EFFECT OF GOAT BREEDS, SEMEN DILUENTS AND FREEZING METHODS ON SPERM FREEZABILITY AND REPRODUCTIVE PERFORMANCE

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ABSTRACT

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Two experiments were designed to study the effect of semen extenders and freezing regimens on post-thaw semen motility and viability index, effect of bucks breed was also included (experiment 1). In experiment 2, semen diluents effects on reproductive performance was conducted. Semen was collected from bucks breeds (Aradi (A), Damascuss (D) and cross (½A½D). Good quality semen was divided into 4 portions, each diluted with one diluent (Milk, Na.Citrate, Tris and Na.Bicarbonate). The diluted semen was packaged into 0.5ml straws then cooled to 5°C. After equilibration, half of the packaged straws were suspended 15cm above liquid nitrogen (LN) for 15min (Freezing regimen1; slow). Other half of straws was suspended at height 15 and 5cm of LN for 10 and 5min, respectively (Freezing regimen2; rapid) before plunged into LN. Frozen semen was thawed for post-thaw motility and viability. In the second experiment, semen with good quality was extended with three types of extenders (Milk, Na.Citrate and Tris). Diluted semen were cooled to 5°C and used for AI. Results revealed that, pre-freeze semen motility was significant higher in Tris, Na.Citrate and Na.Bicarbonate than milk diluent. Post-thaw semen motility and viability were highly significant for milk and Na.Citrate than Tris and Na.Bicarbonate diluents. Post-thaw semen motility was significantly higher in Aradi and Damascus than cross breed. Post-thaw semen motility and viability revealed, significant higher means for Freezing regimen 2 than regimen 1. Milk was significantly higher than Tris and Na.Citrate diluents for fertility and fecundity. It was concluded that, regarding to post-thaw semen viability, fertility%, kidding%, fecundity% and prolificacy%, milk is preferable than Tris and Na.citrate diluents.

تأثير سلالة الماعز ونوع المخففات وكذلك نظم التجميد على كفاءة السائل المنوى للتجميد وتأثير ذلك أيضاً على الأداء التناسلى

كامل مصطفى ، ماهر خليل ، على السيف

الهدف من هذه الدراسة هو معرفة تأثير ممددات السائل المنوى وطرق تجميده وسلالة التيوس على حركة وحيوية الحيامن بعد التجميد والاسالة وكذلك تأثير نوع المخف المستخدم فى التلقيح الاصطناعى على الكفاءة التناسلية للماعز. ولتحقيق هذه الأهداف صممت تجربتين: فى التجربة الأولى: تم تجميع السائل المنوى من تيوس لثلاثة سلالات من الماعز هى العارضى والدمشقى والخليط ، قسمت العينات على أربعة أجزاء ، كلى جزء تم تخفيفه بنوع من الممدات هى الحليب منزوع الدسم والترس وسترات الصوديوم وأخيرا مخفف بيكربونات الصوديوم. بعد التخفيف تم تعبئة السائل المنوى فى قصيبات سعة ½ مللى ثم بردت تدريجيا وتركت فى الثلاجة لمدة لم تقل عن ساعتين لاعطاء فرصة للحيامن للتأقلم والتكييف مع المخفف فى درجة ٥ مئوية ، بعد هذه الفترة تم تقسيم القصيبات الى مجموعتين، الأولى (نظام التجميد البطئ) تم تعرض قصيبات السائل المنوى لبخار النتروجين السائل وعلى مسافة ١٥سم من سطح النتروجين ولمدة ١٥ دقيقة بعدها تم غمس القصيبات فى النتروجين السائل، فى المجموعة الثانية (نظام التجميد السريع) تم تعرض القصيبات لبخار النتروجين لمدة ١٠ دقائق وعلى مسافة ١٥سم من النتروجين ثم تم تقليل المسافة الى ٥سم وتركت لمدة ٥ دقائق أخرى قبل غمسها فى النتروجين السائل. بعد مرور يومين أو أكثر تم فحص قصيبات السائل المنوى بعد اسالتها للتعرف على الحركة الامامية ومعدل الحيوية. فى التجربة الثانية: تم تجميع السائل المنوى من تيوس محسنة وراثيا (لاستخدامها فى التلقيح الاصطناعى لتحسين الصفات الانتاجية لسسلالات الماعز المحلية) ثم خففت بثلاثة أنواع من المخففات وهى التى أعطت أفضل النتائج فى التجربة الأولى، وهذه المخففات هى الحليب والترس وسترات الصوديوم. بعد التخفيف والتبريد والحفظ فى الثلاجة عند درجة ٥ مئوية استخدام السائل المنوى المبرد خلال يومين من تحضيره لتلقيح العنزات اصطناعيا. بعد مرور ٤٥ يوما من التلقيح تم فحص العنزات الملقحة بالسونار للتعرف على نسبة العشار وعند الولادة سجلت النتائج لمعرفة الكفاءة التناسية للعنزات. أسفرت النتائج فى التجربة الأولى أن الحركة التقدمية للحيامن بعد التخفيف والتبريد وقبل التجميد كانت أفضل لمخففات الترس وسترات الصوديوم وبيكربونات الصوديوم عن مخفف الحليب، فى حين أنه بعد التجميد والاسالة كانت خصائص السائل المنوى لمخفف الحليب أفضل من باقى المخففات، أسفرت النتائج أيضا أن خصائص السائل المنوى بعد التجميد والاسالة كانت أفضل فى سلالة العارضى والدمشقى عنها فى الخليط. التجميد السريع كان له تأثير أفضل على خصائص السائل المنوى عن التبريد البطئ. أخيرا وجد أن مخفف الحليب كان له تأثير أفضل على الكفاءة التناسلية عن باقى المخففات الأخرى موضوع الدراسة. ويمكن خلاصة القول بأن مخفف الحليب منزوع الدسم له تأثير ايجابى على تجميد السائل المنوى للتيوس وكذلك على الخصائص التناسلية للعنزات الملقحة.

