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Influence of different extenders on semen quality of the males dromedary camel during incubation at 37°C for up to 6 hours

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Abstract

Fifteen male dromedary camels at 5-10 years of age and 500-600 kg live body weight were used in the present study. Semen was collected, evaluated and extended with different six extenders (Glucose-yolk-citrate: GYC, Fructose-yolk-citrate: FYC, Lactose-yolk-citrate: LYC, Sucrose-yolk: SYC, Tris-yolk-fructose: TYF and Raffinose-yolk-citrate: RYC). The final extension rate was 1semen:4 extender-citrate. The extended semen with different extenders (TYF, GYC, FYC, SYC, LYC and RYC) was incubated at 37°C for up to 6 hours. After each incubation time (0,1,2,4,5 and 6 hours), the percentages of sperm motility, dead spermatozoa, abnormal spermatozoa and acrosomal damage of spermatozoa were determined. Sperm penetration into she-camel cervical mucus during incubation at 37°C for up to 4 hours was also estimated.

The results revealed that, the extended camel semen with TYF, LYC or SYC or SYC extenders was significantly ($P<0.05$) higher the percentage of sperm motility, while significantly ($P<0.05$) lower the percentage of dead spermatozoa, abnormal spermatozoa and acrosomal damage of spermatozoa than GYC, FYC and RYC extenders during incubation at 37°C for up to 6 hours. The advancement of incubation time at 37°C decreased significantly ($P<0.05$) the percentage of motile camel spermatozoa, while significantly ($P<0.01$) increased the percentage of dead spermatozoa, abnormal spermatozoa and acrosomal damage of spermatozoa with all different extenders (GYC, FYC, SYC, LYC, TYF, and RYC). The penetrating ability of the extended camel spermatozoa into she–camel cervical mucus increased significantly with TYF or LYC extender compared to other extenders during incubation at 37 °C for up to 4 hours. However, the advancement of incubation time at 37°C decreased significantly ($P<0.05$) the ability to penetrate cervical mucus of camel spermatozoa of the all used extenders .

(Keyword: Dromedary camels, semen, extenders, incubation, penetration)

Introduction

The Arabian camel (Camelus dromedarius) is an indispensable animal which contributes effectively both as a source of food and welfare to peoples living under harsh desert condition. It is frugal in habits yet highly productive of milk, meat, wool and work. Opportunities to improve reproductive efficiency of the dromedary camel are limited by inherent characteristics, which include long gestation, a restricted breeding season and induced ovulation. The continued use of traditional management adds to the practical difficulties in improving reproductive performance as it is often difficult to be certain that females are pregnant at the end of the breeding season. A further factor in the poor reproductive rate is the level of inbreeding in traditional herds.

On the other hand, freezing semen and artificial insemination (AI) in camel could be employed to overcome some of these problems **(Zhao et al., 1994)**. Artificial insemination with frozen semen is not well developed as a technique for breeding camelids compared to its widespread application in other farm animals. In addition, AI with frozen semen could be used to improve genetic traits such as milk, meat and wool production, and racing ability in camels. The study of spermatozoal morphology has received great emphasis in present day research. It is regarded as an essential factor to be taken into account in analysis of spermatozoa, such as sperm motility pattern, sperm morphology, and the number of spermatozoa in an ejaculate **(Keel and Webster, 1990)**. The process of fertilization involves a complex of biochemical and physiological events that are not measured by the gross physical indicators used in routine semen evaluation **(Jeyendran et al., 1984)**.

A management strategy that promotes 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 fastest way in the modern technology for the application of genetic improvement through

the breeding programs in farm animals. The progress in AI, semen preservation and related techniques in camel has been slow in comparison to other animals which may be 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 development of extenders that will preserve the functional activity of 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 condition. Lack of studies on camel's semen processing and disseminating under desert condition has drawback to clearly monitor productivity of such animals.

Therefore, the present study aimed to evaluate the effects of different extenders on camel's semen quality during incubation at 37°C for up to 6 hours.

Materials And Methods

The present study was carried out in the Private camel's Farm and Abattoirs, Belbies City, Sharkiya Governorate, Egypt, during the period from January, 2012 till November, 2012.

