Effects of region and invidualism traits on sperm freezeability of Angora Goats

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Abstract: It is well accepted that freezability of buck semen depends on many different factors, such as breed, age, season, region and even invidual differences. Therefore, we aim to compare freezability of Angora buck semen (Capra hircus ancryrensis) from different regions (R) and inviduals (I). Semen was collected with artificial vagina from 6 bucks; 2 each from 3 different R in Ankara (Lalahan, Ayaş, Nallıhan). Then, fresh semen was extended individually with Tris based extender, equilibrated (+5 °C/2h), loaded into 0.25 straws, frozen in liquid nitrogen vapour (-120 °C/15 minutes) and stored in liquid nitrogen (-196 °C). Frozen straws were thawed in water bath (37 °C/30 seconds) and percentages of total motility, progressive motility and sperm kinetic parameters (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and hyperactivity) were assesed with computer assisted sperm analyzer (CASA). Viability (SYBR-14/PI), acrosomal integrity (FITC-PNA/PI) and mitochondrial activity (JC-1) were evaluated by fluorescein microscope. There was no statistical difference neither between R groups nor I in fresh semen parameters (P>0.05). In frozen thawed samples, the highest progressive and total motility was recorded as 9.97 ± 4.05; 58.42 ± 9.40 respectively in Nallıhan region. Differences were not statically significant in motility or kinetic parameters in neither R groups nor I (P>0.05). The highest viability was 64.29± 5.28 in Ayaş region (P<0.05). The highest mitochondrial activity was 59.84±5.24 in Nallıhan region and there were no significant differences between neither R groups nor I in terms of acrosome integrity (P>0.05). In conclusion, even though there was no significant difference between I and R in fresh semen parameters, differences observed give valuable information about sperm quality before and after freezing in Angora bucks in different places.

Keywords: Angora goat, CASA, freezability, invidualism, region

Ankara Keçilerinde bölgesel ve bireysel özelliklerin sperma dondurulabilirliğine etkisi

Öz: Teke spermasının dondurulması tür, yaş, sezon, bireysel ve bölgesel farklılıklar gibi birçok faktöre bağlı olduğu çok iyi bilinmektedir. Buradan hareket ederek Ankara Tekesi (Capra Hircus Ancryrensis) dondurulabilirliğini bölgesel (R) ve bireysel (I) olarak karşılaştırılması amaçlandı. Sperma her bir bölgeden 2 teke olacak şekilde toplamda 6 adet tekeden (Ayaş, Lalahan, Nallıhan) suni

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vagina ile alındı. Daha sonra taze sperma bireysel olarak Tris bazlı sulandırıcı ile sulandırıldı, alışıma bırakıldı (+5 C^0/2 saat), 0,25 ml'lik payetlere dolduruldu, sıvı azot buharında (-120 C^0/10 dk) donduruldu ve sıvı azotta (-196 C^0) saklandı. Dondurulmuş payetler su banyosunda (37 C^0/30 sn) çözdürüldü ve progresif motilite, total motilite ve kinetik parametreler (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF and hyperactivity) bilgisayar destekli sperm analiz cihazında (CASA) incelendi. Ayrıca çözüm sonu canlılık (SYBR 14/PI), akrozomal bütünlük (FITC-PNA/PI) ve mitokondrial aktivite (JC-I) floresan mikroskop ile değerlendirildi. Taze sperma parametreleri açısından R ve I'da istatistiksel olarak farklılık bulunmadı (P>0,05). Dondurulmuş çözdürülmüş parametreler açısından istatistiksel olarak farklılık görülmemesine rağmen en yüksek progresif ve total motilite sırasıyla 9,97 ± 4,05; 58,42 ± 9,40 Nallıhan bölgesinde tespit edildi. Ayrıca, en yüksek canlılık oranı 64,29±5,28 ile Ayaş grubunda bulundu (P<0,05). En yüksek mitokondrial aktivite 59,84±5,24 ile Nallıhan bölgesinde bulunurken; akrozom bütünlüğü yönünden R ve I'da istatistiki bir farklılık bulunmadı (P>0,05). Sonuç olarak taze sperma verileri açısından R ve I'da istatistiki bir farklılık görülmemesine rağmen farklı bölgelerde bulunan Ankara Tekelerinde dondurma öncesi ve sonrasında spermanın kalitesi açısından önemli bilgiler vermiştir.