***Key words**: Buck, semen diluents, artificial insemination, fertility.*

INTRODUCTION

The success of artificial insemination (AI) is based on the ability to efficiently collect and cryopreserve spermatozoa from quality bucks for use on does over generations (Amoah and Gelaye, 1990). Three methods of semen preservation (fresh, refrigerated and frozen) are used worldwide in goats (Evans and Maxwell, 1987; Leboeuf *et al.*, 2000). The use of refrigerated semen is a common strategy in circumstances where a particular male is shared by a group of farmers located within a relatively small area. Spermatozoa are likely to suffer a considerable damage and deterioration during dilution and preservation at low temperature; therefore, suitable diluents are a basic necessity for successful preservation of spermatozoa and higher conception rate in field trials using diluted semen (Salamon and Maxwell, 1995). The principal problem in the world wide development of AI in goats is related to the use of frozen semen, since the freezing process reduces the viability of sperm cells (Ritar, 1993). Tris diluent was found to maintain higher quality of semen pre-freeze and post-thaw (Drobnis *et al.*, 1980; Deka and Rao, 1987; Chauhan and Anand, 1990; Sinha *et al.*, 1991; Tuli and Holtz, 1992). Also, Singh and Purbey (1996) concluded that the Tris diluter was superior for freezing goat semen than citrate diluter concerning post-thaw motility. Contrary, Salvador *et al.* (2007) cited that, milk diluent provided higher viability of spermatozoa

than semen diluted in Tris and citrate. Moreover, Drobnis *et al.* (1980) recorded higher extracellular of aspartate aminotransferase (AST) activity released in Tris diluent than skim milk after thawing goat semen. However, Memon *et al.* (1985) and Mukhrejee and Nelson (1987) could not find significant difference between Tris and skim milk extenders for freezing buck semen. The present study aimed to investigate the effect of semen extenders, freezing regimens and bucks breed on post-thaw semen motility and viability index, as well as investigate if semen diluents influenced reproductive performance of goats in arid environment.

