Effect of Different Diluters on The Cooled Camel Semen Quality

A.E.B. Zeidan[2], A.H. Daader[1], A. El-Darawany[1], Doaa M.A. Sarhan1[1] and A.M.Amer*[2]
1. Anim. Prod. Dept., Fac. Agric., Zagazig Univ., Egypt.
2. Anim. Prod. Res. Inst. (APRI), Agric. Res. Cent. (ARC), Dokki, Giza, Egypt.
* Corresponding author: A.M.Amer. Email: doctoramer69@gmail.com

ABSTRACT

The current study sought to determine how several extenders (Tris-Yolk-Fructose: TYF, Glucose-Yolk-Citrate: GYC, Fructose Yolk-Citrate: FYC, and Lactose-Yolk-Citrate: LYC) affected the enzymatic activity and quality of Maghrebi camel semen while stored at 5ºC for up to three days. During four hours of incubation at 37ºC, sperm penetration into she-camel cervical mucus was seen for various extenders. As compared to GYC and FYC extenders, the percentage of motile camel spermatozoa extended with TYF or LYC extenders was significantly higher (P<0.01), while the percentages of dead, abnormal, acrosome, and chromatin damage spermatozoa showed significantly (P<0.01) decreased when stored at 5°C for up to three days. The proportion of dead, aberrant, acrosome, and chromatin damage in camel spermatozoa extended with various extenders increased significantly (P<0.01) with storage at 5°C for up to three days, whereas the percentage of motile spermatozoa dropped. GYC and FYC extenders significantly (P<0.01) increased the activity of seminal Aspartate aminotransferase (AST) or Alanine aminotransferase (ALT) enzymes released into the extracellular medium of the male camel's semen when compared to T YF and LYC extenders during storage at 5°C for up to three days. The quantity of seminal AST and ALT enzyme activity released into the extracellular media of the male camel spermatozoa extended with various extenders increased significantly (P<0.01) with the increase in storage durations at 5°C for up to 4 days. The penetrating ability of camel spermatozoa was highly significantly with camel semen extended with LTC extender. We may ultimately conclude that TYF and LYC extenders are more suited for diluting camel semen when it is being stored at 5°C for up to three days than GYC and LYC extenders. The extended cooled spermatozoa with LYC extender produced the greatest (P<0.01) value of the camel spermatozoa's penetrating capacity into she-camel spermatozoa.

Keywords: Camel, Semen characteristics, Extenders, Storage, Penetration.

INTRODUCTION

Although there are several issues with camel breeding, such as the extended intervals between breeding seasons, governments are generally attempting to increase the number of camels. By employing contemporary laboratory techniques, assisted reproductive technologies (*ARTs*) can aid in the production of more children (Doaa, 2024). Doaa, 2024 also demonstrated that the reproductive biotechnologies currently employed in she-camels include nuclear transfer (cloning), embryo cryopreservation, intracytoplasmic sperm injection (ICSI), artificial insemination for *in vitro* maturation (*IVM*), *in vitro* fertilization (*IVF*), and dynamic follicular synchronization.

Breeding activity in the male dromedary camels in nomadic herds starts at five to six years of age and continues until 14 to 15 years, with some minor differences according to breed and geographical location (El-Wishy, 1988). Although reproductive management of females can influence parameters such as age at first service, conception rate, calving rate and intercalving interval (Khanna, 1990). Genetic materials can be transferred between populations without requiring the transportation of animals thanks to artificial insemination (AI), in vitro fertilization (IVF), embryo transfer (ET) and cryopreservation of gametes (Ferre *et al*. 2020).

According to Matter (2019) and Doaa (2024), dromedary camels have a special ability to adapt to hot, dry climates. Furthermore, Wilson (2005) describes male camels as a seasonal breed, with a noticeable rise in sexual activity during the rutting season.

The quality of semen and its ability to dilute and store are a prerequisite for the success of artificial insemination (AI). Spermatozoa may often be kept for many days at 2 to 5°C to extend their

life. However, even with as little as one day of storage, results are not always obtained (Zeidan and Abbas, 2004).

The present study aimed to investigate the effects of different extenders at the rutting season on Maghrebi camel semen quality and enzymatic activities during storage at 5°C for up to 3 days, the penetrating ability of the diluted camel spermatozoa into she-camel cervical mucus was also assessed.

MATERIALS AND METHODS

1. Experimental Animals and Management

During the rutting season, semen was collected by using of artificial vagina from Five male Maghrebi camels (*Camelus dromedarius*) weighing 500–600 kg of live body weight and aged between 4 and 16 years. Every camel in the herd had a good history of fertility, was in good health, and was clinically free of both internal and external parasites. External genitalia palpation was normal and usual. The size of both testes was about identical, and they could easily move up and down inside the scrotal pouches.

All camels were given full access to clean, fresh water, and they were kept in a yard with a communal feeding trough and a concrete floor with a communal protected water basin. The camels were free to roam throughout the confined space.

2. Semen Collection

Semen was collected directly from Five male camels with buffered citrate solution (0.5 ml NaCl). Semen characteristics was evaluated immediately after collection as the method described by Abd El- Raouf et al. (1975) and Zeidan et al. (2001). The compositions of these extenders are shown in Table (1).

