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INFLUENCE OF SOME CRYOPROTECTANTS IN COMPARING WITH DIFFERENT GLYCEROL LEVELS ON SPERM VIABILITY DURING FREEZING PROCESS OF GOAT SEMEN

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ABSTRACT

A total of 160 good quality ejaculates were collected for freezing from 10 mature Zaraiby bucks (28.4±0.9 kg LBW and 25-35 mo old) in Sakha Research station during July-August period (twice/week for 8 wk) to determine the optimal level of, glycerol alone (1 to 7%, 1st experiment) and comparing the optimal level of glycerol with its combination with dimethyl sulfoxide (DMSO) and different levels (1, 3 and 5%) of ethylene glycol (EG), ethanol and methanol as cryoprotectants in Tris-based extender (2nd experiment), and the effect of the cryoprotectauts on sperm motility percentage (SMP) and recovery rate (RR%) at different freezing process stages (dilution, equilibrium and thawing). Results of the 1st experiment show that SMP in post-diluted and post-equilibrated semen was not affected significantly by glycerol (GL) level ranging between 79-81%. Loss in SMP between dilution and equilibrium ranged between 1-3% for all GL levels, being the lowest for 7% level and the highest for 5% level. Post-equilibrated RR of sperm motility ranged from 96.3 for 5% level to 98.8% for 4 and 7% levels. SMP showed gradual increase ($P<0.05$) from 20 to 48% by increasing GL level from 1 to 6%. Increasing GL level to 7% led to insignificant reduction in SMP (46%). Glycerol at 6% level showed the lowest ($P<0.05$) SMP loss (30%) and highest RR (61.5%) of sperm motility in post-thawed semen. The highest RR (72%) was obtained in 2 h post-thawed semen with 5% GL level, but did not differ significantly from that diluted with 6 and 7% levels. No viable spermatozoa were found 2 h post-thawing in semen diluted with extenders containing low glycerol levels (1 and 2%). Results of the 2nd experiment show that semen diluted with 6% GL showed the best results based on SMP and RR in post-diluted and post-equilibrated semen as compared to the other cryoprotectant levels. Level of 3% EG showed similar results to 6% GL level, while all methanol levels reflected the poorest results as compared to the other levels of cryoprotectants. In post-thawed semen, SMP and RR were the highest ($P<0.05$) and loss in SMP was the lowest ($P<0.05$) for 6% GL (47%), followed by 3% GL+3% DMSO and 6% DMS (39 and 34%, respectively). Non-viable spermatozoa were observed in 2 h post-thawed semen diluted with all levels of EG, ethanol or methanol. However, SMP in 2 h post-thawed semen was affected significantly ($P<0.05$) by diluents containing GL, DMSO or their combination, being 33, 16 and 24%, respectively. Similar trend was obtained for sperm recovery rate in 2 h post-thawed semen, being 70.0, 55.9 and 61.5%, respectively.

The current study indicated that adding glycerol to Tris-based extender at level of 6% for cryopreservation of Zaraiby goat semen frozen in pellets form yielded the highest sperm motility in post-thawed semen.

Keywords: Zaraiby goat, semen, freezing, sperm motility, cryoprotectants, glycerol.

INTRODUCTION

The major physical chemical consequences of freezing are the removal of free water from solution to form ice and the resultant increased concentration of solutes in residual liquid. These event and their effects on the sperm cells are influenced by the level and type of cryoprotectants (Gil *et al.*, 2000, Watson, 2000 and Jorge *et al.*, 2003). Therefore, the cryoprotectants were added to extenders to maintain the sperm for damage during freezing process (Singer *et al.*, 1995). The role of cryoprotectants is a matter of substantial debate and current thinking on nature of protective effects of penetrating and non-penetrating cryoprotectants are discussed by Watson (1995).

