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Viability and Enzymatic Leakage of the Cooled Camel Spermatozoa in Relation to Different Extenders

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Abstract: Five males dromedary camel at 5 to 10 years of age and 400-600 kg body weight, were used. Semen was collected, evaluated and extended with different seven extenders (glucose-yolk-citrate: GYC, fructose-yolk-citrate: FYC, lactose-yolk-citrate: LYC, sucrose-yolk-citrate: SYC, tris-yolk-fructose: TYF, skim-cow-milk: SCM and skim-camel-milk: SLM). The extended semen was cooled to 5°C and stored for up to 3 days. After each storage time (0, 1, 2 and 3 days), the percentages of sperm motility, dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa, were recorded. Activities of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes, were also determined. The results showed that, the extended cooled camel semen with each of FYC, LYC, SYC and TYF extenders was significantly ($P<0.05$) higher the percentage of sperm motility, while significantly ($P<0.05$) lower the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa than GYC, SCM and SLM extenders, during storage at 5°C for up to 3 days. The extended cooled camel semen with LYC, SYC and TYF extenders showed significantly ($P<0.05$) decreased the amounts of AST, ALT and ALP enzymes released into the extracellular medium as compared with GYC, FYC, SCM and SLM extenders, during storage at 5°C for up to 3 days. The advancement of storage time at 5°C for up to 3 days decreased significantly ($P<0.05$) the percentage of motile camel spermatozoa, while increased significantly ($P<0.05$) the percentage of dead spermatozoa, sperm abnormalities, acrosomal damage of spermatozoa and the amount of AST, ALT and ALP enzymes released into the extracellular medium of the extended cooled camel semen with all different extenders (GYC, FYC, SYC, LYC, TYF, SCM and SLM extenders).

Key words: Camel · Cooled semen · Extenders · Enzymes

INTRODUCTION

In the developing countries such as Egypt, increasing camel productivity can help to solve the insufficient amount of animal meat and milk and depends firstly and mostly on reproductive efficiency. A management strategy that promotes maximum reproductive efficiency depends, in turn, on an understanding of reproductive biology of the camel. The artificial insemination (AI) is considered as one of the most important and the fastest way in the modern technology, for the application of genetic improvement through the breeding programs of farm animals. The progress in AI, semen preservation and related techniques in camels has been slow in comparison to other animals due to the difficulty of semen collection, little information in semen characteristics, semen dilution and storage of camel semen. Great attention has been given to the development of extenders that will preserve the functional activity of the spermatozoa (viability and fertilizing ability) during storage at different temperatures. Various media have been recommended for the dilution and preservation of semen. During preservation, several factors may be held responsible for the possible decrease of fertilizing ability of semen during storage under different conditions [1]. Lack of studies on camel's semen processing and disseminating under desert condition has a drawback to clearly monitor the productivity of such animals.

The present study aimed to evaluate the effect of different extenders on camel's semen quality and enzymatic activity under Egyptian desert condition, during storage at 5°C for up to 3 days.

MATERIALS AND METHODS

The present study was carried out in the Private Camel's Farm, Belbies City, Sharkiya Province, Egypt.

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Table 1: Compositions of the different buffered yolk extenders

	(Grams / 100 ml of distilled water)						
Components	GYC	FYC	LYC	SYC	TYF	SCM	SLM
Sodium citrate dihydrate	2.90	2.90	2.90	2.90	-	2.90	2.90
Citric acid monohydrate	0.04	0.04	0.04	0.04	1.675	0.04	0.04
Glucose	1.25	-	-	-	-	-	-
Fructose	-	1.25	-	-	-	-	-
Lactose	-	-	1.25	-	-	-	-
Sucrose	-	-	-	1.25	-	-	-
*Tris aminomethan	-	-	-	-	3.028	-	-
Skim-cow-milk (ml)	-	-	-	-	-	5.00	-
Skim-camel-milk (ml)	-	-	-	-	-	-	5.00
Egg yolk (ml)	10.00	10.00	10.00	10.00	10.00	10.00	10.00

500 IU/ml Penicillin + 500 µg/ml Streptomycin sulphate were added to each extender.
* Tris (Hydroxy methyl) amino methane, Aldrich Chemical Co. Ltd., Gillingham, Dorset-England.GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

Five males dromedary camel at 5 to 10 years of age and 400-600 kg body weight, were used. The camel's were in healthy condition and clinically free of external and internal parasites with a sound history of fertility in the herd. Palpation of the external genetalia showed that they were typically normal.