Experimental animals:

Fifteen male dromedary camels (Camelus dromedarius) at 5-10 years of age and 500-600 kg live body weight were used in the present study. The camels were in healthy condition and clinically free from external and internal parasites with a sound history of fertility in the herd. Palpation of the external genetalia showed typically normal.

Feeding and management:

The rations offered to the camels were calculated according to **Banerjee (1988)**. Two types of ration were used as follows: Green season (from December to May): the average amount give per head/daily was 35 kg berseem (Trifolium alexandrinum) and 7 kg rice straw. Dry season (from June to November), each camel was received about 2 kg commercial concentrate mixture, 2 kg berseem hay and 9 kg rice straw daily. Clean fresh water was offered freely to all camels. Camels were housed in a yard which provided with common feeding through and a concrete floor provided with common sheltered water trough. The camels could more freely in enclosed area.

The present study was planned to evaluate the extended camel semen quality (percentage of sperm motility, dead spermatozoa, abnormal spermatozoa and acrosomal damage of spermatozoa) using six extenders (GYC, FYC, SYC, LYC, TYF and RYC) during incubation at 37°C for up to 6 hours. Composition of the different extenders are shown in Table 1.

Table 1. Composition of the different buffered yolk extenders

Components	(Grams /100 ml of distilled water for each extender)					
	GYC	FYC	LYC	SYC	RYC	TYF
Sodium citrate dehydrate	2.9	2.90	0.04	0.04	2.90	-
Citric acid anyhydrate	0.04			0.04	0.04	1.675
Glucose	1.25	-	-	-	-	-
Fructose	-	1.25	-	-	-	1.25
Lactose	-	-	1.25	-	-	-
Sucrose	-	-	-	1.25	-	-
Raffinose	-	-	-	-	1.25	-
Tris aminomethan	-	-	-	-	-	3.208
Egg yolk (ml)	10	10	10	10	10	10
Penicillin(IU/ml)	500	500	500	500	500	500
Streptomycin (µ/ml)	500	500	500	500	500	500

GYC: Glucose-yolk-citrate, FYC: Fructose-yolk-citrate, LYC: Lactose-yolk-citrate, SYC: Sucrose-yolk-citrate, RYC: Raffinose-yolk-citrate, TYF: Tris-yolk-fructose. Tris (Hydroxymethyl) aminomethan, Aldrich Chemical Co. Ltd., Gillingham, Dorest-England

Camel's semen collection using artificial vagina (AV):

Semen was collected from the male dromedary camel between 08:00 and 10:00 am using artificial vagina (AV). A modified artificial vagina (30 cm long and 5 cm internal diameter, IMV, France) was used as the method described by **Zeidan (2002)** and **Mosaferi et al. (2005)**. 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 is inserted

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to avoid contact with the rubber material. After passing the liner thought 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 imitate the internal cervix and provide more stimulation for the penis to stimulate of erection and natural ejaculate. A shortened of AV without collection funnel was used, for 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 medical Vaseline were smeared on the inner at the entrance of the penis to AV for provide lubrication. A sexually receptive females couching with her front legs tied and tested 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 ejaculation is completed after several thrusts and inter pressed by period 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 so, camel semen immediately after collection was left for liquefaction for about 30-60 minutes to make the sperm motility. Immediately after liquefaction, semen was evaluated and only ejaculates showing active wave motion (<60 %) were pooled . After semen collection, it was placed inside incubator set at 37°C and evaluated immediately.

Semen extension :

Semen was collected, pooled, evaluated for each camel and then extended with different extenders (Glucose-yolk-citrate: GYC, Fructose-yolk-citrate: FYC, Lactose-yolk-citrate: LYC, Sucrose-yolk-citrate: SYC, Tris-yolk-fructose: TYF and Raffinose-yolk-citrate: RYC). Semen extension was carried out by adding the appropriate volume of the extender slowly to the semen. The final extension rate was 1semen:4 extender. Extended semen(in tube) was kept below the level of water in a water bath at 37°C at all times to avoid fluctuations in the temperature of the extended semen .

Percentage of sperm motility (%):

Generally, camel sperm motility (%) was detected as an oscillatory motion the flagellum, but not progressive due to the viscous materials according to **Tibary and Anouassi (1997)**. Sperm motility was estimated by adding one drop of the diluted fresh semen with physiological saline (0.9% sodium chloride) on the dry, clean and pre-warmed (37°C) glass slide.