Glycerol, which was believed to penetrate cells, has been the most widely used as a cryoprotective agent for spermatozoa (Polge *et al.*, 1949). Many studies have been reported on the use of glycerol for semen freezing. Glycerol penetrates the sperm cell membrane, replacing part of its free water, thus reducing the harmful concentration of intracellular electrolytes during freezing (Mann and White, 1957). Conflicted results were obtained by many authors on the effect of different levels of glycerol (2.5 to 9%) on sperm motility of frozen goat semen (Salamon and Ritar, 1982; Deka and Rao, 1986; Moussa, 1987; Tuli *et al.*, 1991 and Singh and Pubery, 1996). Also, Fahy (1986) reported that glycerol concentration for freezing have been between 2.25 and 9%. However, the level of glycerol depends on cooling and freezing rate, diluent composition, method of glycerol addition and in particular on its osmotic pressure (Salamon and Maxwell, 2000). Furthermore, Leboeuf *et al.* (2000) reported that glycerol concentration varied form 3 to 9%, with the optimum of 4% to 7% in the diluents of goat semen.

Dimethyl sulfoxide (DMSO) has been reported to have a cryoprotective effect on bull (Snedeker and Gaunya, 1970) and goat (Amoah and Gelaye, 1997) spermatozoa. While, Molinia *et al.* (1994) revealed that ethylene glycol (EG) exhibited a cryoprotective effect, but DMSO had no effect on pellet-frozen ram spermatozoa. Moreover, a combination of glycerol with either EG (Molinia *et al.*, 1994) or DMSO (Salamon, 1968 and Neubert and Menger, 1981) induced better post thaw motility of ram spermatozoa. Rodrigues *et al.* (2004) found that EG diffuse a cross cell membrane in exchange for cell water. This displacement of water by cryoprotants, in addition to freezing point depression, decreases the possibility of intracellular ice formation and maintains cell volume during freezing, avoiding damage (Demirci *et al.*, 2002).

On the other hand, methanol has been reported to have superior cryoprotective properties for a variety of cell types including fish spermatozoa and embryos (Lahnsteiner *et al.*, 1996 and Chywan and Cheng, 1998). In this respect, Tiersch *et al.* (1994) found that methanol as a cryoprotectant resulted in higher post-thaw sperm motility of catfish than DMSO. Khalifa (2005) found that monohydric and polyhydric alcohols with low concentration levels indicated significant results than high concentration on post-thaw

motility of goat spermatozoa. The date for using methanol as cryoprotectants for goat bucks semen are not available in literature.

Therefore, the current study aimed to determine the optimal level of glycerol alone (1 to 7%) and comparing the optimal level of glycerol with its combination with DMSO and different levels (1, 3 and 5%) of EG, ethanol and methanol as cryoprotectants in Tris-based diluent for cryopreservation of Zaraiby buck semen.

MATERIALS AND METHODS

A total of 10 mature Zaraiby bucks (averaged 28.4±0.9 Kg live body weight and ranged between 25-35 months of age) were used for semen collection in this study. All experimental animals were raised in Sakha Experimental Station under the same environmental conditions. During semen collection period (July-August) all animals were fed ad. libitum on berseem hay in addition to a 250 g/head/day of CFM. Animals were allowed to drink fresh water twice daily. The feeding requirements were calculated according to the recommendations of the Ministry of Agriculture.

Semen collection and dilution:

Total of 160 good quality ejaculates were only used for freezing throughout this study. Semen was selected to be high in sperm concentration and sperm mass motility (not less than 70%). Semen was collected from each buck twice weekly by an artificial vagina between 8.0 and 9.0 a.m. during breeding season (July-August). All ejaculates were transferred immediately after collection to the laboratory. Thereafter, ejaculates of each collection day were pooled, divided into replicates during the first four collection weeks and placed in water bath at 37^{o}C until dilution.

During the first four collection weeks (1st experiment) the pooled semen of each collection day was divided into 7 portions and diluted with Tris-extenders containing 7 levels of glycerol (from 1 to 7%, respectively). However, during the second four collection weeks (2nd experiment), the pooled semen of each collection day was divided into 12 portions and diluted with Tris extenders containing 6% glycerol (the optimal level of glycerol), 3% glycerol plus 3% DMSO, 6% DMSO, as well as 1, 3 and 5% either from EG, ethanol or methanol. Generally, semen was diluted at a rate of 1:8.

