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EFFECT OF GENETIC GROUP, SEMEN DILUENTS AND FREEZING REGIMENS ON SPERM FREEZABILITY AND GOATS REPRODUCTIVITY

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ABSTRACT

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Two experiments were designed to study the effect of bucks breeds, semen extenders and freezing regimens on post-thaw semen motility and viability index (experiment 1). In experiment 2, semen diluents effects on reproductive performance was conducted. Semen was collected from Aradi (A), Damascuss (D) and cross (½A½D) bucks breeds. 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 0.5 ml straws then cooled to 5°C. After equilibration, half of the packaged straws were suspended 15 cm above liquid nitrogen (LN) for 15 min. (Freezing regimen1; slow). Other half of straws was suspended at height 15 and 5 cm of LN for 10 and 5 min, 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 crossbred. Post-thaw semen motility and viability revealed, significant higher means for Freezing regimen 2 than regimen 1. Milk and Tris diluents were significantly higher than Na. Citrate for fertility, fecundity and prolificacy. Aradi does was higher than Damascus does in fertility, fecundity and prolificacy. Also, Aradi does was significantly higher than ½A½D does in fertility, while the reverse trend was observed in fecundity and prolificacy. It was concluded that, regarding to post-thaw semen motility, semen viability, fertility%, fecundity% and prolificacy%, milk diluent is preferable than Tris and Na. citrate. Fertility rate of Aradi goat breed is higher than Damascus and crossbred (½A½D).

***Keywords**: Buck; doe; genetic groups; semen diluents; semen freezing; artificial insemination; reproductive performance.*

INTRODUCTION

Successful AI needs good heat detection and semen handling. Artificial insemination (AI) has the greatest contribution in genetic improvement programs, mainly due to well-established methods for identifying males with the highest genetic merit (Evans and Maxwell, 1987; Leboeuf *et al.*, 2000). The success of AI is based on the ability of collecting and cryopreserving spermatozoa efficiently from proven bucks to be used for inseminating the does over generations (Amoah and Gelaye, 1990). There are three methods of semen preservation (fresh, refrigerated and frozen) to be used worldwide in goats (Evans and Maxwell, 1987; Leboeuf *et al.*, 2000). Spermatozoa are likely to suffer from considerable damages and deteriorations during dilution and preservation at low temperature. Therefore, using suitable diluents are basic necessities to have successful preservation of spermatozoa and higher conception rate in breeding programs (Salamon and Maxwell, 1995).

The main problem of worldwide 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; Ritar and Ball, 1993). Tris diluent

was found to maintain higher quality of semen in pre-freezing and post-thawing (Drobnis *et al.*, 1980; Deka and Rao, 1987; Chauhan and Anand, 1990; Tuli and Holtz, 1992). Also, Singh and Purbey (1996) reported that Tris diluter was superior for semen freezing in goats than citrate diluter concerning post-thaw motility. On the contrary, Salvador *et al.* (2007) cited that milk diluent provided higher viability of spermatozoa than semen diluted in Tris and citrate. The present study aimed to investigate the effect of semen extenders, freezing regimens and genetic groups of bucks on post-thaw semen motility and viability index (experiment 1) and to verify that semen diluents could influence reproductive performance of does in arid environment (experiment 2).

MATERIALS and METHODS

Study area:
A crossbreeding program between Saudi Aradi goats (A) and Damascus goats (D) was carried out at Camel and Range Research Center in Al-Jouf province (Northern region of Saudi Arabia located at latitude of 29°58′11″N 40°12′00″E and longitude of 40.21° W and at 684 m above sea level). The hottest month of the year is August with an average high and low temperature of 41°C and 25°C, respectively, whereas January is the coldest month of the year, with an average high and low temperature of 15°C and 4°C, respectively. The annual rainfall ranges from 0 to 3 mm.

Animals management:
This program permitted to produce three genetic groups of Aradi (A), Damascus (D) and crossbred (½A½D) goats. Animals were housed in semi-shaded/open front barn and fed commercial concentrate and alfalfa hay. The amount of concentrate and hay were calculated according to the nutritional requirements for goats (Kids, does and bucks) depending on the animal ages and production status (National Research Council; NRC, 1981). Water, straw, salt and minerals supplemented in blocks were freely available to all animals.