Table (1). The various buffered yolk extenders compositions (Salisbury et al. 1978).

Components	Grams/100ml of distilled water for each extender			
	GYC	LYC	TYF	FYC
Sodium citrate dehydrate	2.90	2.90	-	2.90
Citric acid anhydrate	0.04	0.04	1.675	0.04
Glucose	1.25	-	-	-
Lactose	-	1.25	-	-
Fructose	-	-	1.25	1.25
Tris aminomethane	-	-	3.208	-
Egg Yolk (ml)	10	10	10	10
Penicillin (IU/ml)	500	500	500	500
Streptomycin (μ/ml)	500	500	500	500

GYC: Glucose- Yolk- citrate. LYC: Lactose- Yolk- citrate. TYF: Tris- Yolk- Fructose. FYC: Fructose- Yolk- citrate. Tris (Hydroxymethyl) aminomelthane, Aldrish Chemical Co. Ltd., Gillingham, Dorset- England.

3.Sperm-Cell Concentration ($\times 10^6$/ml)

The spermatozoa were counted using haemocytometer similar to that recorded by Matter (2019) and Doaa(2024).

4. Semen Extension

Following the procedure outlined by Matter (2019) and Doaa(2024), semen was extracted, combined, and assessed for each camel. It was then prolonged using several extenders (TYF, GYC, FYC, and LYC). The process of semen extension involved gradually introducing the right amount of semen to the extender. In order to prevent temperature changes, the stretched semen (in tube) was

always maintained below the water's surface in a water bath. 1 ml semen to 4 ml extender was the ultimate extension rate.

5. Chilling of Semen at 5ºC:

The test tubes containing extended semen were placed in a 500ml beaker containing water at 30ºC with a thermometer to check the water temperature during the cooling period. Another test tubes containing extended semen only was placed in the beaker to maintain the extended temperature similar to that of the semen (all the test tubes were covered with dark plastic sheaths). The beaker was placed in a refrigerator and gradually cooled till their temperature reached 5ºC over 1.5-2.0 hours Bravo et al. (2000) and Mosaferi et al.(2005).

6. Semen Characteristics

6.1. Sperm motility (%)

According to (Bravo et al. 2000), camel sperm motility was typically observed as an oscillatory motion similar to the flagellum.

6.2. Dead spermatozoa (%)

In accordance with Hackett and Macpherson's (1965) approach, 1.67 gram of Eosin and 10 grams of Nigrosin were dissolved in 100 milliliters of distilled water to perform the eosin/nigrosin staining process.

6.3. Abnormal spermatozoa(%)

In the same smears made for the live/dead spermatozoa ratio, the morphological abnormality of spermatozoa was calculated (Hackett and Macpherson, 1965).

6.4. Acrosome damage(%)

According to Watson (1975), the percentages of acrosome damage were evaluated.

6.5. Chromatin damage(%)

Toluidine blue staining was carried out using the Erenpreiss et al. (2004) procedure.

7. Enzymatic Activity

According to Huang *et al*. (2006), colorimetric analysis was used to evaluate the activity of the Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST) enzymes using the QCA Kit, Amposta, Spain. Samples of extended semen were centrifuged for 15 minutes at 1000 *g*. After being collected, the supernatant fluid (diluted seminal plasma) was stored at -20°C until biochemical examination. According to Huang *et al*. (2006), the sperm-cell concentration ($U/10^6$ spermatozoa) was used to modify all of the ALT and AST enzymes' enzymatic activity in the extracellular medium.

8. Sperm Penetration

The following criteria were used to evaluate sperm penetration into she-camel cervical mucus: Ten she-camels were used to collect cervical mucus throughout the rutting season. A portion of the mucus was drawn into 2 mm internal diameter polyethylene-sealed tubes. According to Matter (2019) and Doaa (2024), semen was extracted and expanded using a GYC, TYC,LYC and FYC extenders .After that, the tubes were put into 2 ml cuvettes, one ml for each. After being placed open-ended into the cuvettes holding the expanded semen, the tubes containing the mucus were incubated for up to four hours at 37ºC. The rank score was used to evaluate sperm penetration, as explained by Hanson et al. (1982) and Eskin et al. (1973).

9. Statistical Analysis

Last squares analysis of variance, as defined by Snedecore and Cochorn (1982), was used to statistically evaluate the data using SAS's General Linear Model (GLA) Procedure (SAS, 2006). The Multiple Range Test by Duncan. (Duncan, 1955) was employed to identify noteworthy variations in means. Prior to statistical analysis, percentage data were converted to arc-sin values. The chi-square test was used to examine the penetration score.

RESULTS AND DISCUSSION

1. Sperm Motility (%)

The motile camel spermatozoa (%) was greatest ($P<0.01$) in the diluted semen that contained LYC or TYF extenders. However, GYC extender had the lowest ($P<0.001$) score (Table 2). This behavior might be explained by the fact that lactose mediates available energy and osmotic equilibrium to the extender, or it could be because lactose protects spermatozoa from osmotic stress better than other sugars. Abd El-Saalam *et al*. (2011) and El-Badry *et al*. (2015) noted similar patterns in dromedary camels. Furthermore, after incubation at 37ºC for up to 6 hours, Zeidan (2002) and Doaa (2024) discovered that the proportion of motile camel spermatozoa was significantly ($P<0.05$) higher with the prolonged semen containing LYC or TYF extenders. However, Matter (2019) found no significant variation when using different extenders (Tris – Lactose, Tris-Sucrose, Tris- Lactose, Skim- milk and sucrose-Yolk) for prefreezing dromedary camel semen. The same subsequent author also noted that LYC extender significantly ($P<0.05$) increased the cryosurvival of camel spermatozoa in the prolonged semen compared to other extenders. As previously mentioned, it is anticipated that the shift from spermatozoa of better to poorer quality would occur gradually over time. These outcomes might be the consequence of lactose components combined positive effects, which could alter the ratios of spermatozoa's motility and freezability (Colas, 1979).