Freezing process:

Extended cooled (5^{o}C) semen was equilibrated for 3 h and frozen in pellets form on solid CO_2 surface according to Nagase and Niwa (1964). Four minutes freezing period on dry ice was adopted according to Sodipe (1989). He suggested 3-4 minutes freezing period to be good enough for pelleting.

Assessment of sperm motility:

Sperm motility was determined using hot-stage microscopy at 37^{o}C. Sperm progressive motility was assessed pre-freezing in post-diluted, post-thawed and 2 hours post-thawed semen. Recovery rate (%) of sperm motility was calculated as the following:

Recovery rate (%) = Initial motility/final motility x 100

Statistical analysis:

Data were statistically analyzed by the methods of General linear model procedure (GLMP) using computer program of SAS (2005). For the effect of different levels of glycerol (1st experiment), total of 280 semen samples were used (5 replicates x 2 collection days x 4 collection weeks x 7 levels). While, 480 semen samples (5 replicates x 2 collection days x 4 collection weeks x 12 levels) were used to determine the effect of different cryoprotectant levels (2nd experiment). Duncan Multiple Range Test was used to test the differences among means (Duncan, 1955). The percentage values of motility were adjusted to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Effect of different glycerol levels (1st experiment):

Sperm motility in post-equilibrated semen:

Results in Table (1) show that sperm motility percentage in post-diluted and post-equilibrated semen was not affected significantly by glycerol level. Loss in sperm motility between dilution and equilibrium processes ranged between 1-3% for all glycerol levels, being the lowest for 7% glycerol level and the highest for 5% level. Also, post-equilibrated recovery rate of sperm motility ranged from 96.3 for 5% glycerol level to 98.8% for 4 and 7% level.

It is of interest to note that the lowest sperm motility percentage in diluted semen was associated with the lowest rate of loss and the highest recovery rate of sperm motility in post-equilibrated semen diluted with 7% glycerol level. While, the opposite was true with 5% glycerol level (Table 1).

Table (1): Effect of glycerol level in Tris-based extender on sperm motility (%) in post-diluted and post-equilibrated semen.

Glycerol level (%)	Sperm motility			
	Post-dilution	Post-equilibrium	Loss (%)	Post-equilibrium recovery rate (%)
1	81.0	79.0	2.0	97.5
2	80.0	78.0	2.0	97.5
3	81.0	79.0	2.0	97.5
4	80.0	79.0	1.0	98.8
5	81.0	78.0	3.0	96.3
6	80.0	78.0	2.0	97.5
7	79.0	78.0	1.0	98.8
±MSE	2.56	6.73	2.45	3.02

Similar result was obtained by Salamon and Ritar (1982), who found that 4.0 or 5.5% glycerol levels in Tris-extender had beneficial effects on post-dilution motility at storage time of only 1.5 h. Also, Moussa (1987) indicated the significant effects of adding different glycerol levels on pre-freezing motility of buck spermatozoa. The highest percentage of motility

spermatozoa was obtained in semen extended by 6 to 8% glycerol extenders in the pre- freezing stage than in 4 and 10% glycerol levels respectively.

Sperm motility in post-thawed semen:

Although sperm motility percentage was not affected by glycerol level in post- diluted and post-equilibrated semen, results in Table (2) indicated significant differences in sperm motility percentage in post-thawed semen as affected by glycerol level. Percentage of sperm motility showed significantly ($P<0.05$) gradual increase by increasing glycerol level from 1% reaching the maximum with 6% glycerol level. However, increasing glycerol level to 7% led to insignificant reduction in sperm motility.

It is worthy noting that the observed loss in sperm motility during freezing showed an opposite trend to that of sperm motility in post-thawed semen and associated with recovery rate of sperm motility in post-thawed semen. Generally, semen diluted with Tris-based extender containing 6% glycerol level significantly ($P<0.05$) showed the lowest loss and highest recovery rate of sperm motility in post-thawed semen (Table2).

Table (2): Effect of glycerol level in Tris-based extender on sperm motility (%) in post-thawed semen.