Anahtar sözcükler: Ankara keçisi, bireysellik, bölge, CASA, dondurulabilirlik

Introduction

The cryopreservation of mammalian sperm is a complex process that involves balancing many factors in order to obtain satisfactory results (10). These factors are mainly environmental; species and individual dependent. Inherent differences exist in the ability of sperm, from different males, to survive cryopreservation. These differences are probably due to inherent differences between both species and individuals within a species in sperm biochemistry and metabolism (8). The susceptibility of spermatozoa to cryoinjury varies between species, although the reasons for this variation are not clear. Some species (bull and fowl spermatozoa) can be frozen with some success wheras some species (boar, ram, rodent) undergo extensive damage despite the development of species specific protocols (14). Although it is accepted that sperm susceptibility to cryoinjury varies between species a more controversial intra-specific variation in post-thaw semen quality has also been documented between individuals (14). Accounts of individual variation in semen freezeability apply across a range of animal models including bulls (12) and boars (13,15). That is probably because of differences regarding sperm biochemistry and metabolism (8), rooting in the genetic variability of individuals (4). Temperature, humidity, nutrition, disease and parasites are some enviromental (regional) factors that can effect animal production (1).

The present study was conducted to determine R and I effects on semen quality in Angora goats (Capra hircus ancryrensis) in Ankara, Turkey.

Materials and Methods

Chemicals: All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Animals: Semen samples from 6 Angora bucks; 2 each from 3 different regions in Ankara (Lalahan, Ayaş, Nallıhan) were used in this study. The bucks were maintained under uniform breeding conditions at Ankara University Faculty of Veterinary Medicine Practice and Research Farm (40°05′ 37.3″ N, 32°37′15.9″ E) throughout the study. Semen samples were collected twice a week using an artificial vagina, during the breeding season. Ejaculates which met the following criteria were evaluated: volume of 0.5–2 ml; minimum

sperm concentration of 1 × 10^9 sperm/ml; motility of 70%. Immediately following collection, the ejaculates were placed in a water bath (35 °C) until evaluation in the laboratory. Semen assessment was performed within approximately 10 min following collection. Semen sample collection was repeated minumum 7 times from each animal.

Semen Processing: A Tris-based extender (Tris 3,63 gr, citric acid 1,82 gr, glucose 0,5 gr, egg yolk 10% (v/v), glycerol 5% (v/v), pH 6.8) was used as the base extender. Each ejaculate was diluted to a final concentration of 200 × 10^6 spermatozoa/ml. Actual sperm concentration was determined using Accucell photometer (IMV Technologie, L'Aigle, France). Diluted samples were filled in 0.25 ml French straws and equilibrated at 5 °C for a period of 2 h and frozen in liquid nitrogen vapour (-120 °C/15 minutes) and stored in liquid nitrogen (-196 °C). After being stored for at least 24 h, straws were thawed individually (37 °C), for 30 s in a water bath for sperm evaluation. Sperm evaluation was performed on all semen samples immediately after thawing.

Evaluation Post-thaw Semen Parameters

CASA Motility: The Sperm Class Analyzer (SCA) CASA system (Microptic S.L., Barcelona, Spain) was used to analyse sperm motility and various kinematic parameters. A 5 µl sample of diluted semen was put onto a pre-warmed slide, covered with a coverslip and sperm motility characteristics were determined with a 100 × objective at 37 °C. The following motility values were recorded: motility (%), progressive motility (%), total sperm motility, VAP (average path velocity, µm s^{-1}), VSL (straight linear velocity, µm s^{-1}), VCL (curvilinear velocity, µm s^{-1}), ALH (amplitude of lateral head displacement, µm), LIN ([VSL/VCL] x 100), WOB ([VAP/VCL] x100), STR ([VSL/VAP] x100). Beat cross frequency (BCF) is determined by measuring the frequency with which the sperm track crosses the cell path in either direction and also it has become possible to evaluate hyperactivated spermatozoa with high velocities and erratic tracks with the use of CASA which has 60-Hz frame rate and high resolution optical system. For each evaluation, at least 220 and most 370 spermatozoa were analysed in six microscopic fields.

