size and gender on maternal immunoglobulins and oxidative status in Damascus goats.

Materials and Methods

Animal material of the study consisted of 51 Damascus goats aged 2-3 years. Twenty of the goats had twin delivery, 16 single delivery and 15 were non-pregnant.

The goats were observed as soon as deliveries started and blood samples were centrifuged after they were taken within the first postpartum 20 minutes from goats delivering. The obtained blood plasmas were preserved at– 20 oC. "blood samples were taken 20 minutes after delivery, centrifuged and stored at at– 20 oC until the measurements."

Plasma IgG (ELISA kit, Catalogue No. 201-07-0065, Shanghai Sunred Biological Technology Co., Ltd. Shanghai, China), IgM (ELISA kit, Catalogue No. 201-07-0069, Shanghai Sunred Biological Technology Co., Ltd. Shanghai, China), TAS (ELISA kit, Catalogue No. RL0017, Rel Assay Diagnostics Kit, Mega Tıp, Gaziantep, Turkey), MDA (ELISA kit, Catalogue No. 21044, Bioxytech-MDA 586, Oxisresearch, USA), and Nitric oxide (ELISA kit, Catalogue No. 780001, Cayman Chemical Company, Ann Arbor, MI, USA) levels were identified with ELISA reader (MWGt Lambda Scan 200, Bio-Tek Instruments, VT, USA).

The data of the study were expressed as mean ± standard error (SE). The data obtained from study groups were evaluated by using ANOVA and Tukey tests. Control of significance between genders was assessed with independent t test. Correlation between parameters was determined by using Pearson's test (SPSS 22.0). The value of $P<0.05$ was accepted as significant.

Approval of the ethics committee

This project was carried out with the approval of Selcuk University Faculty of Veterinary Medicine Ethics Committee dated 26.02.2015 and numbered 2015/27.

Results

As shown at table 1, IgG, IgM and MDA levels according to litter size. It was determined that while IgG and IgM levels were higher in twin pregnant goats compared to non-pregnant (P<0.05), MDA levels were lower in pregnant goats delivering twin than non-pregnant ones (P<0.05). NO level was higher in pregnant goats delivering twin than pregnant delivering singleton and control group (P<0.05).

Table 2 shows the correlation between the measured parameters. While there was a positive correlation between IgG and IgM (r=0.946, P<0.001), no correlation was determined between other parameters (P>0.05).

Table 3 shows the effect of gender on IgG, IgM, TAS, MDA and NO. Gender difference was determined to have no effect on measured parameters (P>0.05).

Discussion

Maternal immunoglobulins ensure offsprings to be protected against disease until their own immune systems develop. Particularly IgG is one of important factors in this protection (Chen et al 1999). Substantial changes are observed in physiological parameters during pregnancy and delivery. As a result of these changes, maternal oxidative status is known to be affected during pregnancy (Rizzo et al 2012, Mutinati et al 2013).

When IgG and IgM levels were evaluated in this study, IgG and IgM levels were identified to increase significantly (P<0.05) in Damascus goats delivering twins compared to those in control group (non-pregnant) and those delivering singleton (Tables 1 and 2). Positive correlation (r = 0.946, P<0.001) was also observed between these two parameters

Table 1. Plasma IgG, IgM, TAS MDA and NO values in Damascus goats delivering singleton twins, and non-pregnant (Control) (Mean ± SE)

	IgG mg/ml	IgM mg/ml	TAS mmol/L	MDA µM	NO µM
Twins birth	23.19±1.07^{a}	14.87±0.53^{a}	0.81±0.09^{a}	1.46±0.46^{b}	7.24±0.52^{a}
Singleton birth	18.86±1.29^{ab}	12.93±0.88^{ab}	0.72±0.12^{a}	2.91±1.16^{ab}	5.00±0.17^{b}
Non-pregnant	16.74±1.79^{b}	11.32±1.28^{b}	0.68±0.12^{a}	5.85±1.60^{a}	3.95±0.26^{b}

IgG: immunoglobulin G, IgM: immunoglobulin M, MDA: malondialdehyde, TAS: Total antioxidant status, NO: Nitric oxide.
a, b: Different letters in the same column are statistically different (ANOVA, Tukey test, P<0.05).

Table 2. Correlation levels between plasma IgG, IgM, TAS, MDA and NO values in Damascus goats delivering singleton, twins, and non-pregnant (Control) (Mean ± SE)

	IgG mg/mL	IgM mg/mL	TAS mmol/L	MDA (µM)
NO (µM)	r = 0.218	r = 0.176	r = -0.064	r = -0.134
	P < 0.129	P < 0.221	P < 0.659	P < 0.349
	NS	NS	NS	NS
MDA (µM)	r = -0.053	r = -0.024	r = -0.112	
	P < 0.712	P < 0.871	P < 0.440	
	NS	NS	NS	
TAS mmol/L	r = 0.063	r = -0.070		
	P < 0.666	P < 0.628		
	NS	NS		
IgM mg/mL	r = 0.946			
	P < 0.001			

IgG: Immunoglobulin G, IgM: Immunoglobulin M, MDA: Malondialdehyde, TAS: Total antioxidant status, NO: Nitric oxide.
NS: Insignificant (Pearson's correlation, P>0.05).