Glycerol level (%)	Sperm motility			
	Post-equilibrium	Post-thawing	Loss rate (%)	Post-thawing recovery rate (%)
1	79.0	20.0^{e}	59.0^{a}	25.3^{e}
2	78.0	26.0^{d}	52.0^{b}	33.3^{d}
3	79.0	37.0^{c}	42.0^{c}	46.8^{c}
4	79.0	41.0^{b}	38.0^{d}	51.8^{b}
5	78.0	47.0^{a}	31.0^{e}	60.3^{a}
6	78.0	48.0^{a}	30.0^{e}	61.5^{a}
7	78.0	46.0^{a}	32.0^{e}	59.0^{a}
±MSE	6.73	7.14	7.50	9.11

$^{a, b.....e}$: Means with different superscripts in the same row are significantly different ($P<0.05$).

Sperm motility in 2 hours post-thawed semen:

Results of incubation of post-thawed semen for 2 hours (Table 3) indicated the highest percentage of sperm motility and the highest recovery rate in 2 h post-thawed semen diluted with Tris-based extender containing 5% glycerol level, but did not differ significantly from that diluted with 6 and 7% levels. It is of interest to observe that, no viable spermatozoa were found 2 h post-thawing in semen diluted with extenders containing low glycerol levels (1 and 2%, Table 3).

Many studies have been reported on the use of glycerol for semen freezing. Glycerol entry into the cells has been presented by metabolic studies of O'Dell and Hurst (1956). Glycerol penetrates the sperm cell membrane, replacing part of its free water, thus reducing the harmful concentration of intracellular electrolytes during freezing (Mann and White, 1957). This may explain the differences in effect of glycerol levels in this

study, being significant on post-thawed semen and non-significant on postdiluted and post-equilibrated semen.

Table (3): Effect of glycerol level in Tris-based extender on sperm motility (%) during semen incubation for 2 h post-thawing.

Glycerol level (%)	Sperm motility			
	Post thawing	2 h post-thawing	Loss rate (%)	2 h post- thawing recovery rate
1	20.0[e]	0.0	20.0[a]	0.0
2	26.0[d]	0.0	26.0[a]	0.0
3	37.0[c]	25.0[b]	12.0[b]	67.5[ab]
4	41.0[b]	26.0[b]	15.0[b]	63.4[b]
5	47.0[a]	34.0[a]	13.0[b]	72.3[a]
6	48.0[a]	33.0[a]	15.0[b]	68.7[ab]
7	46.0[a]	31.0[a]	15.0[b]	67.3[ab]
±MSE	7.14	5.00	2.50	7.31

[a, b.....e]: **Means with different superscripts in the same row are significantly different (P<0.05).**

In accordance with the results of sperm motility presented herein, Deka and Rao (1986) reported that 6.4% glycerol in Tris-based extender revealed higher post-thaw motility for frozen buck semen (66.9%) better than 4 or 9% glycerol (64.0 or 63.7%, respectively). However, Moussa (1987) indicated the significant effects of adding different glycerol levels on post-freezing motility of buck spermatozoa. The highest percentage of motility spermatozoa was obtained in semen extended by 6 to 8% glycerol extenders in the post-freezing stage than in 4 and 10% glycerol levels, respectively. Moreover, some authors found that reduction of glycerol to 3% or 2% and level above 7% decreased the post-thaw motility of spermatozoa in the diluent tested (Ramakrishanan and Ariff, 1994 and Nastri *et al.*, 1994).

Results of Leboeuf *et al.* (2000) indicated that glycerol concentration varied form 3% to 9%, with the optimum of 4% to 7% in the diluents goat buck semen. In this respect, Sinha *et al.* (1991) estimated that the level of glycerol could be lowered to 5% without any decrease effect in post-thawing motility of goat semen.

Recently, Khalifa (2005) found that 3 and 6% glycerol levels showed approximately values on post-thawing and sperm recovery of goat buck spermatozoa (with Tris-yolk fructose "TYF" extender) compared to glycerol levels at 1 and 2%. Means of sperm motility after thawing with glycerol levels 1, 2, 3 and 6 ml/100 extender was 34.50, 47.5, 52.0 and 53.0%, respectively.