Assessment of sperm plasma membrane integrity (viability): This assessment was performed by staining with a sperm viability kit (SYBR-14/PI Molecular Probe: L 7011 Invitrogen, Carlsbad, CA, USA). For the assesment of viability, the method previously described by Bucak et al. (2), which was modified from a study of Garner & Johnson (6) was used. After staining, at least 200 spermatozoa per sample were examined at 1000 × magnification under a fluorescence microscope (Leica DM 2500 Microsystems GmbH, Ernst-Leitz-Straße, Wetzlar, Germany; excitation at 450– 490 nm, emission at 520 nm) to assess sperm membrane integrity. Sperm cells displaying green–red or red staining were considered to have membrane damage, while those displaying green staining were considered to have an intact membrane.

Assessment of sperm acrosome integrity: Sperm acrosome status was assessed by fluorescein isothiocyanate conjugated to Arachis hypogaea (peanut) (L7381 FITC-PNA, Invitrogen) and by PI staining method previously described by Bucak et al. (2), which was modified from a study of Nagy et al. (9). After staining, at least 200 sperm cells were examined at 400 × magnification under a fluorescein microscope (Leica DM 2500; excitation at 450–490 nm, emission at 520 nm) to assess the sperm acrosome integrity. Spermatozoa displaying bright green or patchy green fluorescence were considered as acrosome non-intact or damaged, whereas cells which did not display green fluorescence in their acrosome caps were regarded as acrosome intact.

Assessment of sperm mitochondrial activity: Sperm mitochondrial activity was assessed using staining method (JC-1/PI) previously described by Coyan et al. (3), which was modified from a study of Garner et al (7). After staining, at least 200 sperm cells were examined at 400 × magnification under a fluorescein microscope (Leica DM 2500; excitation at 450–490 nm, emission at 520 nm) to assess the activity. A high level of yellow/orange fluorescence associated with the sperm midpiece (where mitochondria are located) indicated high mitochondrial activity. Mitochondria showing low mitochondrial activity were stained green.

Statistical analysis: The statistical significance of the difference between variables were analyzed with one way analyzis of variance (ANOVA). For the differences in variables which were significant, Duncan test was used as a post-hoc test. All statistical analysis were examined with a minimum 5% margin of error. The descriptive measurements of variables were given in the tables as "Arithmetic Mean($\bar{X}$) ± Standard Deviation (SD)". SPSS® for Windows 14.01 (Licence No:9869264) package programme was used in analysis of the data.

Results

Fresh semen parameters are given in Table 1. Post-thaw viability, mitochondrial activity and acrosome integrity values are presented in Table 2 and Figure 1. Frozen thawed samples motility and kinetic parameters are given in Table 3 and Figure 2; highest progressive and total motility was recorded as 9.97 ± 4.05 and 58,42 ± 9,40 respectively in Nallihan region (P>0.05).

Table 1: Mean (±SD) individual fresh semen parameters

Tablo 1: *Bireysel taze sperma parametre ortalamaları*

Region	Individual	Semen Volume (ml)	Motility (%)	Conc. (x10^6)	Mass act.	pH
Nallıhan	1	0.93±0.37	82.5±10.87	2.009±150.98	4.33±0.52	6.30±0.27
	2	1.15±0.29	83.85±13.93	1.941±147.35	4.46±0.78	6.28±0.29
Lalahan	1	1.17±0.23	85.83±9.75	2.076±376.00	4.42±0.51	6.21±0.21
	2	1.23±0.55	85.00±8.07	1.879±300.47	3.83±0.58	6.39±0.24
Ayaş	1	1.36±0.35	87.78±8.52	1.884±386.01	4.38±0.52	6.34±0.44
	2	1.16±0.17	89.00±9.10	1.987±195.05	4.40±0.70	6.49±0.29
P		-	-	-	-	-

