Slovak J. Anim. Sci., 46, 2013 (2): 39-44

ISSN 1337-9984

THE EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZABILITY OF SEMEN FROM ANGORA, KILIS AND SAANEN GOATS

R. KULAKSIZ[1]*, U. Ç. ARI[1], A. DAŞKIN[2], A. G. ÜNER[3]

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Sciences, Kafkas University, Kars, Turkey
[2]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey
[3]Department of Physiology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydın, Turkey

ABSTRACT

The aim of this study was to investigate effect of different glycerol concentrations on freezability of semen from Angora, Kilis and Saanen goats. Three male goats from each breed were selected and ejaculates were collected with artificial vagina. Three ejaculates from each breed were pooled and extended with skim milk-based extender containing 10 % (v/v) egg yolk and 0 %, 3 %, 5 %, 7 % and 9 % (v/v) glycerol (G0, G3, G5, G7 and G9, resp.) as a cryoprotectant. Extended semen from different goat breeds was equilibrated, cryopreserved and stored in liquid nitrogen. The best post-thaw motility for Angora (51.6 %) and Kilis (75.0 %) goats was obtained with G5 concentration, while the best post-thaw motility for Saanen goat (61.6 %) was obtained with G7 concentration (P<0.001). Similar results were recorded for percentage of live spermatozoa for Angora (58.1 % in G5), Kilis (78.5 % in G5) and Saanen (64.0 % in G7) goats (P<0.001). The lowest abnormal spermatozoa percentages were obtained with G5 concentration for Angora (34.3 %), Kilis (30.8 %) and Saanen (40.8 %) goats (P<0.001). While different glycerol concentrations for goat breeds were considered, it was determined that suitable glycerol percentages for Angora, Kilis and Saanen goats were 5 %, 5-9 % and 7 % respectively. It was concluded that glycerol concentration is an important factor affecting freezability of goat semen from different breeds.

Key words: goat breeds; goat semen; glycerol; cryopreservation

INTRODUCTION

In Turkey there are over 6 million heads of goats. Goat livestock is an important source of milk and meat production in Turkey. Anatolian black goats are the most widespread breed but are low in production. Because native breeds are poor producers, one of the approaches to improve is to adopt a genetic strategy of crossbreeding. Hence, the goats of Saanen may be the breed of choice because of their high milk yield and fecundity. Therefore, cryopreservation of Saanen reared in Turkey is important issue to carry out successful genetic strategy of crossbreeding (Kulaksız and Daşkın, 2010).

Angora and Kilis are two main important goat breeds raised in Turkey. There are several advantages of raising these species, such as their ability of adaptation to harsh conditions of hilly and mountainous districts of Turkey. Angora goats (breed is named after the town Ankara, in Central Anatolia) are reared mostly for mohair production and Kilis goats (originate in Kilis province, which lies on the Syrian border) are the major dairy breeds which have an important role in milk and meat production (Atay *et al*., 2011). These breeds are mainly endangered genetic resources of Turkey. In this context,

***Correspondence:** E-mail: recaikulaksiz@gmail.com
Recai Kulaksız, Department of Reproduction and Artificial Insemination,
Faculty of Veterinary Sciences, Kafkas University, 36300, Paşaçayırı, Kars, Turkey
Tel.: +90 505 3220756 Fax: +90 474 2426807

Received: January 10, 2013
Accepted: April 15, 2013

the cryopreservation of gametes is important because it would allow us to support a genome resource bank for this breed for an indefinite period of time.

Cryoprotectants used for freezing of sperm cells provide protection from cold shock and the other damages during freezing. Optimum adding rates of cryoprotectants become peculiar to species and require determination of some parameters of cell membrane. Glycerol has been extensively used as a cryoprotectant (Purdy, 2006). Recent studies have demonstrated that glycerol remains to be the most effective cryoprotective compound for freezing goat semen and no enhancement was showed by the addition of other compounds (Farshad *et al*., 2009; Bezerra *et al*., 2011). Therefore, glycerol is the most commonly used cryoprotectant for goat semen.