In contrast to the present results, Awad *et al.* (2000) showed that best motility (62.5%) in Baladi goat semen was obtained with 5% glycerol and sperm motility decreased as level of glycerol increased. The differences in sperm motility to respond to glycerol level depends on cooling and freezing rate, diluent composition, method of glycerol addition and in particular on its osmotic pressure (Salamon and Maxwell, 2000). Furthermore, the glycerol concentration may be influenced by the egg yolk level in diluent. Increased concentration of egg yolk may reduce the required concentration of glycerol (Watson, 1995).

Effect of different levels and types of cryoprotectants (2nd experiment): Sperm motility in post-equilibrated semen:

Results in Table (4) show that sperm motility percentage in post-diluted and post-equilibrated semen was affected significantly (P<0.05) by level of different types of cryoprotectants. Sperm motility percentage in post-diluted semen was nearly similar and did not differ significantly among diluents containing 6% glycerol (GL), 6% DMSO or 3% GL+ 3% DMSO, ranging between 80 and 81%. Sperm motility percentage showed insignificantly gradual reduction by increasing level of EG, methanol or ethanol from 1 to 3 to 5%, but its value was slightly lower than those obtained for GL, DMSO or GL+ DMSO.

Table (4): Effect of level of different types of cryoprotectants in Tris-based extender on sperm motility (%) in post-diluted and post-equilibrated semen.

Cryoprotectant level	Sperm motility			
	Post-dilution	Post-equilibrium	Loss rate (%)	Post-equilibrium recovery rate (%)
6% glycerol (GL)	81.0^{a}	79.0^{a}	2.0^{c}	97.5
6% DMSO	80.0^{a}	76.0^{abc}	4.0^{c}	95.0
3% GL+3% DMSO	81.0^{a}	78.0^{ab}	3.0^{c}	96.3
1% EG	80.0^{a}	76.0^{abc}	4.0^{c}	95.0
3% EG	79.0^{ab}	77.0^{abc}	2.0^{c}	97.5
5% EG	78.0^{abc}	75.0^{bcd}	3.0^{c}	96.1
1% ethanol	77.0^{bcd}	74.0^{cde}	3.0^{c}	96.1
3% ethanol	76.0^{cde}	72.0^{def}	4.0^{c}	94.7
5% ethanol	75.0^{de}	71.0^{ef}	4.0^{c}	94.7
1% methanol	76.0^{cde}	70.0^{f}	6.0^{bc}	92.1
3% methanol	74.0^{ef}	66.0^{g}	8.0^{b}	89.1
5% methanol	72.0^{f}	60.0^{h}	12.0^{a}	83.3
±MSE	4.38	5.42	7.50	4.72

$^{a, b.....f}$: Means with different superscripts in the same row are significantly different (P<0.05).

Generally, 6% GL or 3% GL+3% DMSO showed significantly (P<0.05) the highest percentage of sperm motility in post-diluted semen, while, 5% methanol level showed significantly the lowest motility percentage.

The effect of level of different cryoprotectants was more pronounced on sperm motility in post-equilibrated semen, being the highest for 6% GL level (79%) and stilled the lowest for 5% methanol level (60%). It is worthy noting that loss in sperm motility between dilution and equilibrium processes was not affected significantly by all levels of cryoprotectants, except for methanol levels, which showed negative effect on sperm motility in post-equilibrated semen resulting in significantly (P<0.05) the highest loss in sperm motility, being 6, 8 and 12% by increasing methanol level from 1 up to 3%. Such trend in sperm motility in post-diluted and post-equilibrated semen led to the highest recovery rate of sperm motility in post-equilibrated semen diluted with 6% GL and the lowest one for that diluted with 5% methanol (Table 4).

In general, semen diluted with 6% GL showed the best results based on percentage of sperm motility in post-diluted and post-equilibrated semen or based on recovery rate of sperm motility in post-equilibrated semen. Also, 3% EG level showed similar results to 6% GL level. However, all levels of methanol reflected the poorest results as compared to the other levels of cryoprotectants (Table 4).