The present study was carried out to define the effects of different seven extenders (GYC, FYC, LYC, SYC, TYF, SCM and SLM) on semen quality and enzymatic activity, during storage at 5°C for up to 3 days. The compositions of these extenders are shown in Table 1.

Camel's Semen Collection by Artificial Vagina (AV): Semen was collected from five dromedary camel between 08: 00 and 10: 00 a.m. using artificial vagina (AV). A modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France) as described by Zeidan [2] and Mosaferi *et al.* [3]. Ejaculate contact with the rubber liner of the AV was avoided, since Musa *et al.* [4] reported that most rubber liners have a deleterious effect on camel spermatozoa. An additional disposable plastic inner liner is inserted to avoid contact with the rubber material. After passing the liner through the AV, 8 cm of cylindrical form (cut longitudeinally) was placed between outer Jacket of the AV and liner at the end of the AV far from the water valve according to Bravo *et al.* [5]. This was performed to imitate the internal cervix and provide more stimulation for the penis for proper erection and ejaculation. A shortened AV without collection funnel was used, allowing the semen to pass directly into a collection flask. The AV was filled with water at 55-60°C. The temperature inside the inner liner was stabilized at 45–50°C. Few drops of Vaseline were smeared on the inner liner at the entrance to the AV to provide lubrication. A sexually receptive female couching with her front legs tied and teased by the male camel should be used. The olfactory contact should be allowed. The male is left to mount the female from behind on the right side. As soon as, the male camel makes few thrusts, the operator who sits on the right side of the female grasps the males camel sheath and directs his penis into the AV. Ejaculation is completed after several thrusts and interspersed by periods of rest. The ejaculate usually comes in fractions. The collection flask containing the semen is protected by a towel or gauze. Immediately after semen collection, flask containing the semen was incubated in a water bath at 37°C. Fresh camel semen that has a jelly–like consistency is left for liquefaction for about 30-60 minutes to make the sperm attain motility. Only semen samples with no less than 50% sperm motility, were used. After semen collection, it was placed inside incubator set at 37°C and evaluated immediately. Camel or cows milk was obtained freshly after milking of she-camels or cows.

Whole milk extenders were prepared by heating milk to 95°C for 10 minutes and cooled to room temperature and filtered [6].

Semen Extension: Semen was collected, pooled, evaluated and divided into seven equal aliquots and then extended with different seven extenders (GYC, FYC, LYC, SYC, TYF, SCM and SLM). Semen extension was carried out by adding the appropriate volume of the semen slowly to the extender. Extended semen (in tubes) was kept below the level of water in a water bath at all times to avoid fluctuations in the extended semen temperature. The final extension rate was 1 semen: 4 extender.

Chilling of Semen at 5°C: The test tubes containing semen were placed in a 500 ml beaker containing water at 30°C with a thermometer in order to facilitate periodic checking of the temperature during the cooling period. Another test tubes containing extender only were placed

in the beaker to maintain the extender temperature similar to that of the semen (all test tubes were covered with dark plastic sheath). The beaker was placed in a refrigerator and gradually cooled till their temperature reached 5°C during a period of 1.5-2.0 hours.

The percentages of sperm motility, dead spermatozoa, sperm abnormalities were determined according to Salisbury *et al.* [7]. Acrosomal damage of spermatozoa was determined by using a Giemsa stain procedure as the method described by Watson [8] using a phase contrast microscope. Aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) enzymes were determined colourimetrically using the method described by Reitman and Frankel [9]. Alkaline phosphatase (ALP) enzyme was determined colourimetrically using commercial kits purchased from Bio-merieux (Marcy L'E Potile, Charbonnieres, Les Bains, France) according to Graham and Pace [10].

Data were statistically analyzed using least squares analysis of variance according to Snedecor and Cochran [11]. Percentage values were transformed to arc-sin values before being statistically analyzed. Duncan's New Multiple Range-test [12] was used for the multiple comparisons.