There are no more studies about different concentrations of glycerol for cryopreservation of goat semen (Deka and Rao, 1986; Biswas *et al*., 2002; Farshad *et al*., 2009). However, a lot of studies about glycerol concentrations have been carried out about freezability of semen from different species (Pena *et al*., 1998; Rota *et al*., 1998; Baran *et al*., 2000; Buhr *et al*., 2001; Abbas and Andrabi, 2002; Rasul *et al*., 2007; Awad, 2011; Hoffman *et al*., 2011). Moreover, we did not find any study or other information about interaction of goat breed and also other species with glycerol concentrations on freezability of semen until now.

There is no information on the freezability of Kilis goat semen and no study on the effect of different glycerol concentration on the freezebality of Angora, Kilis and Saanen goat semen has been reported. Therefore, the present study was designed to determine the suitable glycerol concentration for cryopreservation of Saanen, Angora, and Kilis goat semen.

MATERIAL AND METHODS

Location

Animals were housed at the Education Research and Practice Farm, Faculty of Veterinary Medicine, University of Ankara, Turkey at 39º57 N, 32º53 E, at an altitude of 850 m.

Experimental animals

Nine healthy male goats of Angora (n=3), Saanen (n=3) and Kilis (n=3) breed, aged between 2 and 3 year, were selected for the study. They were housed in a covered shelter with an open-air run and were allowed to walk freely. Throughout this study the nutrition of the goats remained uniform and constant. Feeding consisted of 750 g commercial concentrate, and 1 kg alfaalfa hay/animal/day; water was provided ‘‘ad libitum’’.

Semen collection, dilution, freezing and thawing

During the breeding season, semen was collected from each goat once a week by means of artificial vagina. Immediately after collection, the ejaculates were placed in a water bath (37ºC) and aliquots were taken for the assessment of semen quality. After individual examination, three ejaculates from the same breed (i.e. Angora, Saanen or Kilis) were pooled and only ejaculates with at least 85 % estimated progressive motility, were used for freezing.

Five extenders were prepared as follows: Skimmed milk-based egg yolk (10 %) (SMEY) added by glycerol 3 % (SMEY3), glycerol 5 % (SMEY5) , glycerol 7 % (SMEY7), glycerol 9 % (SMEY9) and SMEY without glycerol (0 %; control). The pooled ejaculates from different breeds were divided into 5 aliquots, and diluted with SMEG0, SMEG3, SMEG5, SMEG7, or SMEG9 to reach the average semen concentration of $400x10^6$/ml.

The extended semen from different breeds (n=3) and groups (n=5) was separately packaged in 0.25 ml straws, and equilibrated at 4ºC for 2 hours. The straw was frozen in a styrofoam box at 4 cm above the liquid nitrogen (LN) surface for 15 minutes. The frozen semen was stored for 24 hours in LN for further evaluations. The frozen semen straws from different breeds and groups were thawed in a 37ºC water bath for 30 seconds and semen evaluation was carried out as follows.

Semen evaluation

Sperm motility was assessed using a phase-contrast microscope (x 400 magnification, Olympus BH-2, Olympus Optical CO. LTD., Japan), with a warm stage maintained at 37ºC. A wet semen mount was made using 2µL semen placed directly onto a microscope slide and covered by a cover slip. For each sample, at least 5 microscopic viewfields were examined by two trained observers. The mean of the three successive evaluations was calculated as the final motility score (Ax *et al*., 2000).

The viability of sperm in the sample was assessed by means of an eosin-nigrosin staining. The sperm smears were prepared by mixing a drop of semen with two drops of stain on a warm slide and spreading the stain immediately with the aid of a second slide. The viability was assessed by counting 200 sperm cells with a bright-field microscopy (X400, Olympus CX21FS1, Olympus Optical CO. LTD., Japan). The sperm cells showing partial or complete colorization were considered to be non-viable or dead. Only the sperm showing strict exclusion of the stain were considered to be alive (Evans and Maxwell, 1987).