MATERIALS and METHODS

Animals and management:

A crossbreeding program between Saudi goats (Aradi, A) as a native breed with Syrian goats (Damascus, D) was carried out at Camel and Range Research Center (Al-Jouf province, Northern region of Saudi Arabia located at latitude of 29.97° north and longitude of 40.21° Wand at 684 meters above sea level) to improve the productivity of Aradi local goats for meat and milk production through crossbreeding and selection. Genetically selected and improved bucks were used to disperse these valuable genes throughout the goat herds in Saudi Arabia. Animals were housed in semi-shaded/open front barn and fed on a commercial concentrate and alfalfa hay. The amount of concentrate and hay were calculated according to the nutritional requirements for goats (National Research Council; NRC, 1981) which dependent on animal ages and production status. Water, straw, salt and minerals supplemented in blocks were freely available to all animals.

Experiment 1:

Semen preparation and freezing process:

Four semen extenders and two freezing programs were evaluated for freezing buck goat semen. Constituents of these diluents are shown in Table 1. Three to four ejaculates per buck were collected using artificial vagina. Semen samples with motility ≥70% were used for processing. Semen samples for each buck were divided into 4 portions, each portion was added to one diluent (dilution rate 1:15-20; according to semen concentration). The diluted semen samples were packaged into 0.5 ml straws at room temperature and arranged horizontally on freezing racks then gradually cooled to 5 °C within 1-2 hrs. and placed in a refrigerator for equilibration. After equilibration time, half of the total packaged straws were suspended in liquid nitrogen (LN) vapor inside an foam box container at height 15 cm above LN, for 15 min (Freezing regimen 1; slow). Other half of straws were suspended in LN vapor at height 15 and 5 cm of LN, for 10 and 5 min (Freezing regimen 2; rapid) before plunged into LN. Frozen semen straws were stored in LN for 24-72 hrs before thawing. The frozen semen was thawed in a water bath at 37 °C for 2-3 minutes. Pre-freeze semen motility was recorded using microscope fitted with a biotherm stage (37 °C), as well as, the post-thaw motility at 0hr after thawing and reassessed after 1, 2 and 3 hours of thermal stress and the viability index (sum of semen motility/2 at 0 hr. + semen motility at 1 and 2 hr. + semen motility/2 at 3 hr.) according to Milovanov (1962) was determined for each semen sample. Twenty two bucks from three breeds (Aradi, Damascus and Cross ½Ardi x ½Damascus) were used in the study. The effect of semen extenders, freezing regimens and of bucks breed on post-thaw semen motility and viability index were conducted in this experiment.

Experiment 2:

Semen preparation:

Semen samples were collected from genetically selected and improved bucks (to disperse these valuable genes throughout the local goat herds in Saudi Arabia) with good quality were extended with extenders using dilution rate of 1:15 to 1:20 (semen: diluent) according to sperm concentration. Three types of extenders; skim milk, Na. citrate and Tris were used as based extenders for semen dilution, constituents of the diluents are presented in Table 1. The diluted semen samples were gradually cooled within 2hrs to 5°C and stored in a refrigerator as chilled

semen to be used for artificial insemination (Azawi *et al.*, 1993).

Estrous synchronization and artificial insemination (AI):

Intravaginal progestagen release device (CIDR) containing 300 mg progesterone or intravaginal progesterone impregnated sponges containing 30 or 45 mg fluorogesteone acetate (FGA) were administered to 930 does of local different breeds (native and cross breeds of unknown origin), and maintained in situ for 15-17 days. At the day of sponge withdrawal 200-300 IU/eCG was injected intramuscular. AI was done blindly (irrespective to signs of estrus) 48-60 hours after sponge removal, using chilled diluted semen (0.5 ml containing 120-150 $x10^6$ motile spermatozoa). AI was carried out using insemination pipette and vaginal speculum. The hind legs of the doe was lifted and placed at an angle of 45° to the horizontal railing. The vaginal speculum was introduced into the vaginal passage and the cervix was located with the help of light and by gentle sideways or downward manipulation of the speculum. Semen was deposited up to a depth of 0.5-1.0 cm into the cervix (cervical insemination). Pregnancy diagnosis was applied 30-45 days post insemination with the aid of ultrasound scanner. Different breeds of Aradi (A), Damascus (D) and Cross (½A½D) goats were used. Irrespective to goat breeds, Pooled collected data from 2006 through 2010 were performed for statistical analysis to study the effect of semen diluents and mating seasons on the reproductive performance. Parameters of the reproductive performance which done in this study includes: Fertility rate (pregnant does/does inseminatedx100), Kidding rate (kidded does/pregnant does)x 100), Fecundity rate (kids born /pregnant does x 100), and Prolificacy rate (kids born /kidded doesx100).