With regard to extended semen, the percentage of sperm motility was determined using one drop of the extended semen after each storage period for up to 6 hours. The drop of the extended semen was covered by a warmed cover slip and examined immediately after liquefaction using high power magnification (400 x).

Percentage of dead spermatozoa (%):

The eosin/nigrosin staining procedure was carried out by dissolving 1.67 gm eosin and 10 gm nigrosine in distilled water up to 100 ml according to **Hackett and Macpherson (1965)**. Seven drops of the stain were placed into a test tube and warmed to 37°C in a water bath before adding to the semen samples. One drop of the stain was taken by pipette and placed at the end of the warm slide then mixed with one drop of extended semen. A thin smear was made by drawing the edge of a second slide across the mixture. The stained slide was allowed to dry and then examined under oil immersion lens with magnification power 1000 x. The live spermatozoa were clearly defined, bright and unstained (white in colour). Their outlines were clearly defined and their heads were bright and retractile against the backgrounds. The dead spermatozoa were stained pink and their outlines were not clearly defined and its heads were not in sharp contrast with the background. The percentage of dead spermatozoa was calculated from 200 spermatozoa which were counted in each slide in the different microscopical fields using a hand counter. Then the live spermatozoa percentage, were estimated.

Morphology of camel spermatozoa (%):

The morphological abnormalites of spermatozoa (%) were determined in the same smears prepared live/dead spermatozoa ratio.

Percentage of acrosomal damage (%):

Assessment of the percentages of acrosomal damage of spermatozoa and acrosomal damage (%) were done according to **Watson (1975)**. A drop of diluted semen was smeared on a pre-warmed slide and allowed to dry in a current air. The smears were fixed by immersion in buffered formal saline (9 ml sodium chloride + 100 ml formalin + 900 ml

distilled water) for 15 minutes and then washed in running tap water for 15 to 20 minutes. The slides were dried and then immersed in buffered Giemsa solution for 90 minutes and after that they were rinsed briefly in distilled water and dried. A stock of Giemsa solution was prepared from solid Giemsa stain (Northampton, U.K. P460 D) as follows: Giemsa stain (3.80 g) was ground with absolute methanol (AR 64 grade, 375 ml). Glycerol (AR grade, 125 ml) was added and the stain mixture was stored at 37°C for one week.

The percentages of acrosomal damage of spermatozoa were calculated for 100 spermatozoa observed at random on each slide using oil immersion lens (xl000).

Sperm penetration:

Cervical mucus was obtained from she-camel during breeding season. A portion of the mucus was sucked into polyethylene sealed tubes with 2 mm internal diameter to provide a column of 6 cm length as the method described by **Foote (1978)** and then placed into 2 ml cuvettes (1 ml each). The tubes containing the mucus were inserted (open end) into the cuvettes containing the extended semen with GYC, FYC, LYC, SYC, TYF and RYC extenders and incubated at 37 °C for up to 4 hours. Sperm penetration score was judged as the rank score as the method described by **Eskin et al. (1973)** and **Hanson et al. (1982)**.

Data were statistically analyzed by two way ANOVA using General Linear Model (GLM) procedure of SAS **(Goodnight et al., 1982)**. Duncan's New Multiple Range Test **(Duncan, 1955)** was used to detect significant differences among means. Percentage values were transformed to arcsine values before being statistically analyzed.

Results And Discussion

1.Camel semen quality:

1. 1.Percentage of motile camel spermatozoa (%):

Data presented in Table 2 indicated that the percentage of motile camel spermatozoa was significantly (P<0.05) better in the extended semen with TYF or LYC extenders than GYC, FYC, SYC or RYC extenders. Moreover, the percentages of sperm motility extended with FYC or LYC extenders were insignificantly higher than that with GYC or RYC extenders. The highest (P<0.05) value of the percentage of motile camel spermatozoa was recorded with TYF extender and the lowest (P<0.05) value was recorded with GYC extender. Similar trends were reported by **Ahmadi (2001), Zeidan (2002), Zeidan et al. (2014) and Arafat (2015)** in the dromedary camel spermatozoa. These results may be due to the combinations of all beneficial effects of this components and better protection of tris to spermatozoa against to osmotic shock than other sugars. Lactose-yolk-citrate extender in addition to its better buffering capacity, can readily diffuse into sperm cells and serves as an intracellular buffer **(Zeidan, 1994)**. However, **Viudes-de-Castro and Vicente (1996)**, reported that addition of sucrose was significantly increased the percentage of post-thawing motile rabbit spermatozoa and a good sperm motility can be obtained after thawing with sucrose extender.