For the assessment of sperm abnormalities, at least three drops of each sample were added to an Eppendorf container with 1 mL Hancock solution (62.5 mL formalin (37 %), 150 mL saline solution, 150 mL buffer solution

and 500 mL double-distilled water). One drop of this semen mixture was put onto a slide and covered with a cover slip. The percentage of sperm abnormalities was determined by counting a total of 200 sperm under phase-contrast using an immersion objective (Schafer and Holzman, 2000).

Statistical analysis

In the present study, totally 6 replications for each glycerol concentration were carried out. All data from 6 replications were examined for normal distribution with Shapiro-Wilk testand homogeneity of variance - with Levene's test. In case of abnormality of the distribution, logarithmic transformation of data was performed (in order to normalize the distribution). Two-way analysis of variance (ANOVA) was conducted to assess the effect of breed and concentration of glycerol on motility, proportion of live sperm and abnormal spermatozoa. *Post hoc* multiple comparisons were performed using Duncan test. *P* values <0.05 were considered to be significant. The results were presented as the least square of means.

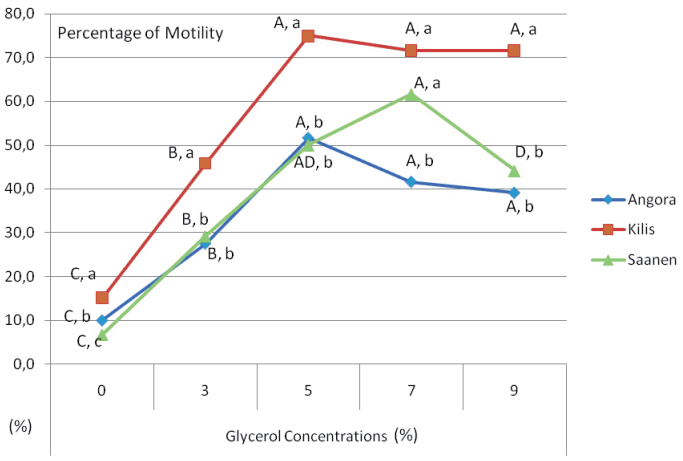
RESULTS AND DISCUSSION

Kilis goat semen had higher motility and viability at different concentrations of glycerol after thawing, compared with Saanen and Angora (P<0.001; Fig. 1 and 2). Motility and viability at 5 % concentration of glycerol was higher than those at other glycerol concentrations (except 7 % glycerol concentration), considered glycerol concentrations in all breeds (P<0.001; Fig. 1 and 2).

When breeds were considered, Saanen goat semen showed higher percentage of abnormal spermatozoa after thawing compared to Angora and Kilis (P<0.001; Fig. 3). Percentage of abnormal spermatozoa at 5 % glycerol concentration was lower than other glycerol concentrations in all breeds, considered glycerol concentrations (P<0.001; Fig.3).

There were statistically significant interactions between concentrations of glycerol and breeds for all spermatologic parameters after thawing (P<0.001; Fig.1, 2 and 3).

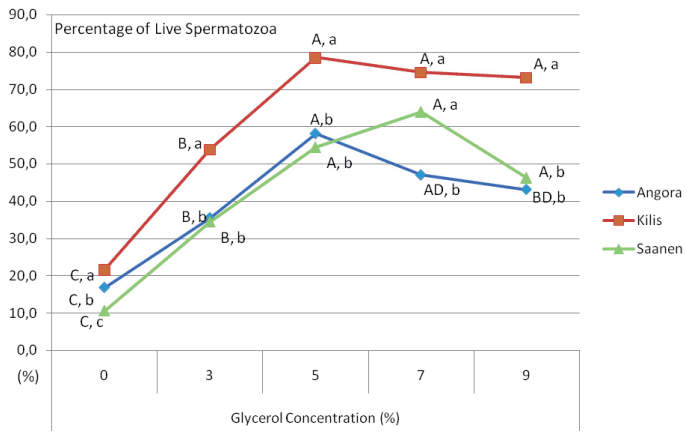
Our results indicated on the interaction between goat breed and glycerol concentration. While 7 % glycerol concentration in Saanen semen cryopreservation provided optimum freezability, Angora goat semen needed 5 % glycerol to provide optimum freezability. Moreover, Kilis goat semen could be successfully cryopreserved using 5, 7 and 9 % glycerol concentrations. In this study, it was determined that 0 and 3 % glycerol concentrations detrimentally affected freezability of semen from three goat breeds. On the other hand, higher concentrations of glycerol (i.e. 7 and 9 %) increased post-thaw semen abnormality in all breeds.