Statistical analysis:

All pooled data (collected from 2006 through 2011) of studied parameters affected post-thaw semen motility and viability and artificial insemination parameters which affected the reproductive traits (fertility, kidding, fecundity, and prolificacy rates) were statistically analyzed using the general linear models (GLM) procedures of the analysis Systems with a subsequent Duncas test was used to compare the mean values resulting from the various treatments. All analyses were carried out using the SPSS 9 for Windows statistical software package.

RESULTS

As shown from Table 2, pre-freeze semen motility was significant higher ($P<0.05$) in Tris diluent (78.49±0.96), sodium citrate (76.72±0.87) and sodium-citrate-bicarbonate (76.08±10.93) than milk diluent (71.88±1.29). Post-thaw semen motility were highly significant ($P<0.05$) for milk (46.44±1.33) and sodium citrate (42.95±0.87) than Tris (38.80±1.40) and Sodium-citrate-bicarbonate (35.81±1.45) diluents. However, the viability index of post-thaw semen motility were significant higher ($P<0.05$) for milk (103.48±3.38), sodium citrate (104.18±3.13) and Tris (95.06±4.06) than sodium-citrate-bicarbonate (91.50±4.23) diluents.

Irrespective to diluent types and freezing protocol, the mean values of pre-freezing semen motility for Aradi (A), Damascus (D) and Cross bucks breed ½A½D were 75.57±0.73, 75.91±0.84 and 76.55±1.35, respectively, with no significant differences (Table 3). The same trends, were observed for VI and its mean values were 101.38±2.47, 95.49±3.68 and 93.48±4.74, for Aradi, Damascus and cross bucks breed, respectively, with no significant differences (Table 3). However, significant higher values ($P<0.05$) of post-thaw semen motility at 0 hr. were achieved by Aradi (42.27±0.91) and Damascus (39.65±1.30) than cross bucks breed (38.11±1.71), irrespective to diluents and freezing regimens (Table 3). The overall means of motility for pre-freeze, post-thaw and viability index were 75.81±0.52, 40.92±0.69 and 98.56±1.89, respectively, irrespective to semen diluents, bucks breeds and freezing regimens.

As presented in Table 4, the effect of freezing protocols on post-thaw semen motility and viability index revealed that highest mean percentages ($P < 0.001$) of post-thaw motility were achieved in freezing regimen 2 at 0 hr. (47.74 ±0.66 *vs* 34.15±1.04), 1 hr. (42.74±0.71 *vs* 28.54±1.00), 2 hrs. (38.59±0.72 *vs* 22.60±0.93), 3 hrs. (31.65±0.88 *vs* 16.12±0.84) and viability index (121.03±1.89 *vs* 76.28±2.59) than freezing regimen 2, irrespective to semen diluents and buck breeds. Analysis of variance (Table 5) showed, significant interactions ($P<0.001$) between diluents and bucks breeds on pre-freeze sperm motility, post-thaw motility and VI. In the same line, the effect of interactions between freezing regimens and diluents were highly ($P<0.001$) significant on post-thaw semen motility and viability index. However, the interaction between bucks individuality and freezing regimens and/or semen diluents on post-thaw semen motility and VI were non-significant.

Regarding the effect of semen diluents used for AI on the reproductive performance; the fertility rate were 45.21% (33/73), 34.40% (215/625) and 57.76% (134/232) for does that had been inseminated with Tris, sodium citrate and milk diluted semen, respectively (Table 6). The corresponding kidding%, fecundity%, and prolificacy% were 90.91%, 143%, 157%; 89.77%, 143%, 159%; and 96.99%, 179%, 185%, for Tris, sodium citrate and milk semen diluents, respectively, with overall means were 41.08%, 92.39%, 156% and 168%, for fertility, kidding, fecundity and prolificacy rates, respectively (Table 6). Statistical analysis revealed that, milk diluent was significantly higher ($P<0.05$) than Tris and sodium citrate diluents for fertility and fecundity rates. However, no significant effect between diluents on kidding and prolificacy rates.