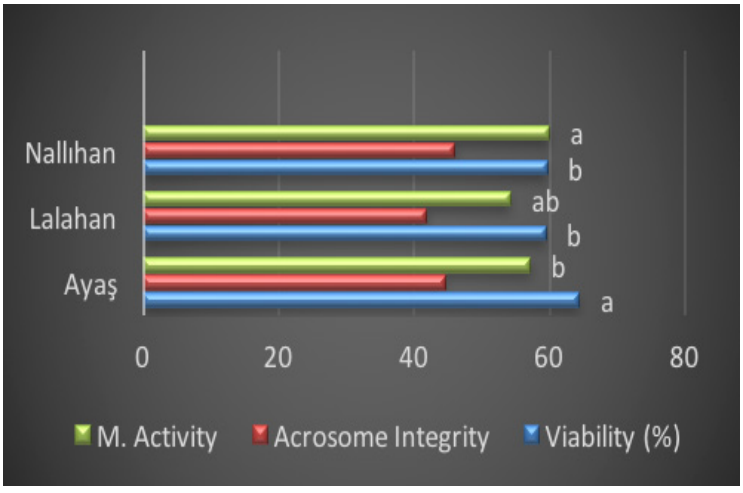
-No significant differences (P>0.05).

Table 2: Mean (±SD) individual fluorescein stain values in frozen thawed buck semen

Tablo 2: *Bireysel dondurulmuş çözdürülmüş teke sperması floresan boyama değerleri*

Region	Individual	Viability (%)	Acrosome Integrity (%)	M. Activity (%)
Nallıhan	1	58.77±5.22^{b}	44.16±5.11	61.22±4.65^{a}
	2	59.32±2.46^{b}	47.55±8.37	58.61±5.69^{ab}
Lalahan	1	57.27±9.76^{b}	39.85±4,35	54.81±4.63^{b}
	2	61.57±5.90^{ab}	43.82±8,74	53.55±6.80^{b}
Ayaş	1	66.61±5.36^{a}	42.24±4.34	57.81±4.75^{ab}
	2	62.27±4.59^{ab}	46.51±8.06	56.53±6.30^{ab}
P		*	-	*

^{*a,b,} *:Different superscripts within the same column demonstrate significant differences (P<0.05)*
-No significant differences between regions (P>0.05).



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

Figure 1: Mean regional flourescein stain values in frozen thawed buck semen

Şekil 1: *Bölgesel dondurulmuş çözdürülmüş teke sperması floresan boyama değerleri*

Table 3a: Mean (±SD) individual CASA motility and kinetic parameters values in frozen thawed buck semen

Tablo 3a: *Bireysel dondurulmuş çözdürülmüş teke sperması CASA motilitesi ve kinetik parametre değerleri*

Region	Individual	Progressive Motility (%)	Casa Motility (%)
Nallıhan	1	9.05±4.28	57.17±7,95
	2	10.40±4.04	59.00±10,25
Lalahan	1	9.08±6.67	48.19±17,12
	2	6.58±5.10	50.24±23,34
Ayaş	1	11.59±5.82	60.11±10.23
	2	7.13±4.83	48.99±18.51
P		-	-

-No significant differences between regions (P>0.05).

Table 3b: Mean (±SD) individual CASA motility and kinetic parameters values in frozen thawed buck semen

Tablo 3b: *Bireysel dondurulmuş çözdürülmüş teke sperması CASA motilitesi ve kinetik parametre değerleri*

Region	Individual	VCL(µm/s)	VSL(µm/s)	VAP(µm/s)	LIN(%)
Nallıhan	1	96.80±7.15	38.93±8.06	60.87±7.51	40.03±6.66
	2	98.51±7.16	40.61±7.20	63.00±6.97	41.05±5.42
Lalahan	1	96.17±8.35	39.44±6.40	60.58±6.65	41.03±5.69
	2	92.05±7.25	35.83±8.09	57.27±8.45	38.70±6.94
Ayaş	1	100.90±5.33	38.06±10.41	61.25±9.36	37.55±9.54
	2	95.24±9.98	36.61±8.63	58.01±8.56	38.39±8.54
P		-	-	-	-

-No significant differences between regions (P>0.05).