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

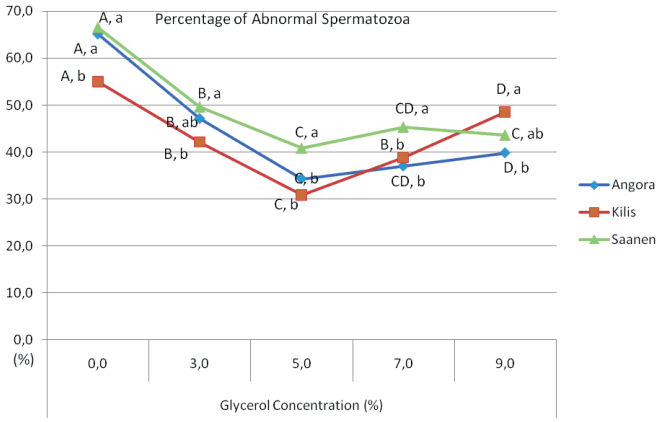
Fig. 1: Post-thawing progressive motility of sperm from Angora, Kilis and Saanen goats at different glycerol concentrations



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

Fig. 2: Post-thawing percentage of live spermatozoa of Angora, Kilis and Saanen goats at different glycerol concentrations



A, B, C, D: Means with different letter are significantly differerent among different concentrations of glycerol for the same goat breed (P<0,001).

a, b, c: Means with different letter are significantly different among goat breeds at the same concentration of glycerol (P<0,001).

Fig. 3: Post-thawing percentage of abnormal spermatozoa for Angora, Kilis and Saanen goats at different glycerol concentrations

There are very limited studies about different concentrations of glycerol for cryopreservation of goat semen. However, a lot of studies have been carried out about freezability of semen from different species (Pena *et al.*, 1998; Rota *et al.*, 1998; Baran *et al.*, 2000; Buhr *et al.*, 2001; Abbas and Andrabi, 2002; Sönmez and Demirci, 2004; Rasul *et al.*, 2007; Awad, 2011; Hoffman *et al.*, 2011, Swelum *et al.*, 2011). Therefore, we had to compare our results with not only those from goat species but also those from other species.

Deka and Rao (1986) showed that the different concentrations (i.e. 4, 6.4, 9 %) of glycerol did not differ in post-thaw sperm motility and abnormalities in goat semen. Results of Deka and Rao (1986) are similar to our results for Kilis goat semen. However, our results for Saanen and Angora goat semen are different. Differences between our results and those by Deka and Rao (1986) may be derived from the differences between goat breeds, extenders used for cryopreservation and also methods of post-thaw semen analyses. Biswas *et al.* (2002) used 5 %, 7 % and 10 % of glycerol for freezing of goat semen. They found that motility and viability of thawed sperm frozen in 7 % glycerol concentration were superior to those of sperm frozen and thawed in

Table 1: Post-thawing progressive motility, viability and abnormality for spermatozoa of Angora, Kilis and Saanen goats at different glycerol concentrations

