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VIABILITY, ENZYMATIC ACTIVITY AND PENETRATING ABILITY OF SPERMATOZOA INTO SHE-CAMEL CERVICAL MUCOUS AS AFFECTED BY DIFFERENT EXTENDERS

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ABSTRACT

Five males camels at 5 to 10 years of age and 500 – 600 kg body weight, were used. Semen was collected, evaluated and extended with 7 extenders (glucose-yolk-citrate : GYC, fructose-yolk-citrate : FYC, lactase-yolk-citrate : LYC, sucrose –yolk-citrate : SYC, tris-yolk-fructose : TYF, skim- cow -milk : SCM and skim- camel -milk : SLM). The extended semen was then incubated at 37°C for up to 12 hours. After each incubation time (0, 1, 2, 4, 6, 8, 10, 12 hours), the percentage of motile spermatozoa, dead spermatozoa, sperm abnormalities and acrosomal damage of the camel spermatozoa were recorded. Aspartate-aminotransferase (AST), alanine – aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes activities, were also determined. The penetrating ability of spermatozoa into she-camel cervical mucous with the different extenders, during incubation at 37°C for up to 4 hours was also assessed.

The result showed that, the extended camel semen with each of LYC, SYC, TYF, SCM and SLM extenders increased significantly ($P < 0.05$) the percentage of sperm motility, while decreased significantly ($P < 0.05$) percentage of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa and activities of AST, ALT and ALP enzymes as compared with GYC and FYC extenders, during incubation at 37°C for up to 12 hours. The highest ($P < 0.05$) percentage of sperm motility was recorded with the extended semen with TYF extender and the lowest ($P < 0.05$) value was recorded with FYC extender. The highest ($P < 0.05$) percentages of dead spermatozoa, sperm abnormalitites, acrosomal damage and activities of AST, ALT and ALP enzymes were recorded with the extended semen with FYC extender and the lowest ($P < 0.05$) values were recorded with TYF extender.

The advancement of incubation time at 37°C for up to 12 hours decreased significantly ($P < 0.05$) the percentage of motile 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 extraellular fluid of the extended camel semen with the all different extenders . The penetrating ability of the extended spermatozoa with TYF, SYC, SCM, SLM and LYC extenders into she-camel cervical mucous was insignificantly better than GYC or FYC extender. While, the penetration score of spermatozoa was significantly ($P<0.05$) decreased with the advancement time of incubation at 37°C with the different extenders.

Key words: Camel semen, enzymes, extenders, incubation, penetration score

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 programmes 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 semen. Great attention has been given to the development of extenders that will preserve the functional activity of the spermatozoa (viability and fertilising ability) during storage at different temperatures. Various media have been recommended for the dilution and preservation of camel semen (Zeidan, 2002 and Zeidan *et al,* 2008). During preservation, several factors may be responsible of the possible decrease in fertilising ability of semen during storage under different conditions (Anand, 1979).

Success of artificial insemination is depends on the quality of obtained semen and its capacity for dilution and storage with minimum loss of fertilising ability in addition, prolongation of semen storage is mainly affected with the proper extender used.

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Now informations is needed to determine if use of semen extenders could improve fertility. Moreover, 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 was aimed to define the effect of different extenders on camel semen quality and enzymatic activities under Egyptian desert conditions, during incubation at 37°C for up to 12 hours. The penetrating ability of spermatozoa into she-camel cervical mucous, during incubation at 37°C for 4 hrs was also assessed.

Materials and Methods

Five males dromedary camel at 5 to 10 years of age and 500 – 600 kg B.wt., were used. The camels were from 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 study the effects of seven different 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) on semen quality and enzymatic activity, during incubation at 37°C for up to 12 hours. The ability to penetrate cervical mucous of camel spermatozoa was also assessed.

Camels semen collection by artificial vagina (AV)

Semen was collected from five dromedary camels between 8 and 10 a.m. using artificial vagina (AV). A modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France) as described by Zeidan (2002) and Mosaferi *et al* (2005) was used. Ejaculate contact with the rubber liner of the AV was avoided, since Musa *et al* (1992) reported that most rubber liners have a deleterious effect on camel spermatozoa. An additional disposable plastic inner liner was inserted to avoid contact with the rubber material. After passing the liner through the AV, 8 cm of cylindrical form (cut longitudinally) 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* (2000). 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 stabilised 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. The ejaculate usually comes in fractions. Ejaculation is completed after several thrusts and interspersed by periods of rest. 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. Semen samples with sperm motility more than 50% were used.