Table 3. Plasma IgG, IgM, TAS, MDA and NO levels in Damascus goats delivering male and female kids (Mean ± SE)

	Male	Female	P
IgG mg/mL	20.96±1.50	16.77±2.00	P>0.05
IgM mg/mL	13.84±1.10	12.04±1.40	P>0.05
TAS mmol/L	0.90±0.20	0.55±0.11	P>0.05
MDA (µM)	3.23±2.10	2.61±1.20	P>0.05
NO (µM)	5.19±0.22	4.80±0.28	P>0.05

(Independent t Test)

(Table 2). While this value was determined as 23.19 mg/mL in twin delivery, it was measured as 18.86 mg/mL in singletons and 16.74 mg/mL in control group. Number of studies on immunoglobulin levels in maternal blood immediately after delivery were found to be limited during the literature review. In the limited number of studies, blood IgG level was reported to be higher in sheep and goats delivering twins compared to those with singleton (Ha et al 1986, Csapó et al 1994). As the results of the present study and results of other studies were evaluated together, it can be asserted that litter size may increase immunoglobulin level, this situation is a physiological reaction to protect twin offspring. Kid gender was determined to have no effect on IgG and IgM amounts ($p<0.05$) in the present study (Table 3). Similarly, Khan and Ahmad (1997) stated in their study that gender was not effective in terms of immunoglobulin levels in newborn lambs. This indicates that gender of kids does not affect maternal immunoglobulin levels.

In multiple pregnancies in sheep, oxidative stress is described as an important risk factor for development of pathologies like pregnancy toxemia (Olfati et al 2013). In the present study, MDA level decreased in twin pregnancy compared to control group ($p<0.05$); however, TAS level did not change ($p>0.05$) (Table 3). Lipid peroxidation occurs as a result of oxidative stress developing in organisms. Despite the fact that the number of studies about gestational oxidative stress in humans is numerous (Shilina et al 1999, Biondi et al 2005), relevant studies were not found for pregnant goats during the pregnancy. Oxidative stress increases depending on elevation of reactive oxygen species resulting from increased placental metabolic activity in early periods of pregnancy. At the same time, placental progesterone stimulates elevation of malondialdehyde (MDA) and blood lipids (Yüksel and Yiğit 2015). MDA is the mostly preferred biological marker for determination of lipid peroxidation (Aydin and Köse 2015). In this study, MDA levels were 5.85 µM in non-pregnant Damascus goats, 2.91µM in those delivering singleton, and 1.46 µM in those delivering twins. Different results were obtained with respect to species of organism and sampling time from studies on MDA levels in mothers during pregnancy. In a study conducted byAbdel-Ghani et al (2016) on pregnant goats, they determined that MDA levels were higher in advancing months of the pregnancy in twin and multiple pregnant goats compared to singleton pregnant goats. In addition, MDA level was observed to increase based on pregnancy period in another study on sheep (Öztabak et al 2005, Gür et al 2011). In a study on Awassi sheep, MDA levels were reported not to differ significantly in early and late pregnancies (Erisir et al 2009). It was observed in studies that homogenous results were not obtained concerning MDA levels. The most important difference between this study and other studies is different sampling time. In this study, low MDA level determined in pregnant goats delivering twins may be associated with high antioxidant capacity, sufficient physiological condition of goats, and particularly antioxidant effect of immunoglobulins (Counts et al 2014, Aydin and Köse 2015). TAS level which is a marker of antioxidant capacity in the organisms was determined to remain unchanged in this study, which confirmed this result (Table 3). It was reported that TAS level could decrease in oxidative damage developing in organisms (Altindag et al 2007).

Nitric oxide (NO) is an important factor regulating uterus and fetoplacental blood flow by contributing to maternal systemic vasodilatation during pregnancy. It is also an endothelial vital factor improving endothelial cell proliferation and inhibiting apoptosis (Çelik and Bülbül 2004). Consequently, an angiogenic and vasodilator marker NO is considered to be the golden standard. A previous study reported that NO values increased in gestational months 2, 3 and 4 in goats with singleton, twin and multiple pregnancy (Abdel-Ghani et al 2016). In the present study, NO level was detected to be higher in goats delivering twin compared to non-pregnant goats and those delivering singleton ($P<0.05$) (Table 1). Possible reason behind why NO levels was higher in goats with twin pregnancy than the other groups may be associated with the fact that it provides vasodilatation which is its major role in the organism due to the need of feeding with more blood in twin pregnancy (Gültekin et al 1996) as well as it is released more in order to occurance easier delivery thanks to its smooth muscle relaxant effect (Çelik and Bülbül 2004).

Conclusion

It can be expressed that litter size and immunoglobulin le-

vels of pregnant Damascus goats may display a positive correlation for protecting each kid, antioxidant capacity in goats may be at sufficient level to meet multiple pregnancy, and gender of the kid has no effect on immunoglobulin level and antioxidant capacity.

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