Experiment 1:
This experiment was conducted to investigate the effect of semen extenders, freezing regimens and genetic groups of bucks on post-thaw semen motility and viability index. Twenty two bucks from three genetic groups of Aradi (A), Damascus (D) and cross ½A½D were used in this study. Four semen diluents and two freezing programs were evaluated for freezing buck semen. Constituents of these diluents are shown in Table 1. Three to four ejaculates per buck were collected using artificial vagina. Semen samples with good motility of ≥70% were used for processing. Semen samples for each buck were divided into four portions, each portion was added to one diluent with dilution rate of 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 a foam box container at height 15 cm above LN for 15 min (Freezing regimen 1; slow freezing). The other half of straws was suspended in LN vapor at height 15 then 5 cm of LN, for 10 then 5 min (Freezing regimen 2; rapid freezing) before plunged into LN. Straws of frozen semen 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) and post-thaw motility at 0 hr after thawing was reassessed after 1, 2 and 3 hours of thermal stress and the viability index was determined for each semen sample.

Experiment 2:
Semen preparation:
In this experiment, estrus synchronization and AI were performed to 928 does. **S**emen samples were collected from genetically selected and improved bucks then extended using dilution rate of 1:15 to 1:20 (semen:diluent) according to sperm concentration to provide 120-150x10^6 as inseminated dose. Three types of extenders (skim milk, Na. Citrate and Tris) were used for semen dilution; constituents of the diluents are presented in Table 1. The diluted semen samples were gradually cooled within 2 hrs to 5ºC and stored in a refrigerator as chilled semen to be used for artificial insemination (Azawi *et al.*, 1993).

Does Preparation for AI:
Intravaginal progestagen release device (CIDR) containing 300 mg progesterone or intravaginal progesterone impregnated sponges containing 30 or 45 mg fluorogesteone acetate (FGA) were taken to 928 does and maintained in situ for 15-17 days. At the day of sponge withdrawal, 200-300 IU/eCG was injected intramuscular. Artificial insemination (AI) was done blindly (irrespective to signs of estrus) 48 to 60 hours after sponge removal, using chilled diluted semen (0.5 ml containing 120-150x10^6 motile spermatozoa) of the three types of diluents. AI was practiced 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 localized with the help of light and by gentle sideways or downward manipulation of the speculum. Semen was deposited at a depth of 0.5-1.0 cm into the cervix (cervical insemination). Non returned rate (NRR) was applied 20 to 25 days after AI using vasectomized bucks to detect does not returned to estrus and confirmed at 30-45 days post insemination with the aid of

ultrasound scanner. Parameters of reproductive performance involved in this study were: fertility rate (pregnant does/does inseminated)x100; kidding rate (kidded does/pregnant does)x100; fecundity rate (kids born /pregnant does)x100; and prolificacy rate (kids born /kidded does)x100.

Statistical analysis:
Data of semen and artificial insemination parameters and doe reproductive traits (fertility, kidding, fecundity, and prolificacy rates) were collected from 2006 to 2011. Such that were statistically analyzed using GLM procedures of SAS program (SAS, 1999). Data of post-thaw semen motility and viability were analyzed using the following linear model:

$$y = Xb + e$$

Where **y** = vector of observed trait, **b**= vector of fixed effects (genetic group, semen diluents and freezing regimens); X is the incidence matrix relating records to the fixed effects; and **e**= vector of random error. Data of doe reproductive traits were analyzed using the same previous model after excluding the effect of freezing regimens.

RESULTS

Effect of semen diluents on post-thaw semen viability
As shown in Table 2, pre-freeze motility was the highest in semen (P<0.05) diluted with Tris extender (78.5), followed by sodium citrate (76.7), sodium-citrate-bicarbonate diluents (76.1), and milk diluent (71.9). Post-thaw motility were significantly high(P<0.05) in semen diluted by milk diluent (46.4), followed by sodium citrate (42.9), Tris (38.8) and sodium-citrate-bicarbonate diluents (35.8). The viability index of post-thaw semen motility was significantly high (P<0.05) in semen diluted by sodium citrate (104.2), descended by semen diluted in milk (103.5), Tris (95.1), and sodium-citrate-bicarbonate diluents (91.5).

Effect of genetic group of bucks on post-thaw semen viability

The mean values of pre-freezing semen motility for Aradi bucks (A), Damascus (D) and crossbred (½A½D) were 75.6, 75.9 and 76.5, respectively, with no significant differences (Table 3). The same trends were observed for viability index where the mean values were 101.4, 95.5 and 93.5, for Aradi, Damascus and ½A½D bucks, respectively, with no significant differences. Post-thaw semen motility at 0hr were Significant higher (P<0.05) in Aradi (42.3) and Damascus (39.6) than cross bucks (38.1). Non-significant differences were observed among genetic groups in viability index of post-thaw semen motility.