Semen extension

Semen was collected, pooled, evaluated and divided into seven equal aliquots and then extended with seven different 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 temperature of the extended semen. The final extension rate was 1 semen: 4 extender.

Percentages of sperm motility, dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa were recorded. Aspartate-aminotransferase (AST), alanine – aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes were also determined. Sperm motility, dead spermatozoa and sperm abnormalities were determined according to Salisbury *et al* (1978). Acrosomal damage of spermatozoa was determined by using a Giemsa stain procedure as the method described by Watson (1975) using a phase contrast microscope. Enzymatic activities (AST and ALT) were determined colourimetrically using the method described by Reitman and Frankel (1957). 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 (1967).

Sperm penetration into camel cervical mucous was assessed by sucking aportion of mucous into polyethylene sealed tubes (2 mm diameter) to provide column of 6 cm length. Semen was extended with

the different extenders (GYC, FYC, LYC, SYC, TYF, SCM and SLM) and then placed into 2 ml cavettos (1 ml each). The tubes containing the mucous were inserted (open end) into the cavettos containing the extended semen with the different extenders and then incubated at 37°C for 4 hrs. Sperm penetration was judged by the rank score as described by Eskin *et al* (1973) and Hanson *et al* (1982).

Data were statistically analysed by analysis of variance according to Snedecor and Cochran (1982). Percentage values were transformed to Arc-Sin values before being statistically analysed. Duncan's New Multiple Range Test (Duncan, 1955) was used for the multiple comparisons.

Results and Discussion

Semen quality

Percentage of sperm motility of the camel spermatozoa (%)

Table 1 showed that the effect of type of extender on the percentage of motility of the incubated camel spermatozoa was significant ($P < 0.05$). The extended camel semen with each of LYC, SYC, TYF, SCM and SLM extenders was significantly higher ($P < 0.05$) the percentage of sperm motility than GYC and FYC extenders. Meanwhile, the effects of extended camel semen with GYC and FYC or LYC, SYC, SCM, SLM and TYF extenders on the percentage of sperm motility were insignificant. It is worth noting that, the percentage of motile camel spermatozoa was superior amongst LYC, SYC, SCM, SLM and TYF extenders than other extenders. The highest ($P < 0.05$) value of the percentage of sperm motility was recorded with TYF extender and the lowest ($P < 0.05$) value was recorded with FYC extender. These results may be due to the combinations of all beneficial effects of tris component. Tris in addition to its better buffering capacity, can readily diffuse into the sperm cell and serve as an intracellular buffer (Bartlett and Van Demark, 1962). This phenomenon may be attributed to the better protection of lactose to spermatozoa against osmotic shock than other sugars or due to mediate available energy and osmotic balance of the extender. Eschborn (1985) stated that tris dilating agent which was mainly used gave the best results for bovine semen. The same later author also recorded that sucrose is the most regularly used protective disaccharides in some reports to prevent freeze-thaw bilayer destabilisation (Strauss *et al*, 1986). Dee Leeuw *et al* (1993) reported that sucrose has the capacity to act as non penetrating cryoprotective agents by direct interaction will the cell membrane. Moreover, camel spermatozoa extended in camel milk tend to had higher motility than GYC or FYC, this can be attributed to that camel milk contained a higher content of antimicrobial factors such as lysozyme, lactoferrin and immunoglobulin's (Elagamy *et al* 1992) and these anti-microbial factors were more heat resistant in camel milk (Elagamy, 2000). Galli *et al* (1993) found that the camel milk contained a high content of polyunsaturated fatty acids which were more prone to freezing stress and their poor freezability may be correlated to the low membrane phospholipids content and its loss during freeze-thawing. Similar trends were reported by El-Badry