Sperm motility in post-thawed semen:

The significant effect of level of different cryoprotectants to maintain sperm motility during freezing was more pronounced in post-thawed semen, being significantly (P<0.05) the highest for 6% GL (47%), followed by 3% GL+3% DMSO and 6% DMS (39 and 34%, respectively), and the lowest for 5% methanol. This was associated with significantly (P<0.05) the lowest SMP loss and the highest recovery rate of sperm motility in post-thawed semen (Table 5). Although sperm motility percentage in post-equilibrated semen diluted with 6% GL did not differ significantly by 6% DMSO, 3% GL+3% DMSO, 1% and 3% EG, the results of sperm motility in post-thawed semen indicated beneficial effect of 6% GL on sperm cryopreservation.

Table (5): Effect of level of different types of cryoprotectants in Tris-based extender on sperm motility (%) in post-thawed semen.

Cryoprotectant level	Sperm motility			
	Post-equilibrium	Post-thawing	Loss rate (%)	Post- thawing recovery rate (%)
6% glycerol (GL)	79.0^{a}	47.0^{a}	32.0^{g}	59.5^{a}
6% DMSO	76.0^{abc}	34.0^{c}	42.0^{f}	44.8^{c}
3% GL+3% DMSO	78.0^{ab}	39.0^{b}	39.0^{f}	50.0^{b}
1% EG	76.0^{abc}	13.0^{de}	53.0^{cd}	30.3^{e}
3% EG	77.0^{abc}	20.0^{ef}	57.0^{ab}	26.0^{ef}
5% EG	75.0^{bcd}	15.0^{gh}	60.0^{a}	20.3^{g}
1% ethanol	74.0^{cde}	26.0^{d}	48.0^{e}	35.1^{d}
3% ethanol	72.0^{def}	18.0^{fg}	54.0^{bc}	25.0^{f}
5% ethanol	71.0^{ef}	19.0^{f}	52.0^{cd}	26.8^{ef}
1% methanol	70.0^{f}	20.0^{ef}	50.0^{de}	28.6^{ef}
3% methanol	66.0^{g}	12.0^{hi}	54.0^{bc}	18.1^{gh}
5% methanol	60.0^{h}	9.0^{i}	51.0^{cde}	15.0^{h}
±MSE	5.42	5.63	7.50	10.77

$^{a, b.....i}$: Means with different superscripts in the same row are significantly different (P<0.05).

Sperm motility in 2 hours post-thawed semen:

Results of incubation of post-thawed semen for 2 hours with level of different cryoprotectants were unexpected, whereas non-viable spermatozoa were observed in 2 h post-thawed semen diluted with EG, ethanol or methanol (Table 6).

However, sperm motility in 2 h post-thawed semen was affected significantly (P<0.05) by diluents containing GL, DMSO or their combination, being 33, 16 and 24%, respectively. Similar trend was obtained for sperm recovery rate in 2 h post-thawed semen, being 70, 55.9 and 61.5%, respectively (Table 6).

In comparing different levels of cryoprotectants for buck semen in the current study, glycerol showed the best results to maintain sperm during different freezing processes when it added to Tris-based extender at a level of 6%.

In accordance with the present results, Singh *et al.* (1995) found that glycerol was better than DMSO as cryoprotective agent for unwashed buck semen and level of 5% glycerol was better than 8% level in Tris-lactose extender.Also, a combination of DMSO with glycerol at a rate of 2%+3% (Salamon, 1968) or 1.5%+7% (Neubert and Menger, 1981) induced better post thaw motility of ram spermatozoa as compared to 5% DMSO alone.

The observed reduction in sperm motility and recovery rate post-thawed semen by increasing level of EG were indicated recently by Khalifa (2005), who found that the percentage of post-thawing motility and sperm recovery were (25.0 and 27.8%), (19.5 and 21.7%) and (10.5 and 11.7%) with EG at 1, 2 and 3%, respectively.

Table (6): Effect of level of different types of cryoprotectants in Tris-based extender on sperm motility (%) during semen incubation for 2 h post-thawing.