Table (2). Using various extenders, the Maghrebi camel spermatozoa's sperm motility (%) was measured and stored at 5ºC for up to three days (Means±SE).

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	70.53±0.81	66.12±0.52	71.62±0.61	72.68±0.92	70.23±0.42^{a}
1	65.81±0.54	43.11±0.31	58.25±0.30	65.91±0.71	58.27±0.22^{b}
2	38.92±0.46	15.74±0.25	29.56±0.18	37.25±0.26	30.36±0.18^{c}
3	16.37±0.23	04.62±0.17	10.27±0.12	18.96±0.23	12.55±0.14^{d}
Mean	47.90±0.17^{A}	32.39±0.89^{C}	42.42±0.58^{B}	48.70±0.81^{A}	42.85

- A – C Values with different superscripts within a row, significantly differ ($P<0.05$).
- a – d Values with different superscripts within a column, significantly differ ($P<0.05$).

TYF : Tris-yolk-fructose, GYC : Glucose- yolk- citrate, FYC : Fructose- yolk- citrate and LYC : Lactose- yolk- citrate

The proportion of motile camel spermatozoa with various extenders (TYF, GYC, FYC, and LYC) was significantly ($P<0.001$) reduced by extending storage at 5ºC for up to three days (Table 2). In the case of dromedary camels, their findings concur with those of Zeidan (2002) and Abd El-Salaam et al. (2011). As storage time increases, sperm motility may decrease because spermatozoa's metabolic activity is decreased, which in turn causes sperm motility to decrease (Zeidan and Abbas, 2004). The decline in adenosine triphosphate content might possibly be the cause of this behavior. A sharp decline in the fructolysis rate coincided with this (Mann and Lutwak-Mann, 1981 and Salamon, 1987).

2. Dead Spermatozoa (%)

The proportion of dead camel spermatozoa during storage at 5ºC for up to three days was significantly impacted by the various extenders (TYF, GYC, FYC, and LYC) (P<0.01). With the GYC extender, the dead spermatozoa (%) was the highest (P<0.001). However, LYC and TYF extenders had the lowest (P<0.05) value (Table 3). The positive effects of tris and the larger molecular weight of lactose extenders, which provide more spermatozoa protection, may be the cause of these outcomes. These findings are consistent with those of Zeidan et al. (2008), who found that the GYC extender produced the greatest (P<0.05) value of the percentage of dead camel spermatozoa, while the LYC extender produced the lowest (P<0.05) value.

Table (3). Dead Maghrebi camel spermatozoa (%) extended with different extenders, during storage at 5ºC for up to 3 days (Means ± SE).

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	26.70±0.14	33.14±0.16	28.27±0.19	24.15±0.18	28.06±0.10^{d}
1	31.48±0.21	52.65±0.28	41.67±0.30	29.81±0.18	38.90±0.23^{c}
2	48.57±0.25	65.28±0.31	66.72±0.45	54.27±0.25	58.71±0.28^{b}
3	67.16±0.30	86.74±0.42	82.43±0.51	66.94±0.28	75.81±0.35^{a}
Mean	43.47±0.25^{C}	59.45±0.18^{A}	54.77±0.16^{B}	43.79±0.14^{C}	50.37

See more details on Table (2)

Additionally, it was shown that when the storage period was extended to 5ºC for up to 3 days (Table 3) in all extenders, the proportion of dead camel spermatozoa rose significantly (P<0.01). In the dromedary camel, similar trends were noted by Zeidan and Abbas (2004), Abd El-Salaam et al. (2011), Matter (2019), and Doaa (2024). These results might be the result of lactic acid buildup, which damages sperm cells (Zeidan, 1999).

The percentages of aberrant camel spermatozoa (%) during storage at 5ºC for up to three days were significantly impacted by the various extenders (TYF, GYC, FYC, and LYC) (P<0.01). The GYC and FYC extenders produced the highest (P<0.01) value of aberrant camel spermatozoa (%).However, LYC and TYF extenders had the lowest (P<0.05) value (Table 4). These findings are consistent with those of Matter (2019) and Doaa(2024) in the spermatozoa of dromedary camels. LYC extenders produced a smaller proportion of aberrant camel spermatozoa than GYC or FYC extenders when incubated at 37ºC for up to 6 hours, according to Zeidan et al. (2008), and Doaa (2024).

Table (4). Abnormal camel spermatozoa (%) were stored at 5ºC for up to 3 days (Means±SE) using various extenders.