Table 1. Percentage of motility of the dromedary camel spermatozoa with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	57.14 ± 1.84	55.71 ± 1.70	62.14 ± 1.01	62.86 ± 1.49	62.86 ± 1.49	61.43 ±2.10	62.14 ±1.49	60.61^{A}±0.69
1	55.71 ±2.30	55.00 ± 1.89	60.71 ± 2.30	62.86 ± 1.49	62.14 ± 1.01	60.71 ±1.30	61.43 ±2.37	59.79^{A}±0.78
2	52.14 ± 2.86	51.43 ± 2.10	58.57 ± 2.1	59.29 ± 1.30	60.00 ±1.54	58.57 ±1.8	60.00 ±2.18	57.14^{B}±0.87
4	46.43 ± 2.37	45.71 ± 2.02	52.14 ± 1.84	52.86 ± 1.49	54.29 ±1.70	52.86 ±2.64	53.57 ±1.80	51.12^{C}±0.85
6	38.57 ± 1.80	37.86 ± 1.84	45.71 ± 2.30	46.43 ±2.10	48.57 ±1.80	45.00 ±2.18	46.43 ±2.10	44.08^{D}±0.91
8	31.43 ± 2.10	29.29 ± 1.70	37.86 ± 2.64	40.00 ± 2.44	40.70 ±2.02	36.43 ±1.80	40.71 ±2.54	36.63^{E}±0.99
10	20.71 ± 2.02	18.57 ± 1.80	26.43 ± 3.03	27.86 ± 3.06	30.00 ±1.54	25.71 ±1.70	28.57 ±2.37	25.41^{F} ± 0.99
12	8.57 ± 1.43	7.14 ± 1.01	10.71 ± 2.02	10.71 ± 1.70	14.29 ±1.70	9.29 ±1.70	11.43 ± 2.10	10.31^{G}±0.67
Means	38.84^{b} ± 2.32	37.59^{b} ± 2.33	44.28^{a} ± 2.42	45.36^{a} ± 2.42	46.61^{a} ±2.25	43.75^{a} ±2.45	45.54^{a} ± 2.38	43.14

a-b: Values with different superscripts with a row, are significantly different (P<0.05).
A-G: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim-camel -milk.

et al (2007). Ahmadi (2001) and Zeidan (2002) found that, the percentage of motile camel spermatozoa was significantly better with LYC than TYF extender, during incubation at 37°C for up to 6 hrs. However, El-Bahrawi (2005) found no significant variation when used different extenders (Tris-lactose, Tris-sucrose, Tris, lactose, skim-milk and sucrose) for pre-freezing dromedary camel semen.

The prolongation of incubation time at 37°C for up to 12 hrs on the percentage of motility of camel spermatozoa increased significantly ($P < 0.05$) at first as compared to 12 hours in different extenders. The advancement of incubation time at 37°C for up to 12 hrs decreased significantly ($P < 0.05$) the percentage of motile spermatozoa. The decrease of sperm motility with advanced incubation time may be attributed to the increase in lactic acid accumulation and that changes in pH of the media which induce the metabolic activity of sperm, consequently the sperm cell motility decrease (Zeidan, 1994). This phenomenon may be attributed to the decrease in the content of adenosine tri phosphate which the could inactivated spermatozoa were apparently in capable of resynthesising. This was accompanied by aprecipitous fall in the rate of fructolysis (Salamon, 1970 and Mann and Lutwak-Mann, 1981).

Percentage of dead camel spermatozoa (%)

Data presented in Table 2 indicated that, the effect of type of extender on the percentage of dead camel spermatozoa was highly significant ($P < 0.05$). The extended camel semen with each of GYC and FYC extenders was significantly ($P < 0.05$) higher percentage of dead spermatozoa than other extenders. However, the effects of extended camel semen with GYC and FYC or LYC, SYC, TYF, SCM and SLM extenders on the percentage of dead spermatozoa were insignificant. The lowest ($P < 0.05$) value of the percentage of dead camel spermatozoa was recorded with TYF extender, while the highest ($P < 0.05$) value was recorded with FYC extender. These results may be due to the beneficial effects of tris and higher molecular weight of lactose or sucrose extenders allowing more protection of spermatozoa. These results are in agreement with those of Zeidan *et al* (2008) who recorded the lowest ($P < 0.05$) value of the percentage of dead camel spermatozoa with LYC extender and the highest ($P < 0.05$) value with GYC extender.