The effect of incubation time (37°C) on the percentage of the dromedary camel sperm motility decreased significantly (P<0.05) in all extenders (TYF, GYC, FYC, SYC, LYC and RYC). These results are in agreement with those of **Ahmadi (2001)** and **Zeidan (2002)** in the dromedary camels. **El-Gaafary and Abd-El-Ghaffar (1994)** found that 1 hour of incubation was required after addition of the stimulator substances for full restoration of motility of bull spermatozoa. Similar trend was reported by **Abbas (1993)** in buffalo and **Zeidan et al. (1998)** in Friesian bulls. The decrease of sperm motility with advanced incubation time may be attributed to the increase of lactic acid accumulation and that changes in pH of the media which induce the metabolic activity of spermatozoa, consequently, the sperm cell motility was decreased **(Zeidan, 1994)**.

1.2. Percentage of dead camel spermatozoa (%):

The results obtained in Table (3) showed that, the mean values of the percentage of dead spermatozoa was significantly (P<0.05) lower in the extended camel semen with TYF and LYC extenders than GYC, FYC, SYC, and RYC extenders. Meanwhile, the percentages of dead spermatozoa extended with TYF or LYC extenders were insignificantly lower than with GYC or RYC extenders. The highest (P<0.05) value of the percentage of dead spermatozoa was recorded with GYC extender and the lowest (P<0.05) value was recorded with TYF extender. Similar trends were reported by **Ahmadi (2001)** and **Zeidan (2002)** in the dromedary camels.

The effect of incubation time up to 6 hours on the percentage of dead of the dromedary camel spermatozoa showed significantly (P<0.01) higher at

the first hour in all extenders (TYF, GYC, FYC, SYC, LYC and RYC). These results are in agreement with those of **Ahmadi (2001), Zeidan (2002)** and **Arafat (2015)** in the dromedary camels.

1.3. Morphology of camel spermatozoa (%):

Data presented in Table (4) revealed that the percentage of abnormal spermatozoa during incubation was significantly (P<0.05) lower with TYF and LYC extenders than GYC, FYC, SYS and RYC extenders. The highest (P<0.05) value of the percentage of abnormal spermatozoa was recorded with GYC extender and the lowest (P<0.05) value was recorded with TYF extender. These results are in agreement with those of **Ahmadi (2001)** and **Zeidan (2002)** in the dromedary camel.

The effect of incubation time at 37°C for up to 6 hour on the percentage of abnormal spermatozoa was significantly (P<0.05) higher at fourth hours than zero hour in different extenders (TYF, GYC, FYC, LYC, SYC, and RYC). Similar trends were reported by **Ahmadi (2001)** and **Zeidan (2002)** in the dromedary camel.

1.4. Percentage of acrosomal damage of camel spermatozoa (%):

Data presented in Table (5) indicated that the mean values of the percentage of acrosomal damage of spermatozoa was significantly (P<0.05) lower with TYF and LYC extenders than GYC, FYC, SYC and RYC extenders. In addition, the percentages of acrosomal damage of spermatozoa extended with FYC or SYC extenders were significantly (P<0.05) lower than that with GYC or RYC extenders.

During different incubation times, the percentages of acrosomal damage of spermatozoa were significantly (P<0.05) lower using TYF extender followed by LYC, FYC, SYC, RYC and FYC extenders, and significantly (P<0.05) higher with GYC extender. **Marinov et al. (1980)** also observed that sucrose lactose diluent gave better results in term of sperm motility and acrosomal proteinase activity. **Zeidan (2002)** also reported that the percentage of acrosomal damage of the dromedary camel spermatozoa was significantly (P<0.01) higher with GYC extender, during incubation at 37°C for up to 6 hours. Similar trends were reported by **Ahmadi (2001)** and **Arafat (2015)** in the dromedary camels.