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	12.82±0.18	16.22±0.25	15.43±0.28	12.14±0.20	14.15±0.28^{d}
1	17.84±0.30	23.16±0.48	18.47±0.46	15.60±0.30	18.67±0.36^{c}
2	19.35±0.39	27.85±0.52	20.53±0.57	17.81±0.36	21.38±0.42^{b}
3	22.70±0.41	34.53±0.57	29.02±0.62	18.64±0.37	26.22±0.54^{a}
Mean	18.17±0.35^{C}	25.44±0.47^{A}	20.86±0.41^{B}	16.04±0.28^{C}	20.12

See more details on Table (2)

The proportion of aberrant camel spermatozoa with various extenders (TYF, GYC, FYC, and LYC) increased significantly (P<0.001) as the storage period was extended to 5ºC for up to 3 days (Table 4). These findings are consistent with those of Zeidan (2002), Matter (2019), and Doaa (2024), they discovered that the percentages of aberrant camel spermatozoa at 5ºC for up to 3 days were significantly (P<0.05) greater than those at 0. These outcomes may be because, in comparison to GYC or FYC extenders, tris or lactose components provide spermatozoa with superior protection against osmotic stress.

4. Acrosome Damage (%)

Different extenders (TYF, GYC, FYC and LYC) had a substantial (P<0.01) impact on the proportion of camel spermatozoa with acrosome damage. According to Table 5, the GYC extender had the greatest percentage of spermatozoa acrosome damage, whereas LYC and TYF had the lowest (P<0.001) value. Zeidan et al. (2008) noted similar patterns in the camels. According to Lenz et al. (1977) and Jones and Stewart (1979), around 50% of the spermatozoa had acrosome enlargement when the bull semen was extended and cooled to 5ºC. The acrosomes (disruption of the plasma and outer acrosomal membranes and dispersion of the acrosomal contents) and middle pieces (breakage of the plasma membrane and a reduction in the electron density of the mitochondrial matrix) of a significant percentage of spermatozoa underwent significant ultrastructural changes as a result of subsequent freezing and thawing.

Table (5). The percentage of acrosome damage (Means ± SE) of camel spermatozoa extended with various extenders during storage at 5ºC for up to three days.

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	5.27±0.02	6.14±0.05	5.07±0.06	4.10±0.02	5.14±0.04^{d}
1	6.17±0.08	8.92±0.11	8.74±0.11	7.13±0.09	7.74±0.09^{c}
2	8.25±0.10	12.36±0.14	9.51±0.13	7.84±0.10	9.49±0.11^{b}
3	10.11±0.12	19.27±0.16	12.65±0.14	10.78±0.11	13.20±0.14^{a}
Mean	7.45±0.08^{C}	11.67±0.09^{A}	8.99±0.10^{B}	7.46±0.04^{C}	8.89

See more details on Table (2)

Furthermore, cold shock from storing semen at low temperatures damaged the structure. Damage to the center-piece plasma membrane, the outer acrosome membrane, and the plasma membrane covering the acrosome were among the alterations. A decrease in the percentage of spermatozoa with intact acrosomes and an increase in the release of enzymes into extracellular media accompany these alterations. Thus, the greatest indicator of the quality of the first semen to date is the morphological features of the sperm acrosome and the enzymatic content in the extracellular media (Abdalla et al., 2011). The proportion of acrosome damage of the camel spermatozoa with various extenders (TYF, GYC, FYC, and LYC) was significantly (P<0.001) enhanced by extended storage at 5ºC for three days (Table 5). The substantial rise in acrosome damage that occurs as storage time at 5ºC for three days progresses might be caused by an increase in lactic acid buildup, which alters the media's pH and osmotic pressure and has a harmful effect on sperm cells (Zeidan, 1999). In a similar vein, Zeidan et al. (2008), Maiada (2011), and Doaa (2024) discovered that storing camel spermatozoa at 5ºC for three days significantly (P<0.05) raised the proportion of acrosome damage. Maxwell and Stojanov (1996) in rams, Zeidan et al. (2008) and Doaa (2024) in camel spermatozoa, and El-Gaafary et al. (1993) in hamsters all observed similar trends.

5. Chromatin damage (%):

In spermatozoa extended with TYF and LYC, the percentages of chromatin damage was significantly (P<0.001) lower (Table 6). However, compared to spermatozoa extended with TYF or LYC extenders, the proportion of chromatin damage in camel spermatozoa increased significantly (P<0.001) in those extended with GYC and FYC. In the camel spermatozoa, similar patterns were noted by **Matter (2019)** and **Zeidan *et al*. (2001)**. Numerous factors, including apoptosis, reactive oxygen species, in vitro handling, extender type, defective spermatogenesis, and cryopreservation stress, can cause damage to DNA spermatozoa **(Baiee *et al*., 2017). Lioyd *et al*. (2012)** verified that sperm DNA integrity was improved in commercial diluent, and that semen stored at 5ºC for up to 48 hours might result in a substantial (P<0.01) increase of DNA fragmentation. **Khalifa *et al*. (2013)** showed similar patterns in ram spermatozoa and **Doaa (2024)** in the camel. **Pradana *et al*. (2016)** discovered, however, that when dog spermatozoa were stored at 5ºC, the chromatin integrity of the sperm was not significantly affected by the kind of extender used.

Table (6). The chromatin damage (%) of Maghrebi camel spermatozoa that were stored at 5ºC for up to three days using various extenders (Means±SE).