RESULTS AND DISCUSSION

Camel Semen Quality During Storage at 5°C

Percentage of Sperm Motility (%): Data presented in Table 2 showed that, the effect of type of extender on the percentage of motility of the cooled camel spermatozoa was significant (P<0.05). The extended semen with each of FYC, LYC, SYC and TYF extenders was significantly (P<0.05) higher the percentage of sperm motility than GYC, SCM and SLM extenders. However, the differences effect of extending among semen LYC, SYC and TYF, FYC, LYC and SYC or between SCM and SLM extenders on the percentage of motile spermatozoa were insignificant. The extended semen with GYC extender decreased significantly (P<0.05) the percentage of sperm motility as compared to the other extenders (GYC, FYC, LYC, SYC, TYF, SCM or SLM). It is worth noting that, the percentage of motile spermatozoa was superior among FYC, LYC, SYC and TYF extenders than other extenders. The highest (P<0.05) value of the percentage of motility of spermatozoa was recorded with TYF extender, while the lowest (P<0.05) value was recorded with GYC extender. These results may be due to the combinations of all beneficial effects of tris components. Tris in addition to its better buffering capacity, can readily diffuse into the sperm cells and serve as an intracellular buffer [13]. Moreover this phenomenon may be attributed to the better protection of lactose to spermatozoa against osmotic shock than other sugars or due to the mediate available energy and the osmotic balance of the extender [14]. Vyas *et al.* [15] showed also that, tris extender was superior than lactose extender for camel semen preservation at 5°C for 48 hours. Zeidan *et al.* [16] confirmed that LYC and TYF extenders were significantly (P<0.01) better maintaining good rabbit semen quality than GYC or skim–milk extender, during storage at 5°C.

The advancement of storage time at 5°C for up to 3 days decreased significantly (P<0.05) the percentage of sperm motility through seven different extenders. These results are in agreement with those of Ahmadi [17] and Zeidan [2] who found that the advancement of incubation at 37°C decreased significantly (P<0.01) the percentage of motility of the dromedary camel spermatozoa. This phenomenon may be attributed to decrease in the content of adenosine triphosphate which caused inactivated spermatozoa apparently in the ability of resynthesizing. This was accompanied with a precipitous fall in the rate of fructolysis [18, 19].

Percentage of Dead Spermatozoa (%): The results obtained in Table 3 showed that the effect of type of extender on the percentage of dead spermatozoa was significant (P<0.05). The extended camel semen with the FYC, LYC, SYC and TYF extenders decreased significantly (P<0.05) the percentage of dead spermatozoa as compared to GYC, SCM or SLM extenders. The differences effect among FYC, LYC, SYC and TYF extenders on the percentage of dead spermatozoa was insignificant. Similarly, the differences effect between SCM and SLM extenders on the percentage of dead camel spermatozoa was insignificant. The extended camel semen with GYC extender increased significantly (P<0.05) the percentage of dead spermatozoa as compared to the other extenders (GYC, FYC, LYC, SYC, TYF, SCM or SLM). The lowest (P<0.05) value of percentage of dead spermatozoa was recorded with TYF extender and the highest (P<0.05) value was recorded with GYC extender. Similar trend was reported by Zeidan [2] who found that, the lowest (P<0.01) value of the percentage of dead of the cooled camel spermatozoa was recorded with LYC extender and the highest (P<0.01) value was recorded with GYC extender. Such findings may be due to the beneficial effects of tris and higher molecular weight of lactose or sucrose extenders allowing

Table 2: Percentage of motility of the dromedary camel spermatozoa with different extenders, during storage at 5°C for 3 days

Storage time (day)	Extenders							
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	53.54+1.82	61.47+1.43	65.02+1.89	65.72+2.02	65.71+1.75	57.14+2.14	56.43+1.81	60.71+0.93^{A}
1	45.74+1.70	52.14+1.49	53.56+1.81	54.26+1.73	57.14+2.45	49.28+2.02	47.15+1.01	51.32+0.83^{B}
2	28.58+1.43	44.28+1.74	45.73+3.69	46.48+1.43	48.57+2.61	40.71+2.32	38.52+2.37	41.84+1.23^{C}
3	15.10+1.54	27.83+2.86	29.26+1.35	29.25+2.39	31.42+2.37	22.86+1.49	20.04+1.09	25.11+1.06^{D}
Means	35.74+2.98^{d}	46.43+2.54^{b}	48.39+2.73^{ab}	48.93+2.70^{ab}	50.71+2.67^{a}	42.50+2.63^{c}	40.54+2.70^{c}	44.75

a-d: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

Table 3: Percentage of dead of the dromedary camel spermatozoa with different extenders, during storage at 5°C for 3 days