Breed	N	Concentration of glycerol (%)				
		0	3	5	7	9
				Motility (%)		
Angora	6	$^{C}10.0\pm1.83^{b}$	$^{B}27.5\pm2.14^{b}$	$^{A}51.6\pm1.05^{b}$	$^{A}41.6\pm1.05^{b}$	$^{A}39.1\pm0.83^{b}$
Kilis	6	$^{C}15.0\pm1.29^{a}$	$^{B}45.8\pm1.54^{a}$	$^{A}75.0\pm1.83^{a}$	$^{A}71.6\pm1.05^{a}$	$^{A}71.6\pm1.05^{a}$
Saanen	6	$^{C}6.6\pm1.05^{c}$	$^{B}29.1\pm0.83^{b}$	$^{AD}50.0\pm1.29^{b}$	$^{A}61.6\pm1.05^{a}$	$^{D}44.1\pm1.54^{b}$
				Viability (%)		
Angora	6	$^{C}16.8\pm2.43^{b}$	$^{B}35.5\pm2.86b$	$^{A}58.1\pm2.04^{b}$	$^{AD}47.0\pm1.93^{b}$	$^{DB}43.1\pm0.95^{b}$
Kilis	6	$^{C}21.5\pm1.09^{a}$	$^{B}53.8\pm0.79a$	$^{A}78.5\pm1.80^{a}$	$^{A}74.5\pm0.56^{a}$	$^{A}73.1\pm0.95^{a}$
Saanen	6	$^{C}10.6\pm0.88^{c}$	$^{B}34.5\pm0.76b$	$^{A}54.5\pm0.62^{b}$	$^{A}64.0\pm1.39^{a}$	$^{A}46.3\pm1.63^{b}$
				Abnormality (%)		
Angora	6	$^{A}65.1\pm1.58^{a}$	$^{B}47.1\pm2.12^{ab}$	$^{C}34.3\pm1.43^{b}$	$^{CD}37.0\pm1.61^{b}$	$^{D}39.8\pm1.52^{b}$
Kilis	6	$^{A}55.0\pm1.44^{b}$	$^{B}42.1\pm1.49^{b}$	$^{C}30.8\pm1.40^{b}$	$^{B}38.8\pm1.14^{b}$	$^{D}48.5\pm0.89^{a}$
Saanen	6	$^{A}66.5\pm0.76^{a}$	$^{B}49.6\pm1.28^{a}$	$^{C}40.8\pm1.08^{a}$	$^{CB}45.3\pm0.84^{a}$	$^{C}43.6\pm1.15^{ab}$

$^{A,B,C,D:}$ Means with different superscripts within the same row are significantly different among different glycerol concentrations for same goat breed (P<0.001).

$^{a,b,c:}$ Means with different superscripts within the same column are significantly different among goat breeds for same concentration of glycerol (P<0.001).

5 % and 10 % glycerol concentrations. Moreover, it was determined that glycerol concentrations did not affect individual freezability of goat semen (Biswas *et al.*, 2002). However, Biswas *et al.* (2002) determined sharp increase and then decrease in post-thaw motility for different concentrations of glycerol. We determined that 5 % and higher concentration of glycerol did not sharply affect post-thaw motility of goat semen for all breeds. Farshad *et al*. (2009) found that post-thaw semen quality was higher at 5 and 7 % glycerol compared with other glycerol concentrations (i.e. 1 %, 3%) in Markoz goat semen. These results are similar with our results for Angora (Markhoz) goat semen cryopreservation.

Sönmez and Demirci (2004) tested different concentrations of glycerol and they determined that 5 % glycerol provided successful cryopreservation of ram semen. Furthermore, higher concentration of glycerol (i.e. 7 %) negatively affected post-thaw semen quality. Although we tried different concentrations of glycerol on different species from small ruminant, the findings of Sönmez and Demirci (2004) are similar to those in our study. However, Awad (2011) compared 3 % and 6 % glycerol concentrations in cryopreservation of ram semen and, interestingly, he did not find any differences between concentrations of glycerol.

In other species, except small ruminants, the studies about glycerol concentrations have been carried out. Rota *et al.* (1998) compared 3 % and 5 % concentrations of glycerol in canine semen cryopreservation. They found that 5 % glycerol is suitable to cryopreserve canine semen, while Cardoso *et al.* (2003) did not find any differences among different concentrations of glycerol (i.e. 4, 6 and 8 %) in cryopreservation of canine semen. On the other hand, Baran *et al.* (2000) investigated interactions between different extenders (TRİS vs. skimmed milk-based) and different glycerol concentrations (4 and 7 %) in canine semen cryopreservation. They determined that 7 % concentration of glycerol had toxic effect on cryopreservation of canine semen extended with TRIS and skim milk based extenders. Pena *et al*. (1998) used different concentrations of glycerol and found that 8 % glycerol provided success for cryopreservation of canine semen.