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	3.11±0.01	5.14±0.02	4.82±0.03	2.78±0.01	3.96±0.04^{d}
1	4.18±0.08	7.11±0.08	6.18±0.08	4.06±0.05	5.38±0.07^{c}
2	5.12±0.08	9.13±0.10	7.53±0.09	5.10±0.06	6.72±0.09^{a}
3	6.74±0.0.09	15.68±0.12	10.17±0.11	6.37±0.08	9.74±0.12^{b}
Mean	4.78±0.03 ^{C}	9.26±0.09 ^{A}	7.17±0.07 ^{B}	4.75±0.05 ^{C}	6.45

See more details on Table (2)

The percentage of camel spermatozoa with chromatin damage in various extenders increased significantly (P<0.001) when storage was prolonged at 5ºC for up to three days (Table 6). These findings concur with Ahmadi (2020) findings regarding camel spermatozoa.

6. Enzymatic Activity

6.1. AST and ALT enzymes activity (U/10^6 Spermatozoa):

Different extenders had a substantial (P<0.001) impact on the male camel spermatozoa's aspartate and alanine-aminotransferase enzyme activity. With GYC and FYC extenders, the AST and ALT enzymes showed the greatest (P<0.01) activity. However, when stored at 5ºC for up to 3 days, the LYC and TYF extenders showed the lowest (P<0.05) activity (Table 7 and 8). The protective mechanism of the advantageous lactose extender components to the sperm cell membrane against any alterations in the plasma membrane may be the cause of these findings, which in turn reduces the quantity of enzymes from the intracellular to extracellular media. Zeidan et al. (2008), Matter (2019), and Doaa (2024) all noted a similar pattern in dromedary camels.

The seminal AST and ALT enzyme activity of the camels with various extenders (TYF, GYC, FYC, and LYC) increased significantly (P<0.001) after storage at 5ºC for up to three days (Tables 7 and 8). The disruption of the cellular sperm barrier may be the cause of the ongoing rise in intracellular AST and ALT enzymatic leakage throughout storage time (Graham and Pace, 1967 and Zeidan et al., 1999). In the male camel spermatozoa, our findings concur with those of Zeidan et al. (2008), Maiada (2011), El-Mahdy (2019), Matter (2019), and Doaa (2024). It seems that increased permeability of the sperm membrane and internal enzyme leaks may be linked to sperm damage during storage.

Table (7). The activity of the enzyme aspartate-aminotransferase (U/10^6) in Maghrebi camel spermatozoa that were stored at 5ºC for up to three days throughout the rutting season using various extenders (means ±SE).

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	20.17±0.18	31.74±0.38	27.18±0.23	20.11±0.17	24.80±0.19^{d}
1	36.19±0.27	52.90±0.64	47.15±0.54	36.52±0.29	43.19±0.51^{c}
2	50.46±0.61	65.17±0.71	54.63±0.63	47.16±0.56	54.35±0.64^{b}
3	63.18±0.74	76.81±0.86	71.46±0.81	60.54±0.74	67.99±0.81^{a}
Mean	42.50±0.29^{C}	56.65±0.72^{A}	50.10±0.57^{B}	41.08±0.26^{C}	47.58

See more details on Table (2)

Table (8). The Alanine-aminotransferase enzyme (U/10^6) activity of camel spermatozoa extended with various extenders during a period of up to three days at 5ºC (Means ± SE).

Storage time (day)	Extenders				Overall mean
	TYF	GYC	FYC	LYC	
0	38.61±0.52	57.83±0.67	44.60±0.62	38.11±0.57	44.78±0.57^{d}
1	61.54±0.72	78.36±0.82	72.41±0.76	60.84±0.69	68.28±0.81^{c}
2	73.18±0.81	81.19±0.92	75.53±0.89	72.46±0.80	75.59±0.86^{b}
3	86.19±0.92	93.18±1.03	90.76±1.01	83.54±0.84	88.41±1.05^{a}
Mean	64.88±0.65^{C}	77.64±0.84^{A}	70.82±0.74^{B}	63.73±0.73^{C}	69.26

See more details on Table (2)

7. Camel Sperm Penetration into Cervical Mucus

The highest (P<0.01) value of the penetrating ability of the camel spermatozoa into she-camel spermatozoa (figure 1) was recorded with the extended spermatozoa with LYC extender. Aitken et al. (1983) found a close correlation between human movement of spermatozoa and their penetrating ability into cervical mucus. Alexander (1981) and Murase et al. (1990) reported that, the duration of sperm motility and penetration distance in the mucus closely correlated to the pregnancy and conception rate. In the dromedary camels, similar results were noted by Zeidan et al. (2008a), Matter (2019), Ahmadi (2020a), and Doaa (2024). The capacity of camel spermatozoa to penetrate she-camel cervical mucus was significantly (P<0.01) reduced by increasing the incubation duration at 37ºC for up to 4 hours with all extenders.