Storage time (day)	Extenders							
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	38.45+1.77	20.14+0.96	19.54+1.31	19.25+1.32	18.57+0.69	31.43+1.67	32.27+0.89	25.66+1.18D
1	41.71+1.84	23.42+1.00	23.18+1.16	22.45+1.46	21.94+0.87	34.82+1.68	35.86+0.67	28.98+1.2C
2	46.29+1.89	29.16+1.28	28.43+1.45	28.02+1.79	26.42+1.13	38.70+1.74	40.88+0.4	33.99+1.67B
3	52.27+1.8	34.55+1.48	34.42+1.65	32.72+1.6	31.58+1.17	47.03+1.76	47.56+0.43	40.02+1.26A
Means	44.68+1.32^{a}	26.82+1.2^{c}	34.42+1.65 ^{c}	26.39+1.27^{c}	24.50+1.06^{c}	38.00+1.38^{b}	39.14+1.14^{b}	32.16

a-c: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

Table 4: Percentage of sperm abnormalities of the dromedary camel spermatozoa with different extenders, during storage at 5°C for 3 days

Storage time (day)	Extenders							
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	20.71+0.99	13.16+0.67	11.45+1.11	10.72+0.75	8.73+0.64	19.57+0.65	26.59+0.92	15.85+0.92^{D}
1	22.85+0.99	15.43+0.37	13.42+1.19	12.59+0.43	12.41+1.15	22.17+0.74	28.70+0.87	18.22+0.91^{C}
2	28.42+1.02	20.54+0.92	20.26+1.27	20.28+1.08	17.57+1.03	27.86+1.18	32.29+0.78	23.94+0.83^{B}
3	31.58+0.78	25.42+0.61	26.17+1.2	26.12+1.31	24.72+1.41	32.40+1.02	37.14+0.83	29.08+0.73^{A}
Means	35.89+0.94^{b}	18.64+0.97^{c}	17.82+1.25^{cd}	17.43+1.27^{cd}	15.86+1.26^{d}	25.50+1.06^{b}	31.18+0.87^{a}	21.06

a-d: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate, TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

more protection of spermatozoa, than other extenders, consequently lowering dead spermatozoa during storage at 5°C.

The advancement of storage time at 5°C for up to 3 days increased significantly (P<0.05) the percentage of dead of spermatozoa with all different extenders. Similar trend was reported by Zeidan [2] in the cooled camel spermatozoa. These findings may be attributed to accumulation of lactic acid which exerts a toxic effect of sperm cell and leakage of interacellular enzymes increased membrane permeabilitis [20].

Percentage of Sperm Abnormalities (%): Data presented in Table 4 showed that, the effect of type of extender on the percentage of abnormalities of spermatozoa was significant (P<0.05). The differences effect among FYC, LYC and SYC extenders or between SCM and SLM extenders on the percentage of abnormalities of spermatozoa was insignificant. Similarly, the differences effect among LYC, SYC and TYF extenders on the percentage of sperm abnormalities was insignificant. The extended camel semen with GYC extender increased significantly (P<0.05) the percentage of sperm abnormalities as compared to the other extenders (GYC, FYC, LYC, SYC, TYF, SCM or SLM). The lowest (P<0.05) value of the percentage of sperm abnormalities was recorded with TYF extender and the highest (P<0.05) value was recorded with GYC extender. These results might be attributed to the better protection of lactose, tris, sucrose or fructose extenders to spermatozoa against osmotic shock than the other sugars, during refrigeration at 5°C. Similar trend was reported by Sieme *et al.* [21] in bactrian camel and Zeidan [2] in the dromedary camel.