Siwelum *et al.* (2011) investigated interactions between 7 % concentration of glycerol and different extenders (TRIS and skim milk based) in bull semen cryopreservation. They found that 7 % glycerol in TRIS improved post-thaw semen parameters compared to skim milk-based extenders in bull semen cryopreservation. Abbas and Andrabi (2002) used range concentrations (2-12 %) of glycerol for cryopreservation of bufallo semen. They determined that 6 or 7 % concentration of glycerol provided successful cryopreservation of bufallo semen. Rasul *et al.* (2007) compared different concentrations of glycerol for freezability of buffalo semen, and they obtained similar results with Abbas and Andrabi (2002). On the other hand, Rasul *et al.* (2007) determined that lower doses of glycerol (0 and 3 %) adversely affected freezability of buffalo semen. These results are similar to our findings, although the species are different.

Buhr *et al.* (2001) tested different doses of glycerol (0, 2, 4, 8 %) for cryopreservation of boar semen. They found that 2 and 4 % concentrations of glycerol provided higher post-thaw motility and acrosomal integrity. Hoffmann *et al.* (2011) showed that different concentrations of glycerol (1-4 %) for cryopreservation of stallion semen extended with skim milk-based extender did not affect post-thaw semen quality.

Briefly, results from other similar studies pointed out that different concentrations of glycerol may have dissimilar effect on freezability of semen from different breeds and species.

CONCLUSION

Whilst the results obtained from the current study were considered, it was concluded that the different concentrations of glycerol may influence the success of cryopreservation of semen from different goat breeds. It was observed that suitable glycerol percentages for Angora, Kilis and Saanen goats were 5 %, 5-9 % and 7 % respectively. However, these results warrant future fertility studies where semen cryopreserved with different concentrations of glycerol will be used.

ACKNOWLEDGEMENT

This study was supported by grants from TUBITAK, Turkey (KAMAG-106G005).

REFERENCES

ABBAS, A. – ANDRABİ, S.M.H. 2002. Effect of different glycerol concentrations on motility before and after freezing, recovery rate, longevity and plasma membrane integrity of Nili-Buffalo bull spermatozoa. *Pakistan Vet. J.*, vol. 22, 2002, p. 1-4.

ATAY, O. – GÖKDAL, Ö. – KONYALI, A. – KESKİN, M. 2011. Türkiye'de yetiştirilen keçi genotipleri. T*arım Günlüğü (Agricultural Agenda)*, vol. 1, 2011, p. 103-109.

AWAD, M. M. 2011. Effects of sub-optimal glycerol concentration and cholesterol-loaded cyclodextrin in a Tris-based diluent on cryopreserved ram sperm longevity and acrosomal integrity. *Small Rum. Res*., vol. 100, 2011, p. 164-168.

AX, R. L. – DALLY, M. A. – LENZ, R. W. – LOVE, C. C. – VARNER, D. D. – HAFEZ, B. – BELLIN, M. E. 2000. Semen evaluation. In: Hafez, B., Hafez, E.S.E. (Eds.), *Reproduction in Farm Animals*, 7th ed. Lippincott Williams and Wilkins, Philadelphia, p. 365-375.

BARAN, A. – AK, K. – ILERI, I. K. 2000. Köpek spermasının farklı oranlarda gliserol içeren sulandıcılarda dondurulması. *İstanbul Üniv. Vet. Fak. Derg.*, vol. 26, 2000, p. 265-279.

BEZERRA, F. S. B. – CASTELO, T. S. – ALVES, H. M. – OLİVEİRA, I. R. S. – LİMA, G. L. – PEİXOTO, G. C. X. – BEZERRA, A. S. D. – SİLVA, A. R. 2011. Objective assessment of the cryoprotective effects of dimethylformamide for freezing goat semen. *Cryobiology*, vol. 63, 2011, p. 263-266.