It was also noticed that the percentage of dead camel spermatozoa increased significantly ($P < 0.05$) with the prolongation of incubation time at 37°C for up to 12 hrs in all different extenders. Similar trend was recorded by Zeidan (1994) who found the incubation time advanced up to 4 hrs increased the percentage of dead spermatozoa significantly ($P < 0.05$). These findings may be attributed to accumulation of lactic acid, which exerts a toxic effect on sperm cell (Zeidan, 1994).

Percentage of sperm camel abnormalities (%)

Table 3 showed that the effect of type of extender on the percentage of camel sperm abnormalities was highly significant ($P < 0.05$). The extended camel semen with GYC or FYC extenders had significantly ($P < 0.05$) higher percentage of sperm abnormalities than other extenders. However, the effects of extended semen with GYC and FYC or

Table 2. Percentage of dead of the dromedary camel spermatozoa with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	33.18 ± 0.91	34.22 ± 1.68	28.14 ± 1.74	28.23 ± 1.75	25.38 ± 1.07	29.11 ± 1.39	26.13 ± 1.88	29.20^{F} ±1.26
1	35.29 ±1.02	36.45 ±1.72	29.43 ±1.84	29.14 ±1.70	27.53 ±1.23	29.86 ±1.53	27.84 ±1.74	30.79^{EF} ± 1.35
2	36.43 ±0.95	37.52 ±1.65	30.58 ±0.81	30.70 ±1.81	28.65 ±1.21	30.73 ±1.36	28.85 ±1.59	31.92^{E} ± 1.35
4	38.45 ±1.13	39.43 ±1.61	32.76 ±0.89	31.68 ±0.87	30.57 ±1.29	33.28 ±1.27	30.67 ±1.52	33.83 E ±1.38
6	40.25 ±1.21	43.15 ±1.70	33.86 ±0.80	32.82 ±0.77	32.40 ±1.45	36.19 ±1.20	33.57 ±1.65	36.03 D ±1.56
8	45.48 ±1.19	48.27 ±0.84	38.55 ±0.90	38.43 ±1.72	36.19 ±1.68	39.50 ±1.13	37.72 ±1.42	40.59 C ±1.69
10	48.57 ±1.31	51.74 ±1.08	42.53 ±1.15	41.80 ±1.67	40.21 ±1.66	42.70 ±1.21	40.54 ±1.57	44.01 B ±1.66
12	53.71 ±1.54	55.10 ±0.98	46.28 ±1.29	45.57 ±1.69	44.72 ±1.77	48.29 ±1.66	45.23 ±1.87	48.41 A ±1.61
Means	41.42^{a} ±2.53	43.24^{a} ±2.72	35.27^{b} ±2.31	34.80^{b} ±2.25	33.21^{b} ±2.36	36.21^{b} ± 2.42	33.82^{b} ±2.39	36.85

a-b: Values with different superscripts with a row, are significantly different (P<0.05).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

LYC and SCM or SYC, SLM and TYF extenders on the percentage of sperm abnormalities were insignificant. The lowest ($P < 0.05$) value of the percentage of sperm abnormalities was recorded with TYF extender, while, the highest ($P < 0.05$) value was recorded with FYC extender. Zeidan (2002) and Zeidan *et al* (2008) found that LYC extender was lower percentage of sperm camel abnormalities than TYF extender during incubation at 37°C for 6 hrs.

The advancement of incubation time at 37°C up to 12 hrs on the percentage of sperm abnormalities increased significantly ($P < 0.05$) the percentage of sperm abnormalities with the all different extenders. These results are in agreement with those of Zeidan *et al* (2008) who found that the effect of incubation time up to 6 hrs had significantly ($P < 0.01$) higher percentage of sperm camel abnormalities. These results might be due to the better protection of lactose, tris and sucrose extenders to spermatozoa against osmotic shock compared to other extenders.