The advancement of storage time at 5°C for up to 3 days increased significantly (P<0.05) the percentage of abnormalities of spermatozoa with all different extenders. Similar trend was reported by Zeidan [2] in the cooled camel's spermatozoa. These findings may be due to the

Table 5: Percentage of acrosomal damage of the dromedary camel spermatozoa with different extenders, during storage at 5°C for 3 days

Storage time (day)	Extenders							
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	17.85+1.14	8.28+0.78	8.70+1.08	6.25+0.64	4.86+0.46	10.27+0.64	11.54+1.34	9.68+0.65^{D}
1	21.43+1.69	9.86+0.89	10.84+1.06	9.17+0.77	5.74+0.52	15.19+0.96	16.19+1.74	12.63+0.82^{C}
2	26.58+1.88	12.71+0.87	14.28+1.27	12.26+0.71	8.14+0.55	21.42+1.31	21.45+2.11	16.69+1.00 ^{B}
3	34.29+1.84	18.15+1.34	20.18+1.61	16.88+0.99	12.40+0.37	27.68+0.89	29.72+2.03	22.76+1.17^{A}
Means	25.04+1.42^{a}	12.25+0.86^{cd}	13.50+1.03^{c}	11.14+0.84^{d}	7.79+0.61^{e}	18.64+1.34^{b}	19.73+1.56^{b}	15.44

a-e: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

Table 6: Effects of different extenders on aspartate-aminotransferase enzyme activity (U/10^{6} spermatozoa) of the dromedary camel spermatozoa, during storage at 5°C for up to 3 days

Storage time (day)	Extenders							
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	44.53+1.56	41.22+1.64	40.96+1.29	40.26+1.23	40.92+1.15	43.18+1.12	43.31+1.32	42.05+0.61^{D}
1	52.60+1.53	47.93+1.17	46.60+1.40	45.67+1.18	44.47+1.24	49.25+1.16	50.85+1.10	48.20+1.10^{C}
2	62.91+1.42	59.72+1.48	51.62+1.28	51.85+1.25	49.32+1.13	57.61+1.20	58.86+1.28	54.98+1.85^{B}
3	73.82+1.45	58.4+1.26	65.35+1.43	56.38+1.38	55.92+1.24	66.84+1.35	69.92+1.22	62.53+2.83^{A}
Means	58.47+6.35^{a}	50.09+3.65^{bc}	48.89+3.31^{c}	48.54+3.53^{c}	47.66+3.25^{c}	54.22+5.14^{ab}	55.74+5.70^{a}	51.94

a-c: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

increase in sperm metabolic activity and consequently, increase lactic acid production which in turn exerts a toxic effect on sperm cell [20].

Percentage of Acrosomal Damage of Spermatozoa (%): Data presented in Table 5 showed that the effect of type of extender on the percentage of acrosomal damage of the cooled camel spermatozoa was significant (P<0.05). The extended semen with TYF extender decreased significantly (P<0.05) the percentage of acrosomal damage of spermatozoa as compared to other extenders (GYC, FYC, LYC, SYC, TYF, SCM or SLM). However, the differences effect among FYC, SYC or LYC or between SCM and SLM extenders on the percentage of acrosomal damage of spermatozoa was insignificant. The extended semen with GYC extender increased significantly (P<0.05) the percentage of acrosomal damage of camel spermatozoa as compared to the other extenders (GYC, FYC, LYC, SYC, TYF, SCM or SLM). The lowest (P<0.05) value of the percentage of acrosomal damage of spermatozoa was recorded with TYF extender and the highest (P<0.05) value was recorded with GYC extender. Marinov *et al.* [22] also observed that sucrose-lactose diluent gave better results in term of sperm motility and acrosomal proteinase activity. Similar trend was reported by Zeidan [2] who found that the lowest (P<0.05) value of the percentage of acrosomal damage of the dromedary camel spermatozoa was recorded with LYC extender and highest (P<0.05) value was recorded with GYC extender, during incubation at 37°C for up to 6 hours.

The advancement of storage time at 5°C for up to 3 days increased significantly (P<0.05) the percentage of acrosomal damage of spermatozoa with all different extenders. These results may be due to increase in lactic acid accumulation that changes both osmotic pressure and pH in the media which in turn exerts a toxic effect on sperm cells. These results are in agreement with those of Ahmadi [17] and Zeidan [2] in the dromedary camels spermatozoa.