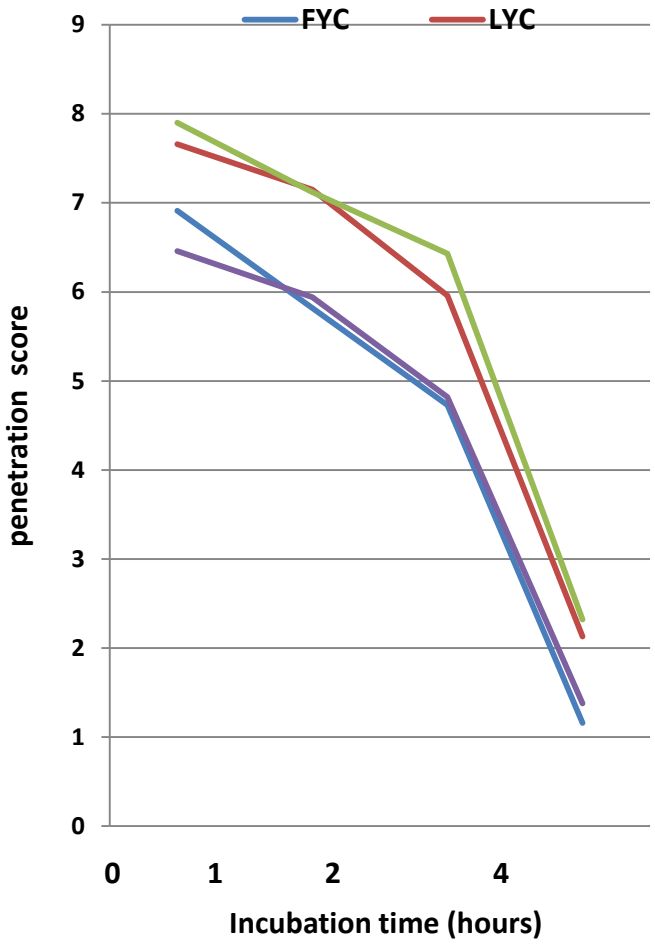

Fig. (1). Penetrating ability of the camel spermatozoa extended with various extenders into she-camel cervical mucus during incubation at 37ºC for up to 4 hours.

CONCLUSION

The goal of the current study was to ascertain how the enzymatic activity and quality of Maghrebi camel semen, which was held at 5ºC for up to three days, were impacted by a number of extenders, including lactose-yolk-citrate (LYC), fructose-yolk-citrate (FYC), glucose-yolk-citrate (GYC), and tris-yolk-fructose (TYF). For different extenders, sperm penetration into she-camel cervical mucus was seen after four hours of incubation at 37ºC.

REFERENCES

Abdalla, E.B.; Zeidan, A.E.B.; Abd El-Salaam, A.M. and Maiada W.A. Allam (2011). Thermoregulation and blood biochemical changes in male dromedary camels during hot-humid and hot dry environments under Egyptian conditions. J. Camel Pract. Res. 18(2): 297-304.

Abd El-Raouf, M.F.; Fateh El-Bab, M. R. and Owaida, M. M. (1975). Studies on reproduction in the camel *(Camelus dromedarius).* V. Morphology of the testis in relation to age and season. J. Reprod. Fert., 43: 109-116.

Abd El-Salaam, A.M.; Absy, G.M.; Gabr, Sh. A. and Zeidan, A.E.B. (2011). Viability, enzymatic activity and penetrating ability of spermatozoa into she-camel cervical mucous as affected by different extenders. J. Camel Pract. and Res. 18 1-9.

Aitken, R.J.; Best, F.; Richardson, D.W.; Schats, R. and Simm, G. (1983). Influence of caffeine on movement characteristics, fertility capacity and ability to penetrate cervical mucus of human spermatozoa. J. Reprod. Fertil., 67: 19-27.

Ahmadi, E.A.A. (2020). Effects of breeds of the male dromedary camel on semen characteristics, sperm survivability and histological changes of the testes. J. EgyptianVet. Med. Asso., 80 (2): 215-236 .

Alexander, N.J. (1981). Evaluation of male infertility with an in-vitro cervical mucus penetration test. Fertil. Steril., 36: 201-208.

Baiee, F.H.; Wahid, H.; Rosnina, Y.; Ariff, O.M.; Yimer, N.; Salman, H.; Tarig, A.A. and Khumran, A.M. (2017). Hypo-osmotic swelling test modification to enhance cell membrane integrity evaluation in cryopreserved bull semen. Pakistan J. Tropical Agri. Sci., 40 (2): 257 268.

Bravo, P.W.; Skidmore, J.A. and Zhao, X.X. (2000). Reproductive aspects and storage of semen in camelidae. Anim. Reprod. Sci., 62: 173-193.

Colas, G. (1979). Fertility in the ewe after artificial insemination with fresh and frozen semen at the induced oestrus and influence of the photoperiod on the semen quality of the ram. Livest . Prod. Sci., 6: 153-166.

Doaa. M.A.Sarhan (2024). Reproductive and physiological changes of dromedary camels during the breeding and non- breeding seasons. Ph.D. Thesis, Fac. Agric., Zagazig Univ., Egypt.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11: 1-42.

El-Badry, D.A.; Scholkamy, T.H.; Abeer, M. Anwer, and Karima, Gh. Mahmoud, M. (2015). Assessment of freezability and functional integrity of dromedary camel spermatozoa harvested from caput, corpus and cauda epididymis. Alexandria J. Vet. Sci., 44: 147- 158.