Cryoprotectant level	Sperm motility			
	Post- thawing	2 h post-thawing	Loss rate (%)	2 h post-thawing recovery rate
6% glycerol (GL)	47.0^{a}	33.0^{a}	14	70.2
6% DMSO	34.0^{c}	19.0^{c}	15	55.9
3% GL+3% DMSO	39.0^{b}	24.0^{b}	15	61.5
1% EG	13.0^{de}	0.0	13	0.0
3% EG	20.0^{ef}	0.0	20	0.0
5% EG	15.0^{gh}	0.0	15	0.0
1% ethanol	26.0^{d}	0.0	26	0.0
3% ethanol	18.0^{fg}	0.0	18	0.0
5% ethanol	19.0^{f}	0.0	19	0.0
1% methanol	20.0^{ef}	0.0	20	0.0
3% methanol	12.0^{hi}	0.0	12	0.0
5% methanol	9.0^{i}	0.0	9	0.0
±MSE	5.63	2.50	1.72	2.59

$^{a, b.....f}$: Means with different superscripts in the same row are significantly different (P<0.05).

With higher levels of EG for buck semen cryopreservation, Awad (1998) reported that the percentages of post-thawing motility and sperm recovery were (36.3 and 52.0%), (31.3 and 44.4%) and (19.0 and 27.0%) with EG at levels of 3, 6 and 12%, respectively. Also, Molinia *et al.* (1994) reported that increasing level of ethylene glycol decreased post-thawed motility and acrosome integrity of spermatozoa. They added that a combination of EG with glycerol has no enhanced cryoprotective effect compared with diluents containing glycerol alone. This phenomena may be due to the effect of toxicity of cryoprotectant combination, which dose not only prevent the use of fully protective level of penetrate in diluents, but also causes further cryoinjur to spermatozoa. The high toxicity of EG compared to glycerol may be due to

that sperm cell membrane are more permeable to EG than glycerol (Szell *et al.*, 1989).

The poorest results of methanol levels presented in this study were indicated by Christensen and Tiersch (1997), who found that post-thaw motility percentage was 26.3, 7.8 and 0.5% with higher levels of methanol (5, 10 and 15%) as cryoprotectnat of channel catfish spermatozoa. Also, Khalifa (2005) reported that the percentage of post-thawing motility and sperm recovery were (30.5 and 35.0%), (33.5 and 38.5%), (22.0 and 25.6%) and (16.5 and 18.4%) with methanol at levels of 0.5, 1, 2 and 3%, respectively.

Generally, monohydric and polyhydric alcohols with low concentration levels indicated significant results than high concentration on post-thawing motility.

In comparable, the best results of 6% glycerol was indicated by Awad (1998), who found that post-thawing sperm motility and recovery rate of Boer buck spermatozoa using 6% glycerol were 40.0 and 52.3% respectively. This was lower than the results obtained herein on Zaraiby goat, which may indicate species differences in sperm freezability.

The current study indicated that adding glycerol to Tris-based extender at level of 6% for cryopreservation of Zaraiby goat semen frozen in pellets form yielded the highest sperm motility in post-thawed semen.

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تـأثير بعـض الحاميـات مقارنـة مـع مسـتويات مختلفـة مـن الجلسـرول علـى حيويـة الحيوان المنوي أثناء عملية تجميد السائل المنوي في الماعز