Enzymatic Activities of the Camels Spermatozoa During Storage at 5°C

Aspartate-aminotransferase Enzyme Activity (U/10^{6} Spermatozoa): Table 6 showed that, the effect of type of extender on aspartate-aminotransferase (AST) enzyme activity in the extended cooled camel spermatozoa was significant (P<0.05). The differences effect among FYC, LYC, SYC and TYF or GYC, SCM and SLM extenders in the activity of AST enzyme was insignificant. The lowest (P<0.05) amount of AST in the extended cooled spermatozoa enzyme released into the extracellular medium was recorded with the extended camel semen with TYF extender and the highest (P<0.05) amount was recorded with GYC extender. These findings may be attributed to the protective mechanism of the beneficial components of tris to sperm cell membrane against any changes in the plasma membrane, consequently lowering the amount of enzymes from the intracellular to extracellular medium. Similar trend was reported by Zeidan [2] in the dromedary camel. However, Ahmadi [17] found

Table 7: Effects of different extenders on alanine-aminotransferase enzyme activity (U/10^6 spermatozoa) of the dromedary camel spermatozoa, during storage at 5°C for up to 3 days

	Extenders							
Storage time (day)	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	31.68+0.85	29.35+1.03	28.41+1.02	28.63+0.54	26.20+1.08	29.85+0.92	30.51+0.88	$29.23+0.66^{D}$
1	35.74+1.03	33.52+1.12	29.60+1.15	28.82+0.95	28.41+1.16	34.83+1.04	35.74+1.12	$32.38+1.25^{C}$
2	43.95+0.96	38.63+1.15	31.24+0.92	32.43+1.12	30.54+1.10	39.90+1.04	40.82+1.13	$36.79+2.01^{B}$
3	50.02+1.25	43.45+1.16	38.13+1.13	36.68+1.22	33.05+1.19	44.26+1.15	47.32+1.14	$41.84+2.30^{A}$
Means	$40.35+4.11^{a}$	$36.24+3.06^{b}$	$31.85+2.17^{c}$	$31.64+1.89^{c}$	$29.55+1.46^{c}$	$37.21+3.12^{ab}$	$38.60+3.59^{ab}$	35.06

a-c: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

Table 8: Effects of different extenders on alkaline phosphatase enzyme activity (U/10^6 spermatozoa) of the dromedary camel spermatozoa, during storage at 5°C for up to 3 days

	Extenders							
Storage time (day)	GYC	FYC	LYC	SYC	TYF	SCM	SLM	Overall means
0	16.48+0.72	14.51+0.67	13.91+0.48	13.90+0.58	13.42+0.36	15.27+0.73	15.90+0.82	$14.77+0.49^{D}$
1	23.67+0.90	19.23+0.82	18.53+1.12	17.68+0.95	15.36+1.06	21.82+1.10	22.67+1.03	$19.85+1.13^{C}$
2	30.23+1.19	24.75+0.89	22.34+1.16	20.25+0.75	18.64+0.22	26.94+1.04	28.83+1.12	$24.57+1.65^{B}$
3	39.45+1.06	30.34+1.12	26.45+1.08	24.81+1.16	22.26+1.20	32.47+1.15	34.70+1.14	$30.07+2.27^{A}$
Means	$27.46+4.89^{a}$	$22.21+3.42^{bc}$	$20.31+2.68^{cd}$	$19.15+2.29^{cd}$	$17.42+1.94^{d}$	$24.13+3.67^{ab}$	$25.53+4.04^{ab}$	22.32

a-d: Values with different superscripts with a row, are significantly different (P<0.05).
A-D: Values with different superscripts with a column, are significantly different (P<0.05). GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, CYS: Sucrose - yolk citrate , TYF: Tris-yolk-fructose, SCM: Skim-cow-milk, SLM: Skim-camel-milk

that, the AST enzyme released into the extracellular medium showed insignificantly lower in the extender camel semen with LYC than TYF extender.

The advancement of storage time at 5°C for up to 3 days increased significantly (P<0.05) the leakage of AST enzyme in the extended camel semen into the extracellular medium with the all different extenders. These results are in agreement with those of Ahmadi [17] who reported that, the incubation time of the extended camel sperm at 37°C for up to 6 hrs showed significantly (P<0.01) increased the amount of AST enzyme released into the extracellular medium in dromedary camel similar to that reported by Zeidan (2). The continuous increase in leakage of the intracellular AST enzyme during incubation time may reflect the breakdown of the cellular sperm membrane [10, 23].