El-Gaafary, M.N.; Rashwan, A.A. and Zenat, A.I. (1993). Investigations on the deep freezing of rabbit semen in straws. Egyptian-American Conf. Physiol. Anim. Prod., El-Fayoum, Egypt, pp 17-25.

El-Wishy,A.B. (1988). Reproduction in the male dromedary (*Camelus dromedaries*). Anim. Reprod. Sci., 17: 217-241.

El-Mahdy, A.A.M. (2019). Some genetic and non-genetic factors affecting blood parameters and relationship with performance in camels .MSc. Thesis, Fac. Agric., Zagazig Univ., Egypt.

Erenpreiss, J.; Jepson, K.; Giwercman, A., Tsarev, I.; Erenpreisa, J. and Spano, M. (2004). Toluidine blue cytometer test for sperm DNA conformation: Comparison with the flow cytometric sperm chromatin structure and TUNE/ assays. Hum. Reprod., 19: 2277-2282.

Eskin, B.A.; Azarbal, S.; Sepic, P. and Slate, W.G. (1973).*In vitro* response of the spermatozoa cervical mucus system treated with prostaglandin ($F_{2\alpha}$). J. Obst. Gynecology, 41: 436-439.

Ferre,L.B.,Kjelland,M.E.,Taiyeb,A.M.,Campos-Chillon,F.and Ross,P.J.(2020). Recent progress in bovine in vitro-derived embryo cryotolerance: Impact of in vitro culture systems, advances in cryopreservation and future consideration. Domest. Anim., 55(6): 659-676.

Graham, E.F. and Pace, M.M. (1967). Some biochemical changes in spermatozoa due to freezing. Cryobiology, 4: 75-81.

Hackett, A.J. and Macpherson, J.W. (1965). Some staining procedures for spermatozoa. Canadian Vet. J., 6: 55-62.

Hanson, E.W.; Overstreet, I.W. and Katz, D.F. (1982). Astudy of the relationship of motile sperm numbers in cervical mucus 48 hours after artificial insemination with subsequent fertility. Amer. J. Obst. Gynecol., 143: 85-90.

Huang, X.J.; Im, K.; Yarimaga, H.S.; Yoon, E.O. and Kim,

H.S. (2006). Aspartate-aminotransaminase (ALT/GPT) detection Techniques. Anal. Biochem., 6:756-782.

Jones, R.C. and Stewart, D.L. (1979). The effects of cooling to 5°C and freezing and thawing on the ultrastructure of bull spermatozoa. J. Reprod. Fertil., 56: 233-238.

Khalifa, T.; Lymberopoulos, A. and Theodosiadou, E. (2013) Association of soybean-based extenders with field fertility of stored ram. (*Ovisaries*) semen:a randomized double-blind parallel group design. Theriogenology, 79: 517-527.

Khanna, A.A. (1990). Sexual behaviour of the male camel *(Camelus dromedarius)* and studies on semen. MVSc. Thesis, Bikaner Univ., Udaipur, India.

Lenz, R.W.; Graves, C.N. and Lodge, J.R. (1977). Influence of incubation in seminal plasma on subsequent metabolic and morphological characteristics of bovine spermatozoa. Theriogenology, 7: 265-275

Lioyd, R.E.; Fazeli, A.; Watson, P.F. and Holt, W.V. (2012). The oviductal protein, heat-shock 70-kDa protein 8, improves the longterm survival of ram spermatozoa during storage at 17ºC in a commercial extender, Reprod.Ferti. Deve., 24:543-549.

.Maiada, W. A. Allam (2011). Reproductive and physiological performance of male dromedary camels during the rutting and non- rutting seasons. MSc. Thesis, Department of Anim. Prod., Fac. Agric., Ain Shams Univ., Egypt.

Mann, T. and Lutwak-Mann, C. (1981). Male Reproductive Function and Semen. Springer-Verlag. Berlin, Heidelberg, New York, USA, pp. 264-268.

Matter, M.A S. (2019). Reproductive and physiological studies on the male dromedary camels during the rutting and nonrutting seasons under Egyptian condition. M.Sc. Thesis, Fac. Agric., Cairo, Al-Azhar University, Egypt.

Maxwell, W.M.C. and Stojanov, T. (1996). Liquid storage of ram semen in the absence or presence of some antioxidants. Reprod. Fertil. Dev., 8: 1013-1020.

Mosaferi,S.;Niasari-Naslaji,A.; Abarghani, A.; Gharahdaghi, A.A.and Gerami, A. (2005). Biophysical and biochemical characteristics of bactrian camel semen collected by artificial vagina. Theriogenology, 63: 92-101.6.

Murase, T.; Okuda, K. and Sato, K. (1990). Assessment of bull fertility using a mucus penetration test and a human chorionic gonadotropin stimulation test. Theriogenology, 34: 801-812.

Plasson, S.J. (1975). *Veterinary Obstetrics and Gynecology*. William and Wilson Company, 4th (Ed.), New York, USA, pp. 415-486.

Pradana, M.Y.W.; Bebas, W. and Puja, K. (2016). The effect of soyabean extender on viability and DNA integrity of Kintamani dog sperm on cold storage . Vet. Sci. Medi. J., 4 (1): 11-16.

Salamon, S. (1987). Artificial Insemintien of Sheep and Goats.LTD Butterworths and Co. (Publishers) Ltd. London and Edinburgh.