Table 2. Mean percentage (%) of motile camel spermatozoa extended with different extenders, during incubation at 37°C for up to 6 hours

Incubation time (hrs)	Extenders						Overall means
	TYF	GYC	FYC	SYC	LYC	RYC	
0	55.00 ± 8.80	49.00 ± 7.31	52.00 ± 8.15	49.00 ± 7.31	51.00 ± 7.81	50.00 ± 7.58	51.00 ± 2.94^{A}
1	55.00 ± 8.60	47.40 ± 6.62	51.40 ± 7.74	48.20 ± 6.98	51.00 ± 7.81	49.00 ± 7.48	50.33 ± 2.86^{A}
2	50.60 ± 7.16	37.00 ± 3.00	41.40 ± 4.24	39.20 ± 4.91	46.20 ± 6.59	39.40 ± 5.68	42.30 ± 2.20^{B}
3	48.60 ± 6.78	37.40 ± 2.60	38.40 ± 3.75	38.20 ± 3.90	45.00 ± 5.00	35.40 ± 2.86	40.50 ± 1.84^{B}
4	40.60 ± 4.94	27.60 ± 3.71	28.00 ± 3.74	30.20 ± 2.65	37.00 ± 3.00	28.60 ± 3.31	32.00 ± 1.64^{C}
5	38.00 ± 4.06	21.60 ± 3.54	24.40 ± 5.23	23.60 ± 3.87	31.40 ± 2.23	25.00 ± 3.16	27.33 ± 1.75^{C}
6	27.60 ± 1.94	16.40 ± 4.06	17.00 ± 4.89	19.00 ± 3.32	26.00 ± 1.87	15.60 ± 4.46	20.27 ± 1.61^{D}
Means	45.06 ± 2.75 ^{a}	33.77 ± 2.55^{c}	36.08 ± 2.88 ^{b}	35.34 ± 2.51 ^{b}	41.08 ± 2.43^{a}	34.71 ± 2.70^{c}	37.67

a-c Values with different superscripts within a row are significantly different (P<0.05).
A-D Values with different superscripts within a column are significantly different (P<0.05).
TYF : Tris-yolk-fructose
GYC : Glucose-yolk-citrate
FYC : Fructose-yolk-citrate
LYC : Lactose-yolk-citrate
SYC : Sucrose-yolk-citrate
RYC : Raffinose-yolk-citrate

Table 3. Mean percentage (%) of dead of extended camel spermatozoa with different extenders, during incubation at 37°C for up to 6 hours

Incubation time (hrs)	Extenders						Overall means
	TYF	GYC	FYC	SYC	LYC	RYC	
0	30.40 ± 7.53	39.80 ± 5.12	36.20 ± 6.00	35.80± 6.17	30.00 ± 6.06	38.20 ± 5.52	35.07 ± 2.33^{C}
1	29.40 ± 7.73	41.00 ± 4.85	37.60 ± 5.68	36.80 ± 5.85	32.60 ± 5.88	38.60 ± 5.45	36.00 ± 2.32^{BC}
2	34.80 ± 6.55	41.40 ± 2.27	38.80 ± 2.97	40.80 ± 4.90	34.40 ± 4.16	39.80 ± 2.69	38.33 ± 1.61^{B}
3	37.00 ± 5.79	43.00 ± 1.84	40.20 ± 2.54	39.80 ± 2.69	40.00 ± 3.75	39.20 ± 1.07	39.87 ± 1.27^{A}
4	37.60 ± 3.14	45.20 ± 1.32	41.00 ± 0.45	41.60 ± 2.11	41.20 ± 2.22	41.20 ± 1.07	41.30 ± 0.83^{A}
5	35.20 ± 1.46	41.20 ± 5.32	40.60 ± 2.73	39.00 ± 2.32	38.60 ± 2.18	40.60 ± 2.80	39.20 ± 1.19^{A}
6	35.60 ± 3.93	41.00 ± 7.75	39.20 ± 7.32	38.40 ± 7.12	38.00 ± 5.78	39.20 ± 7.37	38.57 ± 2.49^{BC}
Means	34.28 ± 1.98^{c}	41.80 ± 0.60^{a}	39.08 ±0.58^{b}	38.88 ± 1.69^{b}	36.40 ± 1.59^{c}	39.54 ± 0.49^{a}	38.33

a-c Values with different superscripts within a row are significantly different (P<0.05).
A-C Values with different superscripts within a column are significantly different (P<0.05).
TYF : Tris-yolk-fructose
GYC : Glucose-yolk-citrate
FYC : Fructose-yolk-citrate
LYC : Lactose-yolk-citrate
SYC : Sucrose-yolk-citrate
RYC : Raffinose-yolk-citrat