Table 1: Composition of semen extenders (g /100 ml distilled water).

Constituents (gm)	Diluents			
	1	2	3	4
Skimmed milk Powder	10	=	=	=
Na. Citrate	=	2.9	=	2.00
Tris	=	=	3.786	=
Citric acid monohydrate	=	=	2.172	=
Sodium Bicarbonate	=	=	=	0.21
KCl	=	=	=	0.04
Glucose	=	=	0.625	0.30
Egg Yolk (v/v)	15%	15%	15%	15%
Glycerol (v/v)	7%	7%	7%	7%
Gentamycin (µg)	50.000	50.000	50.000	50.000
Tylosin (µg)	50.000	50.000	50.000	50.000
Lincospectin (µg)	15.000	15.000	15.000	15.000

Table 2: Effect of semen extenders (diluents) on Pre-freezing and post-thaw semen motility and viability index (Mean±SE)

Semen extenders	Pre-freezing motility	Post-thaw semen motility (%)				
		0hr	1hr	2hrs	3hrs	Viability Index
Milk (1)	71.88±1.29^{b}	46.44±1.33^{a}	39.36±1.29^{a}	31.08±1.27^{a}	19.64±1.18^{b}	103.48±3.38^{a}
Na. citrate (2)	76.72±0.87^{a}	42.95±1.10^{a}	37.87±1.11^{a}	33.11±1.30^{a}	23.44±1.30^{ab}	104.18±3.13^{a}
Tris (3)	78.49±0.96^{a}	38.80±1.40^{b}	34.57±1.46^{ab}	29.07±1.45^{a}	25.12±1.43^{a}	95.06±4.06^{ab}
Na.- citrate-bicarbonate (4)	76.08±10.93^{a}	35.81±1.45^{b}	30.92±1.48^{b}	29.15±1.43^{a}	27.04±1.55^{a}	91.50±4.23^{b}
Total	75.81±0.52	40.92±0.69	35.61±0.69	30.56±0.69	23.85±0.70	98.56±1.89

Means in the same column with different superscripts are significantly differ at P<0.05

Table 3: Effect of Buck breeds on Pre-freezing and post-thaw semen motility and viability index (Mean±SE).

Bucks Breed	Pre-freezing	Post-thaw semen motility (%)				
		0hr	1hr	2hrs	3hrs	Viability Index
Ardi (A)	75.57±0.73^{a}	42.27±0.91^{a}	36.87±0.89^{a}	31.31±0.92^{a}	21.13±0.94^{a}	101.38±2.47^{a}
Damascus (D)	75.91±0.84^{a}	39.65±1.30^{ab}	34.27±1.36^{a}	29.58±1.30^{a}	23.64±1.31^{a}	95.49±3.68^{a}
Cross breed (½A½D)	76.55±1.35^{a}	38.11±1.71^{b}	33.31±1.77^{a}	29.53±1.73^{a}	23.18±1.71^{a}	93.48±4.74^{a}
Total	75.81±0.52	40.92±0.69	35.61±0.69	30.56±0.69	23.85±0.70	98.56±1.89

Means in the same column with different superscripts are significantly differ at P<0.05

Table 4: Effect of freezing regimens on post-thaw semen motility and viability index (Mean±SE).