Percentage of acrosomal damage of camel spermatozoa (%)

Table 4 showed that, the effect of type of extender on the percentage of acrosomal damage was significantly high ($P < 0.05$). The extended camel semen with GYC, FYC extenders had significantly ($P < 0.05$) higher percentage of acrosomal damage than other extenders. However, the effects of extended semen with GYC and FYC or LYC, SCM or SYC, SLM and TYF extenders on the percentage of acrosomal

Table 3. Percentage of sperm abnormalities of the dromedary camel spermatozoa with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	28.14 ±0.91	29.18 ±1.67	18.67 ±1.61	13.43 ±1.59	10.56 ±1.02	20.15 ±1.22	12.65±1.08	18.97^{E} ±2.81
1	28.25 ±1.10	29.73 ±1.57	19.82 ±0.70	14.75 ±1.64	12.83 ±0.99	20.86 ±1.26	13.84±1.03	20.01^{DE} ±2.58
2	30.46 ±0.97	30.54 ±1.65	20.58 ±0.81	15.80 ±0.74	14.42 ±0.92	22.75 ±1.29	15.57±1.19	21.45^{DE} ± 2.59
4	30.86 ±1.03	32.43 ±1.72	22.49 ±0.90	18.82 ±1.55	16.25 ±0.92	24.86 ±1.26	17.48±1.46	23.31^{CD} ±2.43
6	32.68 ±1.02	35.75 ±1.71	25.16 ±0.86	20.51 ±1.57	19.50 ±0.95	28.43 ±1.34	21.88 ±1.49	26.27^{C} ± 2.36
8	34.74 ±1.85	39.52 ±1.75	28.81 ±0.86	25.64 ±1.84	24.86 ±1.67	32.24 ±1.23	32.26±1.22	31.15^{B} ±1.96
10	38.53 ±1.65	43.86 ±0.91	34.58 ±1.78	27.43 ±0.75	26.14 ±1.18	36.17 ±1.48	27.14 ±1.35	33.41^{B} ±2.55
12	45.72 ±1.68	48.50 ±1.69	42.76 ±1.75	31.16 ±0.86	30.72 ±1.21	40.54 ± 1.27	28.16 ± 1.94	38.22^{A} ±3.07
Means	33.67^{a} ± 2.11	36.19^{a} ± 2.53	26.61^{b} ± 2.97	20.94^{c} ± 2.29	19.41^{c} ± 2.53	28.25^{b} ± 2.65	21.12^{c} ± 2.60	26.60

a-b: Values with different superscripts with a row, are significantly different ($P<0.05$).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

Table 4. Percentage of acrosomal damage of the dromedary camel spermatozoa with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	14.25 ±0.89	15.84 ±1.63	8.14 ±1.59	5.29 ±1.42	4.75 ±2.29	8.25 ±1.15	4.86 ±1.55	8.77 F ±1.72
1	15.70 ±0.92	16.56 ±1.53	8.64 ±0.59	6.17 ±1.46	5.14 ±1.42	9.43 ±0.97	5.78 ±1.61	9.63 F ±1.78
2	16.52 ±1.87	18.44 ±1.65	10.70 ±1.52	6.78 ±1.42	5.82 ±1.26	10.80 ±0.96	6.52 ±1.65	10.80EF ±1.89
4	18.49 ±0.9	20.72 ±1.71	12.81 ±1.40	7.82 ±1.59	5.23 ±1.29	13.76 ±1.99	7.41 ±1.53	12.32DE ±2.14
6	20.14 ±1.03	23.27 ±1.68	15.17 ±1.34	9.74 ±0.52	6.81 ±1.34	15.51 ±0.97	8.76 ±1.61	14.20CD ±2.31
8	24.48 ±1.02	27.13 ±1.83	17.22 ±1.42	11.50 ±1.43	10.46 ±1.53	20.25 ±1.15	10.85 ±1.63	17.41C ±2.58
10	29.57 ±1.69	30.52 ±0.81	22.35 ±1.87	15.45 ±1.53	12.51 ±1.37	24.14 ±1.32	13.51 ±1.48	21.15B ±2.82
12	34.86 ±1.51	36.15 ±1.59	27.30 ±1.57	19.51 ±1.72	18.14 ±1.67	29.42 ±1.36	18.24 ±1.58	26.23A ±2.92
Means	21.75a ±2.58	23.58a ±2.55	15.29b ±2.39	10.28c ±1.76	8.61c ±1.65	16.45b ±2.67	9.49c ±1.60	15.06

a-b: Values with different superscripts with a row, are significantly different ($P<0.05$).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

damage were insignificant. The lowest (P < 0.05) value of the percentage of acrosomal damage was recorded with TYF extender, while, the highest (P < 0.05) value was recorded with FYC extender. Similar trends were reported by Zeidan *et al* (2008). However, Zeidan (2002) found that the mean value of percentage of acrosomal damage of camel spermatozoa was insignificantly better with LYC extender, during incubation at 37°C for up to 6 hrs.