Table 4. Mean percentage (%) of abnormal extended camel spermatozoa with different extenders, during incubation at 37°Cfor up to 6 hours

Incubation time (hrs)	Extenders						Overall means
	TYF	GYC	FYC	SYC	LYC	RYC	
0	10.20 ± 0.12	19.00 ± 0.83	16.40 ± 0.81	16.20 ± 0.02	12.80 ± 1.59	18.60 ± 0.40	15.53 ± 0.92 ^{E}
1	10.40 ± 0.122	19.20 ± 0.75	17.00 ± 0.61	16.60 ± 0.12	13.20 ± 1.77	18.80 ± 0.31	15.87 ± 0.91 ^{E}
2	12.20 ± 0.43	20.40 ± 0.93	18.80 ± 0.91	18.00 ± 0.26	14.80 ± 1.74	20.00 ± 0.19	17.37 ± 0.92 DE
3	13.20 ± 0.53	22.00 ± 0.77	20.00 ± 0.97	19.00 ± 0.26	16.00 ± 2.02	21.60 ± 0.20	18.63 ± 0.95 CD
4	14.20 ± 0.28	23.20 ± 0.78	21.40 ± 0.32	20.40 ± 0.21	17.20 ± 2.08	23.80 ± 0.88	20.03 ± 0.97 BC
5	15.80 ± 0.32	24.60 ± 1.67	23.20 ± 0.56	22.00 ± 0.22	18.80 ± 2.08	24.40 ± 0.01	21.47 ± 0.96 ^{B}
6	18.40 ± 0.16	27.00 ± 0.77	25.60 ± 0.73	24.80 ± 0.32	21.40 ± 2.29	27.20 ± 0.59	24.07 ± 0.97 ^{A}
Means	**13.48 ± 0.65** c	**22.20 ± 1.06** a	**20.34 ± 0.91** b	**19.57 ± 0.64** b	**16.31 ± 0.83** c	**22.06 ± 0.88** a	18.99

a-c Values with different superscripts within a row are significantly different (P<0.05).
A-E Values with different superscripts within a column are significantly different (P<0.05).
TYF : Tris-yolk-fructose
GYC : Glucose-yolk-citrate
FYC : Fructose-yolk-citrate
LYC : Lactose-yolk-citrate
SYC : Sucrose-yolk-citrate
RYC : Raffinose-yolk-citrate

Table 5. Mean percentage (%) of acrosomal damage of camel spermatozoa extended with different extenders, during incubation at 37°C for up to 6 hours

Incubation time (hrs)	Extenders						Overall means
	TYF	GYC	FYC	SYC	LYC	RYC	
0	4.60 ± 0.51	10.40 ± 0.96	6.40 ± 0.81	6.40 ± 0.40	6.20 ± 0.66	10.20 ± 0.59	7.37 ± 0.63 ^{E}
1	5.00 ± 0.63	10.60 ± 0.77	6.60 ± 0.68	6.80 ± 0.40	6.20 ± 0.26	10.40 ± 0.40	7.60 ± 0.59 DE
2	6.00 ± 0.20	11.40 ± 0.57	8.00 ± 0.71	8.60 ± 0.47	7.00 ± 0.84	11.60 ± 0.98	8.77 ± 0.57 CD
3	6.40 ± 0.24	12.40 ± 0.36	8.80 ± 0.73	9.60 ± 0.16	7.60 ± 0.67	12.60 ± 0.68	9.57 ± 0.56 ^{E}
4	8.00 ± 0.32	13.40 ± 0.07	10.20 ± 0.58	10.90 ± 0.68	8.40 ± 0.68	13.40 ± 0.51	10.72 ± 0.48 B
5	8.80 ± 0.58	14.20 ± 0.80	11.00 ± 0.95	12.34 ± 0.86	9.00 ± 0.84	14.20 ± 0.20	11.59 ± 0.50 B
6	10.20 ± 0.86	16.20 ± 0.58	13.20 ± 0.66	13.60 ± 0.67	10.40 ± 0.87	15.40 ± 0.24	13.17 ± 0.50 A
Means	**7.00 ± 0.37** c	**12.66 ± 0.58** a	**9.17± 0.46** b	**9.75 ± 0.50** b	**7.83 ± 0.36** c	**12.54± 0.45** a	9.82