Effect of freezing regimens on post-thaw semen viability
The effect of freezing protocols on post-thaw semen motility and viability index revealed that, the percentages of post-thaw semen motility (47.7 vs 34.1) and viability index (121.0 vs 76.3) were significantly higher (P<0.001) in rapid freezing regimen comparable to slow freezing regimen (Table 4).

Effect of semen diluents on reproductive traits
Regarding the effect of semen diluents on reproductive performance, the fertility rates were 52.99±3.27, 45.83±5.91 and 36.01±1.93 for does inseminated by semen diluted with milk, Tris and sodium citrate respectively (Table 5). The corresponding percentages of kidding, fecundity, and prolificacy were 95.97±1.77, 185.4±6.45 and 193.28±5.69 for does inseminated with milk diluent; 90.91±5.08, 181.82±14.73, and 200.00±11.74 for Tris diluent; and 90.18±1.99, 154.02±4.85, and 170.79±3.84 for Na. Citrate diluent, respectively. These results revealed that milk and Tris diluents were significantly higher in fertility, fecundity and prolificacy rates (P<0.05) than Na. Citrate diluent, while the effect of diluent on kidding rate was non-significant (Table 5).

Effect of genetic group on reproductive traits
As shown in Table 6, local Aradi does was higher than Damascus does in fertility (47.95±2.26 vs 30.43±3.03), fecundity (164.96±4.87 vs 157.1±9.00) and prolificacy (181.22±3.84 vs 171.88±7.54). Also, Aradi does was significantly higher than ½A½D does in fertility, while the reverse trend was observed in fecundity and prolificacy (Table 6). But, kidding rates were nearly similar in the three genetic groups studied.

Interactions
Interactions between diluents and genetic groups of bucks were significantly affected pre-freeze sperm motility, post-thaw motility and viability index (P<0.001). Also, the interactions between freezing regimens and diluents were significantly high on post-thaw semen motility and viability index (P<0.001).

Table 1: Composition of semen diluents (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 diluents (Means ±SE) on Pre-freezing and post-thaw semen motility and viability index

Semen diluent	Pre-freezing motility	Post-thaw semen motility				
		0hr	1hr	2hrs	3hrs	Viability index
Milk	71.88±1.29^{b}	46.44±1.29^{a}	39.36±1.29^{a}	31.08±1.27^{a}	19.64±1.18^{b}	103.48±3.38^{a}
Na citrate	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	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	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}

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

Table 3: Effect of genetic groups of bucks (Means ±SE) on pre-freezing and post-thaw semen motility and viability index

Genetic group	Pre-freezing motility	Post-thaw semen motility				
		0hr	1hr	2hrs	3hrs	Viability index
Aradi (A)	75.57±0.73^{a}	42.27±0.87^{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±1.34^{a}	95.49±3.68^{a}
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}

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

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

Post-thaw hours	Post-thaw semen motility	
	Slow freezing regimen	Rapid freezing regimen
0 hr	34.15±1.04 ^{b}	47.74±0.66 ^{a}
1 hr	28.54±1.00 ^{b}	42.74±0.71 ^{a}
2 hrs	22.60±0.93 ^{b}	38.59±0.72 ^{a}
3 hrs	16.12±0.84 ^{b}	31.65±0.88 ^{a}
Viability Index	76.28±2.59 ^{b}	121.03±1.89 ^{a}

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

Table 5: Effect of semen diluents on doe reproductive performance

Diluent	No. of treated does	Fertility %	Kidding %	Fecundity %	Prolificacy %
Milk	234	52.99±3.27 [a]	95.97±1.77 [a]	185.48±6.45 [a]	193.28±5.69 [a]
Tris	72	45.83±5.91 [ab]	90.91±5.08 [a]	181.82±14.73 [a]	200.00±11.74 [a]
Na Citrate	622	36.01±1.93 [b]	90.18±1.99 [a]	154.02±4.85 [b]	170.79±3.84 [b]