Salisbury, G.W.; Van Demark, N.L. and Lodge, J.R. (1978). *Physiology of Reproduction and Artificial Insemination of Cattle*. WH Freeman and Company, San Francisco, USA.

SAS (2006). SAS Users Guide: Statistics Analysis System . Inst. Inc. Cary, NC, USA.

Snedecore,G.W.and Cohran, W.G. (1982). Statistical Methods.6th ed. Iowa stateUniversity Press. Ames. TOWA, U.S.A.

Watson, P.F. (1975). Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Rec., 97: 12-15.

Wilson, R.T. (2005). *The Camel*, 1st (Ed.), Longman Publishing, London and New York, USA,PP.

Zeidan, A.E.B. (1999). Effect of age on some reproductive traits of the male one- humped camels (*Camelus dromedarius*). Zagazig Vet. J., 27: 126-133.

Zeidan, A.E.B. and Abbas, H.E. (2004). Physiological and biochemical changes in the male dromedary camels during the rutting and non- rutting seasons. Zagazig Vet. J., 32: 37-48

Zeidan, A.E.B. (2002). Semen quality, enzymatic activities and pentrating ability of spermatozoa into she-camel cervical mucus as affected by caffeine addition. J. Camel Pract. Res., 9: 153-161.

Zeidan, A.E.B.; Habeeb, E.A.M.; Ahmadi, E.A.; Amer, H.A. and Abd El-Razik,M.A.(2001).Testicular and physiological changes of the male dromedary camels in relation to different ages and seasons of the year. Proc. 2nd Intern. Conf. Anim. Prod. Health in Semi-Arid Areas, El- Arish North Sinai, Egypt, pp. 147-160.

Zeidan, A.E.B.; Abou-Aiana, R.M.; Ahmadi, E.A.A.; Abd El- Aziz, N. A.; Sayah, M.S.; Ammar, A.H. and Abd El- Salaam, A. M. (2008). Viability and enzymatic leakage of the cooled camel spermatozoa in relation to different extenders. American-Eurasian J. Agric. Environ., Sci., 4:142-149.

Zhang,B.R.;Buhr,M.;Kroetsch,T.andLeibo,S.P.(2001). Glycine betaine improves survival of fresh bovine spermatozoa. Reprod. Fertil. Dev., 13: 187-192.

تاثير المخففات المختلفة على جودة السائل المنوى المبرد للجمال

علاء السيد بلاسى زيدان[2] . احمد حسن دعادر[1] .عبد الحليم الدروانى[1]. دعاء سرحان[1] . احمد موسى عامر[2]

[1] قسم الانتاج الحيوانى – كلية الزراعة – جامعة الزقازيق- مصر
[2]معهد بحوث الانتاج الحيوانى – مركز البحوث الزراعية – الجيزة - مصر

الملخص العربى

تهدف الدراسة لمعرفة تاثير مخففات الترس TYF والجلوكوز GYC والفركتوز FYC واللاكتوز LYC على خصائص السائل المنوى للجمال اثناء الحفظ بالتبريدعلى درجة 5 درجة مئوية لمدة ثلاثة ايام كما تم اختبار مقدرة الحيوانات المنوية للجمال على النفاذ داخل مخاط عنق الرحم وذلك اثناء التحضين على درجة 37 درجة مئوية لمدة اربع ساعات ولقد اظهرت النتائج ما يلى

وجود زيادة معنوية فى النسبة المئوية لحيوية الحيوانات المنوية اثناء الحفظ فى مخففى الترس واللاكتوز عنها فى مخففى الجلوكوز والفركتوز بينما وجدت زيادة معنوية فى النسبة المئوية للحيوانات المنوية الميتة والغير طبيعية ومتحطمة الاكروسوم والكروماتين فى مخففى الجلوكوز والفركتوز عنها فى مخففى الترس واللاكتوز وبزيادة وقت الحفظ بالتبريدعلى درجة 5 درجة مئوية لمدة ثلاثة ايام فلقد نقصت النسبة المئوية لحيوية الحيوانات المنوية فى حين زادت النسبة المئوية للحيوانات المنوية الميتة والغير طبيعية ومتحطمة الاكروسوم والكروماتين فى جميع المخففات كما اظهرت النتائج زيادة لنشاط انزيمى ال AST وال ALT فى مخففى الجلوكوز والفركتوز عنها فى مخففى الترس واللاكتوز بينما زاد هذا النشاط فى جميع المخففات بزيادة وقت الحفظ بالتبريدعلى درجة 5 درجة مئوية لمدة ثلاثة ايام كما لوحظ اختراق الحيوانات المنوية لمخاط عنق الرحم بدرجة اكبر لممددات ال LYC وال TYF عنها فى ممددات ال GYC وال وذلك خلال أربع ساعات من التحضين على درجة حرارة 37 درجة مئوية.

وفى النهاية يمكن ان نوصى باستخدام مخففى الترس واللاكتوز لانهما الافضل عن مخففى الجلوكوز والفركتوز وذلك عند تخفيف السائل المنوى للجمال وحفظه بالتبريد فى درجة حرارة 5 مئوية.

الكلمات الدالة: الابل، الخصوبة، الترس، اللاكتوز