Post-thaw hours	Post-thaw semen motility (%)		
	Regimen 1 (Slow freezing)	Regimen 2 (Rapid freezing)	Overall
0 hr	34.15±1.04^{b}	47.74±066^{a}	40.92±0.69
1 hr	28.54±1.00^{b}	42.74±0.71^{a}	35.61±0.69
2 hrs	22.60±0.93^{b}	38.59±0.72^{a}	30.5±0.69
3 hrs	16.12±0.84^{b}	31.65±0.88^{a}	23.85±0.70
Viability Index	76.28±2.59^{b}	121.03±1.89^{a}	98.56±1.89

Means in the same raw with different superscripts are significantly differ at P<0.001

Table 5: ANOVA showing the effect of semen diluents, bucks individuality Freezing pograms on Pre-freeze, post-thaw semen motility and VI

Source of variation	df	Pre-Freezing Semen motility		Post-thaw Semen motility									
				0hr		1hr		2hrs		3hrs		Viability Index	
		MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Diluents (D)	3	1040.99	9.06***	920.12	6.65***	607.34	4.55**	302.13	2.34 NS	1702.46	11.87***	1212.50	1.36 NS
Bucks Individualit y (BI)	21	412.38	3.59***	568.47	4.11***	688.27	5.16***	670.60	5.20***	538.26	3.75***	5429.11	6.10***
Freezing Regimens (FR)	1			15275.29	110.33***	14444.01	108.20***	17892.62	138.84***	17460.31	121.71***	145780.31	163.72***
D x BI	56	328.103	2.86***	247.58	1.79***	256.98	1.93***	269.85	2.09***	324.86	2.26***	2056.17	2.31***
D x FR	3			1112.52	8.04***	1002.84	7.51***	1217.44	9.45***	1066.62	7.44***	9554.54	10.73***
BI x FR	21			188.60	1.36 NS	139.05	1.04 NS	103.48	0.80 NS	66.04	0.46 NS	765.54	0.86 NS
D x BI x FR	56			93.03	0.67 NS	115.28	086 NS	84.62	0.66 NS	93.63	0.65 NS	638.34	0.72 NS
Error	344	114.85		138.45		133.50		128.87		143.46		890.44	

** P<0.01 *** P < 0.001 NS = Non-Significant

Table 6: Effect of semen diluents on goats reproductive performance.

Diluents	Fertility %	Kidding %	Fecundity %	Prolificacy %
Milk	57.76 (134/232)[a]	96.99 (129/133)[a]	179 (238/133)[a]	185 (238/129)[a]
Tris	45.21 (33/73)[b]	90.91 (30/33)[a]	143 (47/33)[b]	157 (47/30)[a]
Na. Citrate	34.40 (215/625)[b]	89.77 (193/215)[a]	143 (307/215)[b]	159 (307/193)[a]
Overall	41.08 (382/930)	92.39 (352/381)	156 (593/381)	168 (593/352)

Means in the same columns with different superscripts are significantly differ at P<0.05.

DISCUSSION

In the present study, the pre-freeze semen motility were significant higher (P<0.05) in Tris (78.49±0.96) than skim milk diluted semen (71.88±1.29). However, the post-thaw semen motility and viability index were highly significant (P<0.05) for skim milk (46.44±1.33 and 103.48±3.38, respectively) than other diluents. This means, under our conditions, milk extender appeared to provide higher in vitro spermatozoa viability. These findings to be in harmony with those recorded by Chehadeh et al (2001) they observed that Tris was the better diluent for maintaining goat sperm motility (77.08 %) after semen dilution than Milk (66.92%), CEGLY diluent (66.00%) and Sodium Citrate (64.00%) extenders. Also, Dorado *et al.* (2007) detected that, TRIS extender provided more effective preservation of total motility, velocity parameters and amplitude of lateral head displacement after freezing than milk extender. However, Salvador *et al.* (2007) compared the effect of three extenders for buck semen conservation; skimmed Milk, sodium Citrate and Tris-based diluents on the in vitro viability of Murciano-Granadina goat spermatozoa stored at 5 °C. Milk diluents provided higher in vitro viability of spermatozoa than semen diluted in Tris. Moreover, Maha-Ziada *et al.* (1998), Hassan

(1990); Staish-Kumar *et al.* (1994) and Mohammed *et al.* (1998) obtained satisfactory post-thaw motility by freezing buffalo semen in milk diluent more than that in Tris or sodium citrate diluents. In addition, Dorado *et al.* (2007) observed, that, the percentage of acrosome intact spermatozoa was significantly higher in samples diluted with milk extender than Tris diluent, at the same time, semen doses cryopreserved in milk extender provided greater pregnancy rates after insemination compared to those in Tris extender (52.4% *vs* 42.9%).