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استخدم في هذه الدراسة ١٦٠ قذفه جيده جمعت من عشرة ظباء زاريبي بالغة متوسط وزنها ٢٨,٤±٠,٩ كيلوجرام وزن حي وعمر بين ٢٥-٣٥ شهر بمحطة البحوث بسخا خلال الفترة من يوليو إلى أغسطس كانت تجمع مرتين أسبوعيا لمدة ٨ أسابيع. تم تصميم تجربتين الأولى كانت لتحديد انسب مستوى من الجلسرول (بمستويات من ١-٧%). والثانية لمقارنة انسب مستوى من الجلسرول في التجربة الأولى مع مجموعتين من الـ ("DMSO" Dimethyl sulfoxide) ومستويات مختلفة (١ و ٣ و٥ %) من إثيلين جليكول والإيثانول والميثانول في مخفف الـ Tris الأساسي ، ودراسة تأثير ذلك على النسبة المئوية لحيوية الحيوانات المنوية (SMP) ونسبة الاسترداد (RR%) أثناء مراحل عملية التجميد المختلفة (التخفيف والموازنة والذوبان). لقد أوضحت نتائج التجربة الأولى أن حيوية الحيوانات المنوية (SMP) في السائل المنوي بعد التخفيف وبعد الموازنة لَم تتأثر معنوياً بمستوى الجلسرول حيث تراوح بين ٧٩-٨١%. تراوحت نسبة الفقد في حيوية الحيوانات المنوية بين التخفيف والموازنة بين ١-٣% في جميع مستويات الجلسرول ، وأن اقل نسبة فقد كانت عند مستوى ٧% وأعلى نسبة كانت عند مستوى ٥%. تراوح معدل الاسترداد (RR) بعد الموازنة بين ٩٦,٣ عند مستوى ٥% إلى ٩٨,٨% عند مستويات ٤ و٧%. لوحظت زيادة تدريجية معنوية ($P<0.05$) في نسبة حيوية الحيوانات المنوية (SMP) من٢٠ إلى ٤٨% بزيادة مستوى الجلسرول من ١ إلى ٦%. وأن زيادة مستوى الجلسرول إلى ٧% أدى إلى خفض معدل الحركة للحيوانات المنوية قليلاً (٤٦%). كما أوضحت النتائج أن مستوى ٦% جلسرول أعطى اقل نسبة فقد (٣٠%) وأعلى نسبة استرداد (٦١,٥%) في حيوية الحيوانات المنوية بعد الإسالة وكانت الفروق معنويةً ($P<0.05$). وأن أعلى نسبة استرداد (٧٢%) حدث بعد الإسالة بساعتين كانت عند مستوى ٥% جلسرول ، لكن هذا لَم يختلف معنوياً عن السائل المنوي المخفف بمستويات ٦ و٧% جلسرول. في حالة التركيز المنخفض من الجلسرول (١ و٢%) كانت الحيوانات المنوية غير حيوية بعد ساعتين من الإسالة. أظهرت نتائج التجربة الثانية أن السائل المنوي المخفف بـ ٦% جلسرول أعطى أفضل النتائج من حيث حيوية الحيوانات المنوية ونسبة الاسترداد بعد التخفيف وبعد الموازنة بالمقارنة مع مستويات المخففات الأخرى. تشابه مستوى ٣% من الإيثلين جليكول مع مستوى ٦% جلسرول وأعطى نفس النتائج ، بينما كانت كل مستويات الميثانول اقل النتائج بالمقارنة مع المستويات الأخرى للحاميات. كانت حيوية الحيوانات المنوية ونسبة الاسترداد في السائل المنوي بعد الإسالة أعلى معنوياً ($P<0.05$) وكانت نسبة الفقد اقل معنوياً ($P<0.05$) عند مستوى ٦% جلسرول (٤٧%) ، ثم عند مستوى ٣% جلسرول + ٣% DMSO وعند مستوى ٦% DMS (٣٩ و٣٤% على التوالي). كانت الحيوانات المنوية غير حيوية في السائل المنوي المخفف عند كل المستويات من الإيثانول أو الميثانول بعد ساعتين من الإسالة. عموماً تأثرت حيوية الحيوانات المنوية (SMP) معنوياً ($P<0.05$) بعد الإسالة بساعتين في السائل المنوي المخفف بالجلسرول وDMSO أو بإضافتهم معاً وكانت ٣٣ و ١٦ و٢٤% على التوالي. حدث نفس الشيء ، أيضاً بالنسبة لمعدل استرداد الحيوانات المنوية بعد ساعتين من الإسالة ، حيث كان٧٠ و ٥٥,٩ و٦١,٥% على التوالي.

الخلاصة: يتضح من نتائج هذه الدراسة أن زيادة مستوى الجلسرول المضاف إلى مخفف الـ Tris حتى ٦% لحماية السائل المنوي لتيوس الماعز الزاريبي أثناء عملية التجميد أدى إلى زيادة حيوية الحيوانات المنوية في السائل المنوي بعد الإسالة.