a-c Values with different superscripts within a row are significantly different (P<0.05).
A-E Values with different superscripts within a column are significantly different (P<0.05).
TYF: Tris-yolk-fructose
GYC: Glucose-yolk-citrate
FYC: Fructose-yolk-citrate
LYC: Lactose-yolk-citrate
SYC: Sucrose-yolk-citrate
RYC: Raffinose-yolk-citrate

With regard to incubation time, the percentages of acrosomal damage of the spermatozoa were significantly (P<0.05) higher of six hours than zero hour in all extenders (TYF, GYC, FYC, SYC, LYC and RYC).

The significant increase in acrosomal damage of spermatozoa with the advancement of incubation 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 (2001), Zeidan (2002), Miada(2011)** and **Arafat (2015)** in the dromedary camels.

2. Sperm penetration into she-camel cervical mucus:

Sperm penetration into she-camel cervical mucus during incubation at 37°C for up to 4 hours was illustrated graphically in Figer 1. The mean penetrating ability of camel spermatozoa into she-camel cervical mucus increased significantly (P<0.05)semen in TYF and LYC extenders. Extended semen with TYF or LYC extenders showed significantly (P<0.05) the highest penetrating ability of spermatozoa into cervical mucus, followed by EYC or SYC extenders, while semen extended with RYC or GYC extenders was recorded with RYC or GYC extenders was recorded the lowest penetrating score. However, the advancement of incubation time at 37 °C for up to 4 hours was significantly (P<0.05) decreased the penetrating ability of spermatozoa into she-camel cervical mucus with the different extenders. **Aitken et al. (1983)** found a close correlation between human movement of spermatozoa and their penetrating ability into cervical mucus. **Murase et al. (1990)** reported that, the duration of sperm motility and penetration distance in the mucus was closely correlated to the pregnancy and conception rate. Similar findings were recorded by **Zeidan (2002)** and **Maiada (2011)** in the dromedary camel.

In conclusion, the extended camel semen with Lactose-yolk-citrate (LYC), Tris-yolk-fructose (TYF) and Sucrose-yolk-citrate (SYC) extenders showed better sperm motility ,longevity of the dromedary camel spermatozoa, during incubation at 37°C for up to 6 hours . Therefore , it can be recommended to extended camel semen with TYF or LYC extender for maintaining of survival rate of the camel spermatozoa, as well as, sperm motility, penetration into she-camel cervical mucus for artificial insemination programs to enhance of fertilizing ability of the dromedary camel spermatozoa, under Egyptian environmental condition.