The prolongation of incubation at 37°C for up to 12 hrs increased percentage of acrosomal damage significantly (P < 0.05) with the all different extenders. These results are in agreement with those of Zeidan *et al* (2008) in the dromedary camel. The significant increase in acrosomal damage with the advancement of incubation time at 37°C for up to 12 hours may be due to the increase in lactic acid accumulation that changes both osmotic pressure and pH in the media which in turn exerts a toxic effects on sperm cells.

Enzymatic activities (U/10^6 spermatozoa)

Tables 5, 6 and 7 showed that, the effect of type of extender on AST, ALT and ALP enzymes activities was highly significantly (P < 0.05). The differences effect between GYC and FYC extenders on activity of AST , ALT and ALP enzymes were insignificant. AST enzyme activity was insignificantly higher in camel semen extended with LYC than SCM and SYC extenders. Similarly, the differences effect between SYC and TYF extenders on the AST enzyme activity

Table 5. Activities of seminal aspartate – aminotransferase enzyme (U/ 10^6 spermatozoa) of the dromedary camels with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	46.34 ±1.35	45.93 ±1.75	42.73 ±1.42	41.27 ±1.65	40.28 ±1.75	42.63 ±2.17	41.32 ±1.58	42.93^{G} ±0.89
1	49.53 ±1.48	48.82 ±1.48	43.90 ±2.62	42.46 ±2.35	42.31 ±2.17	43.85 ±2.18	42.94 ±1.85	44.83^{FG} ±1.15
2	52.27 ±1.65	53.80 ±1.95	46.70 ±2.18	43.93 ±1.75	43.56 ±2.65	45.62 ±1.18	44.23 ±1.62	47.16^{EF} ±1.58
4	54.60 ±1.82	56.64 ±2.54	49.55 ±2.13	45.64 ±2.38	46.23 ±1.53	47.71 ±2.17	46.91 ±2.25	49.61^{DE} ±1.64
6	59.92 ±1.68	60.75 ±1.83	52.26 ±1.66	47.90 ±2.42	49.16 ±2.32	50.28 ±2.16	49.86 ±2.41	52.88^{D} ±2.04
8	65.28 ±1.85	65.43 ±2.15	56.64 ±1.72	50.28 ±2.75	50.13 ±2.15	54.80 ±2.31	53.56 ±2.30	56.59^{C} ±2.43
10	69.82 ±2.12	70.64 ±2.32	62.35 ±2.18	54.63 ±2.11	53.12 ±2.36	59.38 ±2.35	57.72 ±2.17	61.09^{B} ±2.62
12	76.50 ±2.01	78.69 ±2.25	66.82 ±2.71	58.75 ±2.15	59.35 ±2.13	64.52 ±2.16	63.80 ±2.36	66.92^{A} ±2.97
Means	59.28^{a} ±3.73	60.09^{a} ±3.93	52.62^{b} ±3.08	48.11^{c} ±2.16	48.02^{c} ±2.21	51.10^{bc} ±2.77	50.04^{bc} ±2.77	52.75

a-b: Values with different superscripts with a row, are significantly different (P<0.05).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