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

Table 6: Effect of genetic group on doe reproductive performance

Genetic group	No. of treated does	Fertility %	Kidding %	Fecundity %	Prolificacy %
Aradi (A)	488	47.95±2.26[a]	91.03±1.87 [a]	164.96±4.87 [ab]	181.22±3.84 [a]
Damascus (D)	230	30.43±3.03 [b]	91.43±3.37 [a]	157.14±9.00 [c]	171.88±7.54 [a]
½A½D	210	36.67±3.33 [b]	96.10±2.22 [a]	180.52±8.48 [a]	187.84±7.69 [a]

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

DISCUSSION

Cryopreservation of semen has become a valuable tool for the preservation of genetic reserves of endangered species or sires of superior breeding value (Sabine Schafer-Somi, *et al.*, 2006). The results of this study have indicated that there is a difference between extenders regarding their pre-freeze motility, post-thaw motility and viability index of goat spermatozoa. Best results were obtained with a milk extender which appeared to provide higher in vitro spermatozoa viability. These findings are in harmony with those recorded by Salvador *et al.* (2007) who compared the effect of three extenders (skimmed milk, sodium Citrate and Tris-based diluents) on the in vitro viability of Murciano-Granadina goat spermatozoa stored at 5°C. They stated that semen diluted by milk provided higher in vitro viability of spermatozoa than semen diluted in Tris. Hassan (1990); Mohammed *et al.* (1998) and Ziada *et al.* (1998) obtained satisfactory post-thaw motility by freezing buffalo semen in milk diluent more than that in Tris or sodium citrate diluents. Moreover, Dorado *et al.* (2007, 2010) observed that percentage of acrosome intact spermatozoa was significantly higher in semen samples diluted with milk extender than semen diluted by Tris extender, and at the same time semen cryopreserved in milk extender provided better pregnancy rates after insemination compared to those semen cryopreserved in Tris extender (52.4% vs 42.9%). Contrary, Chehadeh *et al.* (2001) observed that Tris was the best diluent for maintaining goat sperm motility (77.1 %) followed by milk (66.9%), Cegly (66.0%) and sodium citrate (64.0%) extenders. However, the post-thaw semen motility of milk diluent was sharply declined from 46.4 at 0 hr to 19.6 at the third hour after thawing throughout the incubation period at 37ºC. 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). Nunes *et al*. (1982) identified a protein (SBUIII) from the goat bulbourethral gland, which decreased survival of cooled or frozen goat sperm diluted in milk-based media. It also induced the acrosome reaction and subsequent cell death of spermatozoa incubated in milk medium at 37°C (Pellicer-Rubio *et al.,* 1997). On the other hand, post-thaw semen motility of Tris diluent herein was slowly decreased from 38.80 at 0hr to 25.1 at the third hour after thawing. Fischer (1990) and Shahram Bohlooli *et al.* (2012) found that Tris was the best in preserving acrosomal integrity and motility after thawing. 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. Salamon and Ritar (1982) considered tris hydroxyl methyl 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. The fructose content of yolk Tris diluent may also help in maintaining the osmotic pressure, and providing nutrient for sperm metabolism.

Non-significant differences were observed among genetic groups in pre-freeze semen motility and post-

thaw viability index. Similarly, William (1982) showed, the effects of age and goats breed on initial motility, Prefreeze motility and Post-thaw motility were negligible. Furstoss *et al.* (2009) evaluate the semen of 13151 and 9206 ejaculates of 758 Alpine and 535 Saanen bucks, respectively reporting that there is no significant effect for genetic groups of bucks on post-thaw semen motility. But, results herein showed significant differences in values (P<0.05) of post-thaw semen motility at 0 hr; being 42.3 for Aradi, 39.6 for Damascus and 38.1 for crossbred bucks of ½A½D. Similarly, Karatzas *et al.* (1997) using does of Greek breed (Capra prisca) that were synchronized and inseminated from bucks of Alpine, Saanen, and Damascus found that fertility and kidding rates were lower in does inseminated from semen of Damascus bucks than semen of Alpine and Saanen bucks.