BISWAS, D. – BARI, F. Y. – SHAMSUDDIN, M. – RAHMAN, M. M. – RAHMAN, M. M. 2002. Determination of glycerol percentages for preserving the Black Bengal Buck (*Capra hircus*) spermatozoa for long time. *Pak. J. Biol. Sci*., vol. 5, 2002, p. 715-718.

BUHR, M. M. – FISER, P. – BAILEY, J. L. – CURTIS, E. E. 2001. Cryopreservation in different concentrations of glycerol alters boar sperm and their membranes. *J. of Andrology*, vol. 22, 2001, p. 961-969.

CARDOSO, R. C. S. – SİLVA, A. R. – UCHOA, D. C. – DA SİLVA, L. D. M. 2003. Cryopreservation of canine semen using a coconut water extender with egg yolk and three different glycerol concentrations. *Theriogenology*, vol.59, 2003, p. 743-751.

DEKA, B. C. – RAO, A. R. 1986. Effect of glycerol level in tris-based extender and equilibration period on quality of frozen goat semen. *Theriogenology*, vol. 2, 1986, p. 231-237.

EVANS, G. – MAXWELL, W. M. C. 1987. Salamon's Artificial Insemination of Sheep and Goats. Butterworths, Sidney, p. 107-141.

FARSHAD, A. – KHALİLİ, B. – FAZELİ, P. 2009. The effect of different concentrations of glycerol and DMSO on the viability of Markhoz Goat spermatozoa during different freezing temperatures steps. *Pak. J. Biol. Sci*., vol. 12, 2009, p. 239-245.

HOFFMAN, N. – OLDENDOF, H. – MORANDINI, M. – ROHN, K. – SIEME, H. 2011. Optimal concentrations of cryoprotective agents for semen from stallions that are classified 'good'or 'poor' for freezing. *Anim. Reprod. Sci.*, vol. 125, 2011, p.112-118.

KULAKSIZ, R. – DAŞKIN, A. 2010. *In vitro* evaluation of Saanen buck semen frozen in different extenders supplemented with various antioxidants. *Ankara Üniv. Vet. Fak. Derg.*,vol. 57, 2010, p. 151-156.

PENA, A. I. – BARRIO, F. – QUINTELA, L. A. – HERRADON, P. G. 1998. Effect of glycerol treatments on frozen-thawed dog sperm longevity and acrosomal integrity. *Theriogenology*, vol. 50, 1998, p. 163-174.

PURDY, P. H. 2006. A review on goat sperm cryopreservation. *Small Rumin. Res*., vol.63, 2006, p. 215-225.

RASUL, Z. – AHMED, N. – ANZAR, M. 2007. Antagonist effect of DMSO on the cryopreservation ability of glycerol during cryopreservation of buffalo sperm. *Theriogenology*, vol. 68, 2007, p. 813-819.

ROTA, A. – LINDE-FORSBERG, C. – VANNOZZI, J. – ROMAGNOLI, S. – RODRIGUEZ-MARTINEZ, H. 1998. Cryosurvival of dog spermatozoa at different glycerol concentrations and freezing/thawing rates. *Reprod. Dom. Anim*., vol. 33, 1998, p. 355-361.

SCHAFER, S. – HOLZMANN, A. 2000. The use of transmigration and spermac stain to evaluate epididymal cat spermatozoa. *Anim. Reprod. Sci*., vol. 59, 2000, p. 201-211.

SONMEZ, M. – DEMIRCI, E. 2004. The effect of ascorbic acid on the freezability of ram semen diluted with extenders containing different proportions of glycerol. *Turk. J. Vet. Anim. Sci*., vol. 28, 2004, p. 893-899.

SWELUM, A. A. – MANSOUR, H. A. – ELSAYED, A. A. – AMER, H. A. 2011. Comparing ethylene glycol with glycerol for cryopreservation of buffalo bull semen in egg-yolk containing extenders. *Theriogenology*. vol. 76, 2011, p. 833-842.