Table 3c: Mean (±SD) individual CASA motility and kinetic parameters values in frozen thawed buck semen

Tablo 3c: *Bireysel dondurulmuş çözdürülmüş teke sperması CASA motilitesi ve kinetik parametre değerleri*

Region	Individual	STR(%)	WOB(%)	ALH(%)	BCF(%)	Hyperactive (%)
Nallıhan	1	63.45±5.38	62.80±5.32	4.07±0.40	8.27±0.78	20.75±2.50
	2	64.05±4.75	63.84±3.77	4.07±0.40	7.94±0.58	21.68±5.03
Lalahan	1	64.79±4.23	63.05±4.99	4.04±0.52	8.44±0.65	20.05±6.36
	2	61.95±5.68	62.03±5.97	4.03±0.40	7.53±0.68	16.43±6.21
Ayaş	1	61.01±7.32	60.76±7.76	4.43±0.51	7.91±0.80	22.49±4.78
	2	62.40±7.87	60.87±6.00	4.31±0.66	8.21±0.87	18.46±6.92
P		-	-	-	-	-

-No significant differences between regions (P>0.05).

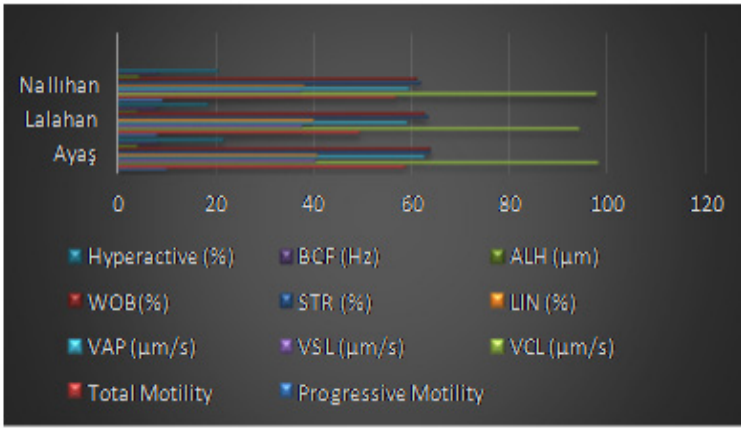


Figure 2: Mean regional sperm values in frozen thawed goat semen

Şekil 2: *Bireysel dondurulmuş çözdürülmüş teke sperması CASA motilitesi ve kinetik parametre değerleri*

Discussion and Conclusion

In the present study, we investigated individual and regional differences between fresh and freezed-thawed semen. There was no significant difference between bucks in fresh semen parameters as given in Table 1 (P>0.05). Dominguez-Rebolledo et al. (2011) worked in the red deer and they found individual variability might improve the development of optimized sperm work protocols. Although Loomis and Graham (8) found that differences are probably due to inherent differences between both species and individuals within a species in sperm biochemistry and metabolism, in our study we did not find any significant difference between individuals in terms of fresh semen paramters. If this variation occurs between individuals, these differences could probably be seen in the same species in different regions.

There were no statistical difference between individual and regional groups in

some spermatological parameters (fresh semen parameters and freezed-thawed CASA motility). But in florescein stainings in Table 2, the highest viability was 64.29±5.28 in Ayaş region (P<0.05). Roca et al. (11) indicated that individual variations are the main influencing factor for freezability of spermatozoa. Fraser et al. (5) showed that differences in the quality of cryopreserved boar semen are determined genetically. Similar to these studies, in Figure 1 the highest mitochondrial activity belongs to the Nallıhan group with 61.22±4.65 (P<0.05). In frozen thawed semen the highest viability was observed in Ayaş group with 66.61±5.36 (P<0.05). Age, breed, management, nutrition and differences among technicians could be effective on these minor sperm defects (1).