Table 6. Activities of seminal alanine – aminotransferase enzyme (U/ 10^6 spermatozoa) of the dromedary camels with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	32.87 ±1.02	32.85 ±1.15	31.72 ±0.84	27.64 ±1.18	27.45 ±0.82	31.27 ±1.22	31.46 ±1.17	30.75F ±0.86
1	34.26 ±0.88	34.68 ±1.11	33.25 ±0.92	30.42 ±1.30	29.34 ±0.95	32.46 ±1.18	32.62 ±1.13	32.43^{EF} ±0.74
2	37.85 ±0.74	38.26 ±0.78	35.42 ±1.13	32.20 ±1.04	30.72 ±0.75	33.95 ±1.52	34.18 ±1.10	34.65^{E} ±1.05
4	40.48 ±1.04	42.85 ±1.14	38.90 ±1.12	35.34 ±1.17	32.15 ±1.33	36.84 ±1.16	38.15 ±1.22	37.82^{D} ±1.32
6	44.62 ±1.25	46.76 ±1.58	42.63 ±1.65	37.13 ±1.54	35.28 ±1.26	40.28 ±1.32	40.34 ±1.43	41.01^{CD} ±1.52
8	48.28 ±1.42	51.65 ±1.32	44.32 ±1.48	40.25 ±1.32	38.25 ±1.17	42.67 ±1.45	43.26 ±1.17	44.10^{C} ±1.73
10	51.64 ±1.38	55.68 ±1.45	47.60 ±1.25	44.12 ±1.73	40.23 ±1.18	45.96 ±1.28	47.12 ±1.64	47.48^{B} ±1.90
12	55.96 ±1.38	58.27 ±1.42	50.91 ±1.45	47.22 ±1.38	43.62 ±1.35	49.28 ±1.24	49.53 ±1.36	50.68^{A} ±1.90
Means	43.25^{ab} ±2.94	45.13^{a} ±3.39	40.59^{bc} ±2.45	36.79^{d} ±2.40	34.63^{d} ±2.01	39.09^{c} ±2.32	39.58^{c} ±2.37	39.87

a-b: Values with different superscripts with a row, are significantly different (P<0.05).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

Table 7. Activities of seminal alkaline phosphatase enzyme (U/ 10^6 spermatozoa) of the dromedary camels with different extenders, during incubation at 37°C for 12 hours.

Incubation time (hrs)	Extenders							Overall means
	GYC	FYC	LYC	SYC	TYF	SCM	SLM	
0	13.93 ± 0.85	13.92 ± 0.71	12.85 ± 0.36	12.61 ± 0.93	11.87 ± 0.42	13.46 ± 0.94	12.25 ± 0.34	12.98^{F} ± 0.31
1	15.23 ± 0.54	15.54 ± 0.34	14.25 ± 0.41	13.82 ± 0.81	12.45 ± 0.75	14.90 ± 0.61	13.97 ± 0.28	14.31^{F} ± 0.39
2	17.90 ± 0.82	18.61 ± 0.53	15.94 ± 0.78	15.24 ± 0.54	13.63 ± 0.57	16.67 ± 0.45	14.96 ± 0.37	16.14^{EF} ± 0.66
4	20.32 ± 0.65	23.84 ± 0.57	17.68 ± 0.75	17.92 ± 0.48	14.74 ± 0.53	19.23 ± 0.64	16.41 ± 0.46	18.59^{E} ± 1.11
6	24.63 ± 1.02	26.98 ± 0.97	21.40 ± 1.03	20.32 ± 0.88	16.28 ± 1.01	23.54 ± 0.83	19.67 ± 0.45	21.83^{D} ± 1.34
8	28.90 ± 0.47	30.25 ± 1.02	25.62 ± 0.65	23.51 ± 0.95	17.86 ± 0.91	26.98 ± 0.48	21.96 ± 0.38	25.01^{C} ± 1.62
10	34.52 ± 1.02	36.63 ± 0.92	29.27 ± 0.86	26.94 ± 0.94	19.24 ± 0.73	30.39 ± 1.04	24.13 ± 1.05	28.73^{B} ± 2.25
12	39.76 ± 1.00	40.32 ± 1.03	33.64 ± 0.95	30.42 ± 0.74	21.67 ± 0.96	34.94 ± 1.02	27.78 ± 1.02	32.65^{A} ± 2.52
Means	24.40^{ab} ± 1.30	25.76^{a} ± 3.41	21.33^{b} ± 2.66	20.10^{bc} ± 2.27	15.97^{d} ± 1.22	22.51^{b} ± 2.75	18.89^{c} ± 1.92	21.28

a-b: Values with different superscripts with a row, are significantly different (P<0.05).
A-F: 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. SYC: Sucrose-yolk-citrate.
TYF: Tris-yolk-furctose. SCM: Skim-cow - milk. SLM: Skim- camel -milk.