Alanine-aminotransferase Enzyme Activity (U/10^6 Spermatozoa): The results obtained in Table 7 showed that the effect of type of extender on alanine-aminotransferase (ALT) enzyme activity in the extended cooled camel spermatozoa was significant (P<0.05). The differences effect among LYC, SYC and TYF or GYC, FYC, SCM and SLM extenders in the activity of ALT enzyme in the extended cooled semen was insignificant. Similarly, the difference effects among FYC, SCM and SLM extenders in the activity of ALT enzyme were insignificant. The lowest (P<0.05) amount of ALT released into the extracellular medium was recorded with the extended camel semen with TYF extender and the highest (P<0.05) amount was recorded with GYC extender. Similar trend was reported by Graham and Pace [10] and Zeidan *et al.* [16]. Zeidan [2] also found that, the ALT enzyme released into the extracellular medium showed significantly (P<0.01) lower in the extended camel semen with LYC than TYF extender.

The advancement of storage time at 5°C for up to 3 days increased significantly (P<0.05) the leakage of ALT enzyme in the extended camel semen into the extracellular medium with all different extenders. Similarly, Zeidan [2] found that, the incubation time of the extended camel sperm at 37°C for up to 6 hours increased significantly (P<0.01) the amount of ALT enzyme.

Alkaline Phosphatase Enzyme Activity (U/10^6 Spermatozoa): Table 8 showed that, the effect of type of extender on alkaline phosphatase (ALP) enzyme activity in the extended cooled camel spermatozoa was significant (P<0.05). The differences effect among LYC, SYC and TYF or GYC, SCM and SLM, FYC, LYC and SYC or FYC, SCM and SLM in the activity of ALP enzyme in the extended cooled semen was insignificant. The lowest (P<0.05) amount of ALP enzyme released into the extracellular medium was recorded with the extended camel semen with TYF extender and the highest (P<0.01) amount was recorded with GYC extender. Zeidan [2] reported that, the ALP enzyme activity showed significantly (P<0.01) lower in the extended camel

semen with LYC than TYF extender in the dromedary camel.

The advancement of storage time at 5°C for up to 3 days increased significantly ($P<0.05$) the leakage of ALP enzyme in the extended camel semen into the extracellular medium with all different extenders. These results are in agreement with those of Ahmadi [17] who found that the incubation time at 37°C for up to 6 hours of the extended camel semen showed significantly ($P<0.05$) increased the amount of ALP released into the extracellular medium. Similar trend was reported by Zeidan [2] in dromedary camel. The phosphatase enzyme leakage from the sperm cells into the seminal plasma due to cold shock during storage at 5°C increased the enzymes level [24]. Such increase in the enzyme activity after storage, however, may be a sign for increasing the cell damage which occurred during storage process [25].

CONCLUSION

The extended camel semen with fructose-yolk-citrate (FYC), lactose-yolk-citrate (LYC), sucrose-yolk-citrate (SYC) or tris-yolk-fructose (TYF) extenders showed better sperm motility, longevity of the dromedary camel spermatozoa and maintaining enzymatic activities during storage at 5°C. Therefore, it can be recommended to collection and extended camel semen with each of FYC, LYC, SYC or TYF extenders during storage at 5°C for artificial insemination programms to enhance the fertilizing ability of spermatozoa, particularly under Egyptian desert condition.