In recent years, the development of freezing techniques for semen cryopreservation has become a major resource for the preservation of genetic material in most domestic species (Songsasen and Leibo 1997a,1997b; Ollero *et al.*, 1998; Colenbrander *et al*., 2003; Cremades *et al.*, 2005). Freezing and thawing rates have been shown to influence the post-thaw survival of a number of cells including sperm cells from various species (Mazur, 1985 and Watson, 1990). In the present study, the effect of freezing protocols on post-thaw semen motility and viability index revealed that percentages of post-thaw motility and viability index were favorable (P < 0.001) in rapid freezing regimen comparable to slow freezing regimen. These findings are in agreement with Mohammed *et al.* (1998) who recorded maximum post thaw motility of 63.3 (P<0.01) associated with the highest viability (153.3) when Friesian semen was rapidly frozen at 2 cm above LN for 15 min. while the minimum motility (16.7) and the lowest viability index (44.2) were observed when the semen was relatively slow frozen at 8 cm above LN for 10 min. The same authors added that the highest motility (63.3) of post-thaw buffalo semen (P<0.01) and the highest viability index (161.7) were obtained when the semen was frozen at 2 cm above LN for 10 min, while the lowest motility and viability were obtained when the semen was frozen at 8 cm above LN for 10 min. On the basis of post-thaw motility, Bhandari *et al.* (1982) reported that the 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 fast freezing (10 min) gave low survival of sperm for cattle and buffalo semen where post-thaw motility was 47.2 and 30.3, respectively, while slow freezing (15 min) gave high survival of sperms where post-thaw motility was 57.2 and 34.3 for cattle and buffalo semen, respectively.

Interactions between genetic groups of bucks; freezing protocols on one side and semen diluents on the other side were significantly affects pre-freeze sperm motility, post-thaw motility and viability index (P<0.001). These findings are greatly in accordance with Mohammed *et al.* (1998) who found that interactions between freezing regimens and diluents were highly affected (P<0.01) viability index and AST of post-thaw buffalo spermatozoa. In opposition to our results, Dhami and Sahni (1995) and Mohammed *et al.* (1998) found non-significant interactions between diluents and cooling rates on post-thaw bovine semen motility and viability. However, the sperm cryosurvival appears to depend on biochemical composition of the diluents used, thermal behaviour, osmotic resistance and 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).

Also, the interactions between freezing regimens and diluents were significantly high on post-thaw semen motility and viability index (P<0.001). These findings are greatly in accordance with Mohammed *et al.* (1998) who found that interactions between freezing regimens and diluents were highly affected (P<0.01) viability index and AST of post-thaw buffalo spermatozoa. Contrary, Dhami and Sahni (1995) and Mohammed *et al.* (1998) found non-significant interactions between diluents and cooling rates on post-thaw bovine semen motility and viability.

Regarding the effect of semen diluents on reproductive performance, the results revealed that milk and Tris diluents were significantly higher (P<0.05) in fertility, fecundity and prolificacy rates than Na. Citrate diluent. These results are in accordance with those reported by Dorado *et al.* (2007, 2010), where Tris-glucose and skim milk extenders were used to compare the ability of extenders for maintaining the sperm viability after cryopreservation. They reported that Tris extender gave good in vitro performance compared to milk extender, although this improvement was not reflected in results of fertility rates, where semen cryopreserved in milk extender provided better pregnancy rates after intra-cervical insemination compared to those semen cryopreserved in Tris extender (52.4% versus 42.9%). Similarly, Mara *et al.* (2007) using three types of diluents for semen dilution reported that percentages of pregnant goats were 71.4, 61.4 and 48.8 % for skim milk, tempol and tempol+hyaluronic acid diluents, respectively, while kidding rates were 66.7% for skim milk diluent, 61.4% for tempol diluent and 48.8% for tempol+HA diluent, without significant differences among treatment groups. Das *et al.* (1985) reported that conception rate of goat semen diluted and preserved in milk diluter was comparatively higher (84 %) than that of egg yolk citrate (76 %) and Tris diluter (76 %). In Norwegian dairy goat breed with applying AI for 514 does during natural oestrus, Nordstoga *et al.*

(2010) found that 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 (commercial extender) resulted in 31.7% NRR and a kidding rate of 19.8%.

The effect of genetic group on reproductive performance showed that, Aradi goat breed was higher than Damascus in fertility and fecundity. Also, Aradi goat breed was significantly higher ($P<0.05$) than crossbred ½A½D in fertility. In accordance with the present finding, Joshi and Shrestha (2003) found that the prolificacy rate of indigenous Khari breed was 1.97, while the crosses of Khari x Jamunapari, Khari x Barbari and Khari x Saanen were 1.79, 2.09 and 2.60, respectively. It seems that the first cross (½A½D) showed higher values in fecundity, kidding and prolificacy, explaining the effect of heterosis. Similarly, Gaddour *et al*. (2007) recorded that local goat breed of southern Tunisia had higher rates than Damascus goat (used as ameliorative breed) in fertility (92.71 vs 95.00), prolificacy (153.43 vs 136.86) and fecundity (142.50 vs 129.71). Najari *et al.* (2002) concluded the same aspect for other goat breeds. Duricic *et al.* (2012) recorded that no significant differences between goat breeds for kidding and prolificacy rates were recorded.