were insignificant. Meanwhile, the differences effect between GYC and LYC, SYC and TYF or among LYC, SCM and SLM extenders were insignificant on the activity of ALT enzyme. Regarding ALP, the differences effect among GYC, LYC, SYC and SCM or between SYC and SLM extenders on ALP enzyme activity was insignificant. The lowest (P < 0.05) amount of AST, ALT and ALP enzymes released into the extracellular medium was recorded with the extended semen with TYF extender and the highest (P < 0.05) amount was recorded with FYC 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 trand was reported by Zeidan (2002), Abd El-Salaam (2007) and Zeidan *et al* (2008) in the dromedary camel.

The time advancement of incubation at 37°C for up to 12 hours increased significantly (P < 0.05) the leakage of AST, ALT and ALP enzymes into the extracellular medium with the all different extenders. These results are in agreement with those of Zeidan (2002) and Zeidan *et al* (2008) who reported that, the incubation time of the extended camel sperm at 37°C for up to 6 hrs showed significantly (P < 0.05) increased the amount of AST enzyme released into the extracellular medium in dromedary camel. The continuous increase in leakage of the intracellular AST, ALT and ALP enzymes during incubation time may reflect the breakdown of the cellular sperm membrane (Graham and Pace, 1967 and Zeidan *et al*, 1998). In addition, the phosphatase enzymes leakage from the sperm cells may be a sign for increasing the cell damage which occurred during storage process (Brown *et al,* 1969).

Camel sperm penetration into cervical mucous

Fig 1 shows that the penetrating ability of the extended spermatozoa with TYF, SYC, SCM, SLM and LYC extenders into she-camel cervical mucous was insignificantly better than GYC and FYC extenders. However, incubation time at 37°C for up to 4 hrs was significantly (P < 0.05) decreased the

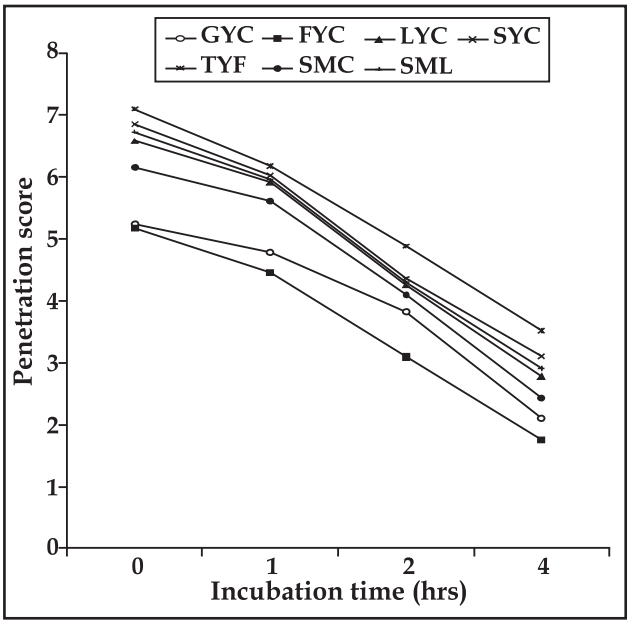

Fig 1. Penetration score values of the extended spermatozoa with the different extenders into she-camel cervical mucous, during incubation at 37°C for 4 hours.

penetration score. Aitken *et al* (1983) found a close correlation between human spermatozoa movement and their penetrating ability into cervical mucous. In addition, Alexander (1981) and Murase *et al* (1990) reported that the duration of sperm motility and penetration distance in the mucous closely correlated to the pregnancy and conception rate. Similar findings were recorded by Zeidan (2002) with the dromedary camels.

Conclusion

In conclusion, extended camel semen with TYF, SYC, LYC, SCM and SLM extenders showed better sperm motility, viability and maintaining enzymatic activities and penetrating ability of spermatozoa into she-camel cervical mucous than GYC and FYC extenders, during preservation at 37°C for 12 hours. Therefore, it can be recommended to collect the dromedary camel semen with each of TYF, SYC, LYC, SCM and SLM extenders for artificial insemination to enhance of fertilising ability of she-camels.

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