REFERENCES

1. Anand, S.R., 1979. Buffalo sperm metabolism. An assessment of motility, live sperm counts and leakage of acrosomal enzymes from buffalo spermatozoa during dilution and preservation. In. Buffalo Reprod. Artif. Insemin. Proc. Seminar, FAO/SIDA/Govit. of India. N.D. R. I., Karnal, India, pp: 284-291.
2. Zeidan, A.E.B., 2002. Semen quality, enzymatic activities and penetrating ability of spermatozoa into she-camel cervical mucus as affected by caffeine addition. J. Camel Practice Res., 9: 153-161.
3. Mosaferi, S., Niasari-Naslaji, A. Abarghani, A.A. Gharahdaghi and A. Gerami, 2005. Biophysical and biochemical characteristics of bactrian camel semen collected by artificial vagina. Theriogenology, 63: 92-101.
4. Musa, B., H. Sieme, H. Merkt, and B.E.D. Hago, 1992. Artificial insemination in dromedary camels. Proc. 1st Inter. Camel Conference, Dubai, U.A.E., pp: 179-182.
5. Bravo, P.W., J.A. Skidmore and X.X. Zhao, 2000. Reproductive aspects and storage of semen in camelidae. Anim. Reprod. Sci., 62: 173-193.
6. Kumar, S., K.L. Sahni, B.N. Benjamin and G. Mohan, 1993. Effect of various levels of yolk on deep freeizng and storage of buffalo semen in different diluters without adding glycerol. Buffalo J., 1: 79-85.
7. Salisbury, G.W., N.L. Van-Demarkand J.R. Lodge, 1978. Extenders and extension of unfrozen semen. In: Physiology of Reproduction and Artificial Insemination of Cattle. 2nd Edn., W. H. Freemen and Company, San Francisco, USA, pp: 473-474.
8. Watson, P.F., 1975. Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Rec., 97: 12-15.
9. Reitman, S. and S. Frankel, 1957. A colorimetric methods for the determination of serum glutamic oxaloacetic transaminase. American J. Clinical Pathol., 2: 56-61.
10. Graham, E.F. and M.M. Pace, 1967. Some biochemical changes in spermatozoa due to freezing. Cryobiology, 4: 75-81.
11. Snedecor, G.W. and W.G. Cochran, 1982. Statistical Methods. 7th Edn., Iowa State University Press, Ames., U.S.A., pp: 593.
12. Duncan, D.B., 1955. Multiple Range and Multiple F-Test. Biometrics, 11: 1-42.
13. Bartlett, F.D. and N.L. Van Demark, 1962. Effect of diluent composition on survival and fertility of bovine spermatozoa stored in carbonated diluents. J. Dairy Sci., 45: 360-362.
14. Foote, R.H., 1964. Water: The chief ingredient in semen extenders. Artificial Insemination Digest, 12: 10-14.
15. Vyas, S., P. Goswami, A.K. Rai and N.D. Khanna, 1998. Use of tris and lactose extenders in preservation of camel semen at refrigerated temperature. Indian Vet. J., 75: 810-812.
16. Zeidan, A.E.B., A.I. Aboulnaga, Z.A. Ibrahimand M.A.M. Hamedi, 2002. Quality, enzymatic activity and fertility rate of the cooled rabbit semen supplemented with caffeine. Egyptian J. Rabbit Sci., 12: 27-41.
17. Ahmadi, E.A.A., 2001. Physiological and reproductive studies on camels. Ph. D. Thesis, Fac. Agric., Zagazig University, Zagazig, Egypt.

18. Salamon, S., 1970. The survival of ram spermatozoa following pellet freezing below -79°C. Australian J. Biol. Sci., 23: 459-468.
19. Mann, T. and C. Lutwak-Mann, 1981. Male Reproductive Function and Semen. Springer-Verlag, Berlin Heidelderg, New York, pp: 264.
20. Zeidan, A.E.B., 1994. New aspects in freezing cattle semen. Ph.D. Thesis, Fac. Agric., Zagazig Univ., Zagazig, Egypt.
21. Sieme, H., H. Merkt, B. Musa, B. Hago and T. Willem, 1990. Liquid and deep freeze preservation of camel semen using different extenders and methods. Proc. the workshops in it possible to improve the reproductive performance of the camel? Paris, pp: 273-284.
22. Marinov, P., A. Torniov, V. Dikovand P. Corvalan, 1980. Acrosomal proteolytic activity as a method for evaluation of the cryoprotective action of diluents for freezing of ram semen. Proc. 9th Inter. Cong. Anim. Reprod. Artif. Insemin., Madrid, Spain, 5: 521-525.
23. Zeidan, A.E.B., M.N. El-Gaafary and F.E. El-Keraby, 1998. Effect of new packaging method for frozen-bull semen in pellets form on some biochemical changes and conception rate. Inter. Conference on Animal Production and Health in Semi-Arid Area, El-Arish, North Sinani, Egypt, pp: 223-234.
24. Walton, A., 1957. Cold shock of spermatozoa. Proc. R. Soc. B., 147: 508.
25. Brown, K.I., E.F. Grahamand B.G. Crabo, 1969. Some factors affecting extension and storage of turkey spermatozoa. Poultry Sci., 48: 1791.