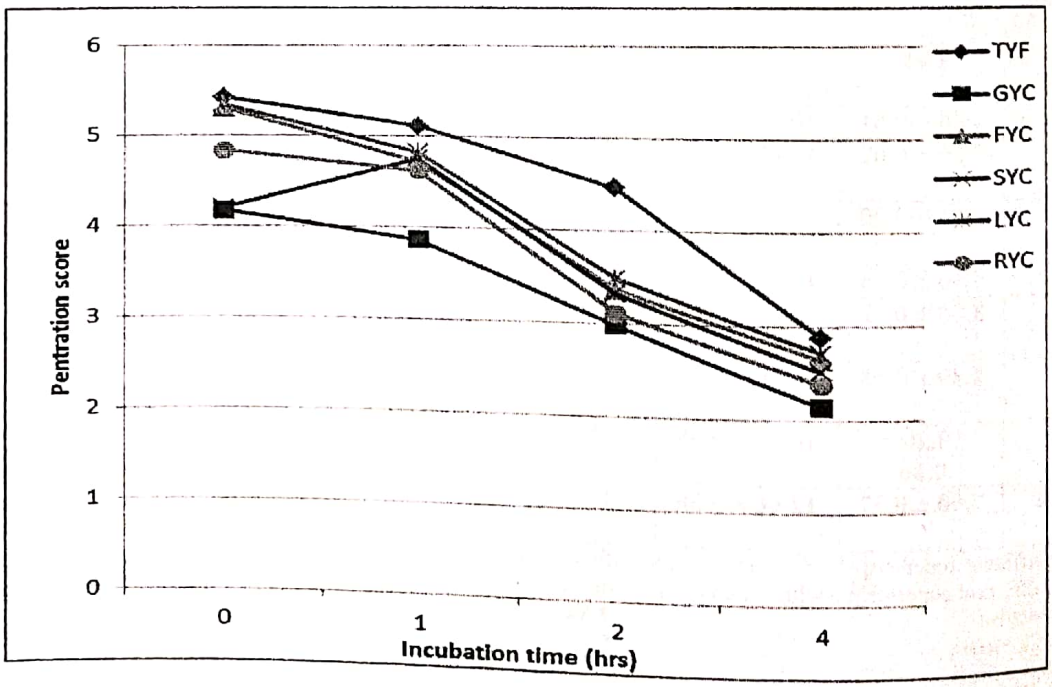

Fig. 1. Penetration score values of the extended camel spermatozoa with the different extenders into she-camel cervical mucus, during incubation at 37°C for upto 4 hours

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الملخص العربى

تاثير المخففات المختلفة على نوعية السائل المنوى في ذكور الجمال العربية اثناء التحضين على درجة حرارة 37 مئوية لمدة 6 ساعات

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استخدم في هذه الدرراسة 15ذكر جمل عربى تتراوح اعمارها من 5 الى 10 سنوات واوزانها من 500 الى 600 كجم وزن حى .حيث تم جمع وتقييم وتخفيف السائل المنوى بستة مخففات(جلوكوز- صفار بيض-سترات GYC وفركتوز- صفار بيض-سترات FYC ولاكتوز- صفار بيض-سترات LYC وسكروز- صفار بيض-سترات SYC وترس –صفار بيض- فركتوزTYF ورافينوز- صفار بيض-سترات RYC) وكان معدل التخفيف النهائى 1 سائل منوى : 4 مخفف .بعد ذلك تم تحضين السائل المنوى المخفف بالمخففات المختلفة (TYF, GYC, FYC, SYC, LYC , RYC)على درجة حرارة 37 درجة مئوية لمدة 6 ساعات وبعد كل فترة تحضين1و2و3و4و5و6 ساعات تم تقدير كلا من النسبة المئوية لحيوية الحيوانات المنوية والحيوانات المنوية الميتة والحيوانات المنوية الشاذة والحيوانات المنوية شاذة الاكروسوم وكذلك تم تقديرنفاذ يةالحيوانات المنوية داخل عنق الرحم للنوق وذلك اثناء التحضين على درجة حرارة 37 درجة مئوية لمدة 4 ساعات .حيث اوضحت النتائج ان السائل المنوى للجمال المخفف بمخففات SYC,LYC,TYF كان اعلى بدرجة معنوية ($P<0.05$) في النسبة المئوية لحيوية الحيوانات المنوية بينما كانت النسبة المئوية للحيوانات المنوية الميتة والحيوانات المنوية الشاذة والحيوانات المنوية شاذة الاكروسوم اقل بدرجة معنوية($P<0.05$) عن مخففات GYC, FYC , RYC وذلك اثناء التحضين على درجة حرارة درجة مئوية 37 لمدة 6 ساعات . ومع تقدم فترة التحضين على درجة حرارة 37 درجة مئوية انخفضت النسبة المئوية لحيوية الحيوانات المنوية بدرجة معنوية ($P<0.05$) بينما زادت النسبة المئوية للحيوانات المنوية الشاذة والحيوانات المنوية شاذة الاكروسوم بدرجة معنوية ($P<0.05$) وذلك في كل المخففات المستخدمة . زادت قدرة الحيوانات المنوية للابل المخففة بمخفف TYF, SYC, LYC للنفاذية داخل مخاط عنق الرحم للنوق بدرجة معنوية ($P<0.05$)مقارنة بالمخففات الاخرىGYC, FYCN , RYC وذلك اثناء التحضين على درجة حرارة 37 درجة مئوية لمدة 4 ساعات في حين انخفضت قدرة الحيوانات المنوية للابل على اختراق مخاط عنق الرحم للنوق معنويا($P<0.05$) وذلك في جميع المخففات المستخدمة.