In the present study, post-thaw semen motility of milk diluent was sharply declined from 46.44±1.33 at 0 hr. to 19.64±1.18 at 3 hr. after thawing throughout the incubation period at 37 °C. This may be attributed to the rapid deterioration of buffering capacity of milk, to increased microbial growth and raising the acidity of the biological medium (Dhami and Sahni 1995). On the other hand, post-thaw semen motility of Tris diluent herein was slowly decreased from 38.80±1.40 at 0hr to 25.12±1.43 at 3 hr. after thawing. Salamon and Ritar (1982) considered Tris hydroxymethyl aminomethane, which is an important component of Tris diluent, is principally responsible for prolonging the preservation time by creating a buffer zone in and outside of the spermatozoa. In addition, the fructose content of the yolk Tris diluent may also help in maintaining the osmotic pressure, and providing nutrient for sperm metabolism.

Regarding to buck breeds present in this study and its effect on post-thaw semen motility and viability, irrespective to diluents and freezing protocol, the mean values of post-thaw semen motility at first, second and third hrs., after thawing, as well as, the viability index for different buck breeds showed non-significant differences. In accordance, Furstoss *et al.* (2009) evaluate genetic influencing characteristics of young buck semen production. They indicated that, no significant effect between buck breeds on the post-thaw semen motility However, results herein, showed a significant higher values ($P<0.05$) of post-thaw semen motility at 0 hr. were achieved by Aradi (42.27±0.91) and Damascus (39.65±1.30) than cross bucks breed; ½A½D (38.11±1.71). Similarly, Karatzas *et al.* (1997) used Greek breed (Capra prisca) were synchronized and inseminated with bucks of Alpine, Saanen, and Damascus breeds for studying the fertility. Buck breeds used for preparing frozen-thawed semen affected the fertility level of the does. The kidding rate was lower in does inseminated with semen prepared from Damascus bucks breed than bucks of Alpine and Saanen breeds. Moreover, Perez and Mateos (1996) used a group of 19 bucks of two Spanish breeds to study the effect of photoperiod on semen. There were differences between breeds in semen characteristics, with higher semen production and better semen quality ($P<0.01$) observed in Malaguena than Verata bucks breed. Generally, the overall means of motility for post-thaw and viability index were 40.92±0.69 and 98.56±1.89, respectively. These results are similar with have been reported in Zaraibi (Chehadeh *et al.*, 2001) and Cashmere bucks breed (Ritar and Ball 1993).

Spermatozoa experience physical and chemical stresses during cooling and freezing as a result of ice formation and osmotic changes in the medium. Sperm cryosurvival appears to depend on intrinsic properties of the sperm plasma membrane, such as biochemical composition, thermal behaviour, osmotic resistance and the physical stresses determined by the freezing protocol (Hammerstedt *et al.*, 1990; De Leeuw *et al.*, 1991). Sperm survival in such conditions can be modified by the rate at which they are cooled (Fiser and Fairfull, 1990; Bwanga *et al.*, 1991). The effect of freezing protocols on post-thaw semen motility and viability index were indicated in this study. Irrespective to semen diluents and buck breeds, the highest mean percentages ($P < 0.001$) of post-thaw motility and viability index were achieved in freezing regimen 2 than regimen 1. These findings are in agreement with Mohammed *et al.* (1998), recorded maximum ($P<0.01$) post-thaw motility (63.33+1.66) associated with highest viability (153.33+4.16) when Friesian semen was rapidly

frozen at 2 cm above LN for 15 min., meanwhile, the minimum motility (16.67±6.67) accompanied with lower viability index (44.17+15.45) were observed when relatively slow frozen at 8 cm above LN for 10 min. Similarly, the highest (P<0.01) motility (63.33±1.66) of post-thaw buffalo semen accompanied with highest viability index (161.67±7.12) obtained when frozen at 2 cm above LN for 10 min and the lowest motility and viability were obtained when frozen at 8 cm above LN and maintained for 10 min. On the basis of post-thaw motility, similarly, Bhandari *et al.* (1982) reported fastest timing of freezing was the best for semen survival than slow freezing, which is in similar trend to our study. Contrary, Jansen (1989) reported that fastest freezing gave low survival of sperm when fast freezing was applied (10 min) for cattle and buffalo semen, the post-thaw motility was 47.16+1.81 and 30.33±3.31, respectively, and when slow freezing (15 min) was employed post-thaw motility was 57.19±1.24 and 34.33+3.19, for cattle and buffalo semen, respectively. Anyhow, the cooling rates should not be too fast to cause cell death due to cold shock or too slow to cause death due to osmotic shock.