In the present study, about 8% of the does not returned to estrus after 21-25 days of AI and recorded as pregnant didn't kidded as results of pseudopregnancy. High percentage of pseudopregnancy (9%) was appeared in Aradi and Damascus goats, whereas a lower percentage was recorded in ½A½D doe (4%). Similarly, pseudopregnancy appeared to be 3-20% (Mialot *et al.,* 1991; Hesselink 1993; Leboeuf *et al.,* 1994). Pseudopregnancy was related to reproduction method (3.8% in 1493 FGA/PMSG treated goats ys 2.5% in 3774 naturally mated goats, (Mialot *et al.* 1991), with parity (1 % of nulliparous ys 18% of multiparous; Hesselink 1993), and with age (10% of 280 less than 5 years old does vs 32% of 34 does more than 6 years old; Hesselink 1993).

Conclusions

Fertility and fecundity rates were significantly higher ($P<0.05$) in milk diluted semen than Tris and sodium citrate diluents although in vitro evaluation of pre-freeze semen motility in milk diluent was significantly lower than Na. citrate and Tris diluents. The cooling rate for semen should not be too fast to cause cell death due to cold shock or too slow to cause death due to osmotic shock. The first generation of crossing Damascus x Aradi showed high percentages of fertility, kidding, fecundity and prolificacy; indicating an important heterotic effect on these traits.

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تأثير المجموعات الوراثية ونوع المخفف ونظم التجميد على قابلية السائل المنوي للتجميد وكذلك الكفاءة التناسلية للماعز

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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 ثلاثة سلالات من الماعز هى العارضى والدمشقى والخليط ، قسمت العينات على أربعة أجزاء ، كل جزء تم تخفيفه بأحد الممدات التالية وهى: الحليب منزوع الدسم والترس وسترات الصوديوم ومخفف بيكربونات الصوديوم. بعد التخفيف تم تعبئة السائل المنوى فى قصيبات سعة ½ملى ثم بردت تدريجيا وتركت فى الثلاجة لمدة لا تقل عن ساعتين لإعطاء فرصة للحيامن للتأقلم والتكييف مع المخفف فى درجة 5 مئوية ، بعد هذه الفترة تم تقسيم القصيبات الى مجموعتين، الأولى (نظام التجميد البطئ) تم تعرض قصيبات السائل المنوى لبخار النتروجين السائل وعلى مسافة 15سم من سطح النتروجين ولمدة 15 دقيقة بعدها تم غمس القصيبات فى النتروجين السائل، أما المجموعة الثانية (نظام التجميد السريع) تم تعرض القصيبات لبخار النتروجين لمدة 10 دقائق وعلى مسافة 15سم من النتروجين بعدها تم تقليل المسافة الى 5سم وتركت لمدة 5 دقائق أخرى قبل غمسها فى النتروجين السائل. بعد مرور يومين تم فحص قصيبات السائل المنوى بعد اسالتها للتعرف على الحركة الامامية ومعدل الحيوية. **التجربة الثانية**: تم تجميع السائل المنوى من تيوس محسنة وراثيا (لاستخدامها فى التلقيح الاصطناعى لتحسين الصفات الانتاجية لسلالات 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 5 مئوية استخدم السائل المنوى المبرد خلال يومين من تحضيره لتلقيح العنزات اصطناعيا. بعد مرور 45 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. وجد أيضا أن نظام التجميد السريع كان له تأثير أفضل على خصائص السائل المنوى عن نظام التبريد البطئ. وجد أن مخفف الحليب والترس كان لهما تأثير أفضل على الكفاءة التناسلية عن مخفف سترات الصوديوم. وأخيرا وجد أن سلالة الماعز العارضى يليها الخليط كانتا أفضل من حيث الكفاءة التناسلية عن سلالة الماعز الدمشقى. ويمكن خلاصة القول بأن مخفف الحليب منزوع الدسم ونظام التجميد السريع كانا لهما تأثير إيجابي على تجميد السائل المنوى للتيوس. سلالة الماعز العارضى والخليطة وتركيبها الوراثى ½ عارضى½دمشقى كانتا أفضل من حيث الكفاءة التناسلية عن سلالة الماعز الدمشقى.