In the present study, there were no significant difference between individuals but after thawing; the lowest progressive motility was recorded as 6.58±5.95 in Lalahan group (Table 3) and the highest progressive motility was 9.97±4.05 in Nallıhan group. The highest progressive motility was observed in Ayaş group with 11.59±5.82. Moreover, there was no statistical difference between groups (P>0.05) in terms of kinetic parameters (VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF) (Table 3). These variabilies can be explained by differences in biochemistry, metabolism and environment.

As a result, individual and regional differences are very important for semen freezing quality. It gives valuable information about post-thaw semen quality and freezability. Further studies should be conducted on semen metabolism of animals bred in different regions.

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References

1. Brito LFC, Silva AEDF, Rodrigues LH, Vieira FV, Deragon LAG, Kastelic JP (2002): *Effects of environmental factors, age and genotype on sperm production and semen quality in Bos indicus and Bos taurus AI bulls in Brazil*. Anim Reprod Sci, **70,** 181–190.

2. Bucak MN, Ataman MB, Başpınar N, Uysal O, Taşpınar, M, Bilgili A, Öztürk C, Güngör S, İnanç, ME, Akal E (2015): *Lycopene and resveratrol improve post-thaw bull sperm parameters: sperm motility, mitochondrial activity and DNA integrity.* Andrologia, **47,** 545-552.

3. Çoyan K, Başpınar N, Bucak MN, Akalın Peker P (2011): *Effects of cysteine and ergothioneine on post-thawed Merino ram sperm and biochemical parameters.* Cryobiology, **63,** 1–6.

4. Dominguez-Rebolledo AE, Martinez-Pastor F, Bisba AF, Ros-Santaella JL, Garcia-Alvarez O, Maroto-Morales A, Soler AJ, Garde JJ, Fernandez-Santos MR (2011): *Response of thawed epididymal Red Deer spermatozoa to increasing concentrations of hydrogen peroxide, and importance of individual male variability.* Reprod Dom Anim, **46,** 393–403.

5. Fraser L, Chandra SP Strzeżek J (2008): *Identification of amplified fragment length polymorphism markers associated with freezability of boar semen – a preliminary study*. Med Weter, **64,** 646-649.

6. Garner DL, Johnson LA (1995): *Viability assessment of mammalian sperm using SYBR-14 and propidium iodide*. Biol Reprod, **53,** 276–284.

7. Garner DL, Thomas CA, Joerg HW, Dejarnette JM, Marshall CE (1997): *Fluorometric assessments of mitochondrial function and viability in cryopreserved bovine spermatozoa*. Biol Reprod, **57,** 1401–1406.

8. **Loomis PR, Graham JK** (2008): *Commercial semen freezing: Individual male variation in cryosurvival and the response of stallion sperm to customized freezing protocols*. Anim Reprod Sci, **105**, 119–128

9. Nagy S, Jansen J, Topper EK, Gadell BM (2003): *A triple-stain flow cytometric method to assess plasma and acrosome-membrane integrity of cryopreserved bovine sperm immediately after thawing in presence of egg-yolk particles*. Biol Reprod, **68,** 1828–1835.

10. *Purdy PH* (2006): *A review on goat sperm cryopreservation*. Small Rum Res, **63**, 215–225.

11. Roca J, Hernandez M, Carvajal G, Vazquez JM, Martinez EA (2006): *Factors influencing boar sperm cryosurvival*. J Anim Sci, **84,** 2692-2699.

12. Thomas CA, Garner DL, DeJarnette M, Marshall CE (1997): *Fluorometric assessments of acrosomal integrity and viability in cryopreserved bovine spermatozoa*. Biol Reprod, **56,** 991-998.

13. Thurston LM, Watson PF, Mileham AJ, Holt WV (2001): *Morphologically distinct sperm subpopulations defined by fourier shape descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation.* Journal of Andrology, **22,** 382-394.

14. Thurston LM, Siggins K, Mileham AJ, Watson PF, Holt WV (2002a): *Identification of amplified restriction fragment length polymorphism markers linked to genes controlling boar sperm viability following cryopreservation*. Biol Reorod, **66,** 545–554.

15. Thurston LM, Watson PF, Holt WV (2002b): *Semen Cryopreservation: A genetic explanation for species and individual variation?.* CryoLetters, **23,** 255-262.

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