Analysis of variance showed, significant interactions (P<0.001) between diluents and bucks breeds on pre-freeze sperm motility, post-thaw motility and viability index. Also the interactions between freezing regimens and diluents were highly (P<0.001) significant on post-thaw semen motility and viability index. Our findings greatly accordance with Mohammed et al (1998), they found the interactions between freezing regimens and diluents had highly (P<0.01) significantly effect on viability index and AST of post-thaw buffalo spermatozoa. In opposition to our results, Dhami and Sahni (1995); Mohammed *et al.* (1998) found non-significant interactions between diluents and cooling rates on post-thaw bovine semen motility and viability. They ascribed this to variables which work independently of one another in the freezability and post-thaw longevity of bovine spermatozoa. Irrespective to bucks breed, the bucks individuality herein study had a significantly affect (P<0.01) for pre-freeze and post-thaw semen motility, as well as viability index. These results supported by study performed by Dorado *et al.* (2010) in which a Significant differences were found between semen extenders (P<0.001), bucks (P<0.05) and even ejaculates (P<0.05). Moreover, Corteel *et al.* (1987) observed, irrespective of the process used for freezing, differences between males regarding the freezability and fertility of semen, so they could be classified as 'good freezers' or 'bad freezers'. This variability is relatively independent of prior semen quality, and the semen of certain individuals consistently freezes with less cryoinjury than that of others. Spermatozoa acquire cold shock sensitivity as they traverse the epididymal tubules (Watson, 1981), and this is believed to be related to changes in membrane lipids during the epididymal transit. Differences in either ejaculation frequency, previously indicated by Boue and Corteel (1992), or in epididymal transit time and sperm mixing in the epididymis may provide a potential mechanism for variability in response to subsequent temperature fluctuation, explaining why ejaculates within individuals can vary in their responses to freeze-thawing (Watson, 1995).

The present work focused on investigating the influences of diluents used for semen extenders on the reproductive traits. In this study, although vitro evaluation of pre-freeze semen motility in skim milk was significantly lower than Na. citrate and Tris diluents, but though, the fertility and fecundity rates were significantly higher (P<0.05) in milk diluted semen than Tris and sodium citrate diluents. These results are in accordance with that reported by Dorado *et al.* (2007), where Tris-glucose and skim milk extenders were used to compare the ability of extenders to maintain sperm viability after cryopreservation. Tris extender results are better in vitro performance compared to milk, though these improvements were not reflected in fertility results, where, semen doses cryopreserved in milk extender provided greater pregnancy rates after intra-cervical insemination compared to those in Tris extender (52.4% versus 42.9%). Similarly, Mara *et al.* (2007) used three types of diluents; skim milk (SM), TEMPOL diluent and TEMPOL+hyaluronic acid (HA)

for goat semen dilution and consequently used as chilled semen for AI. The percentages of pregnant goats were 71.4%, 61.4% and 48.8% for the three diluents, respectively. Kidding rates were 66.7%, 61.4% and 48.8% for SM, TEMPOL and TEMPOL+HA, respectively without significant differences among treatment groups. In the same line, Nordstoga *et al.* (2010) testing the effect of two different extenders for Norwegian buck semen. Semen was diluted either in a milk-based extender containing egg yolk (M) or in Andromed (commercially extender). AI were done to 514 does during natural oestrus. Spermatozoa diluted in milk extender resulted in a 25-day non-return rate (NRR) and kidding rates of 37.3% and 24.5%, respectively, while semen diluted in Andromed diluent resulted in 31.7% NRR and a kidding rate of 19.8%.

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