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EFFECT OF EXTENDERS ON MOTILITY AND VIABILITY OF CHILLED-STORED CAMEL SPERMATOZOA (*Camelus dromedarius*)

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ABSTRACT

The present study was designed to compare between Tris-fructose yolk and Green buffer-yolk extenders on preserving dromedary camel spermatozoa at 5°C. Forty eight ejaculates were collected from seven mature dromedary camels using a modified bull artificial vagina with a neoprene liner and a camel collecting glass. Two aliquots from each ejaculate were extended 1:1 in the Tris-fructose egg yolk and Green buffer-yolk extenders. The percentage of progressively motile spermatozoa was determined after dilution at 35°C, after cooling to 5°C and after 24, 48 and 72 hours in both extenders and the viability indices were computed. Smears from the extended semen stained with Spermac® and vital stain were examined for abnormal acrosome %, live sperm % and percentages of sperm abnormalities. The Green buffer-yolk extender was superior to Tris – fructose yolk in the mean values of motility at 0 and 24 hours ($P<0.01$) of preservation at 5°C. No motile spermatozoa were observed in Tris-fructose yolk extender at 48 hours of storage at 5°C. Green buffer-yolk was more beneficial ($P<0.05$) to camel spermatozoa than the Tris-fructose yolk extender in the percentage of abnormal acrosome and the percentage of live sperm at 24 and 48 hours of chilled storage of semen. There was a highly significant ($P<0.01$) difference in the rate of increase of abnormal acrosome between the Tris – fructose yolk (193.00 %) and Green buffer-yolk (79.01%) extenders.

Key words: Acrosome, dromedary, extender, motility, semen

Artificial insemination has been the most powerful tool for livestock improvement ever available to the breeder; however, this technique has not been developed as a routine method for breeding camelids compared with its fast and universal application in other farm animals (Zhao, 2000). Difficulties associated with artificial insemination in dromedary camels are poor post ejaculation sperm motility and lack of standard techniques for freezing semen (Skidmore, 2004). Different extenders have been designed and used in cattle (Vishwanath and Shannon, 2000), sheep (Paulenz *et al,* 2002) and bactrian camels (Chen *et al,* 1990) to protect and maintain spermatozoa during processing and storage (Wani *et al,* 2008). All new extenders or preservation methods for semen need to be tested before practical application in the field (Amann, 1989). The most widely used extender for dromedary camel semen is green buffer (GB, IMV Technologies) as it has been specifically designed for camel semen (Morton *et al,* 2010).

The present study is designed to compare the efficacy of two extenders, Tris-fructose yolk and Green buffer- yolk, on preserving dromedary camel spermatozoa at 5°C.

Materials and Methods

Experimental animals

The study was conducted at the Camel Research Center belonging to King Faisal University. Seven adult male dromedary camels aged 6 - 15 years and weighing 450 – 800 kg were used. The animals were in a healthy condition with sound history of fertility in the herd. Camels were maintained under standard conditions of feeding and management. They had no contact with the females during the period of the experiment (February to March).

Extenders

Two extenders were used in this study. The first was Tris-fructose egg yolk that prepared as described by Zeidan *et al* (2008). The second was Green buffer® (I.M.V., L'Aigle France), prepared as manufactory instructions. The extenders were maintained in water-bath at 35°C prior to collection of semen.

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Table 1. Effect of extenders on viability of the chilled stored camel semen (mean ± SEM).

Extenders	Sperm motility after dilution at 35°C	Sperm motility during incubation at 5°C				Viability indices
		0 hour	24 hours	48 hours	72 hours	
Tris – fructose yolk	61.43 ± 5.95	19.29[a] ± 8.05	2.86[a] ± 1.84	0.00 ± 0.00	0.00 ± 0.00	4.11[a] ± 1.40
Green buffer® yolk	62.14 ± 3.43	53.57[b] ± 3.22	38.57[b] ± 8.78	25.00 ± 7.48	11.43 ± 6.34	26.27[b] ± 5.40

Means with dissimilar superscripts in the same column are significantly different at P<0.01

Semen collection

Semen was collected as previously described by Skidmore and Billah (2006) using a modified bull artificial vagina with a neoprene liner (IMV Technologies) and a camel collecting glass (IMV Technologies). Collecting glasses containing semen were stored at a 35°C water-bath until evaluation, dilution and processing (which occurred within 10 min of collection).

Semen preservation and evaluation

A total of 42 semen ejaculates from the seven male camels (6 from each) were used in this experiment. Immediately after collection, the ejaculates were evaluated for volume, motility and sperm concentration. Thereafter, two aliquots from each ejaculate were extended 1:1 in the Tris-fructose egg yolk and Green buffer® egg yolk extenders. The percentage of progressively motile spermatozoa was determined after dilution at 35°C, after cooling to 5°C and after 24, 48 and 72 hours in both extenders using automated sperm analyser (Sperm Vision® 3.5 Minitube of America, Inc). The viability indices (Milovanov *et al*, 1964) were computed from the following equation:

$$VI = \Sigma [M \times (T-R/2)]$$

where; VI is the viability index, Σ is a sign for the sum total, M is the percentage of sperm motility, T is the time of next determination of motility and R is the time of previous determination of motility.

Smears from the extended semen stained with Spermac® and vital stain were examined (1000 x) for abnormal acrosome %, live sperm % and percentages of sperm abnormalities. Smears were examined at each time the progressive sperm motilities were determined.

Statistical analysis

Analysis of data was performed by *t*-test and analysis of variance (ANOVA) using a commercial software (Statistica for windows, 1993).

Results

Table 1 shows the effect of extenders on the viability of the chilled stored camel semen. There was a highly significant (P<0.01) difference between the viability of spermatozoa stored in the Tris – fructose yolk (4.11 ± 1.40) and Green buffer-yolk (26.27 ± 5.40). The Green buffer-yolk was observed to be superior to Tris – fructose yolk in the mean values of motility at 0 hour (P<0.01) and 24 hours (P<0.01) of preservation at 5°C. No motile spermatozoa were observed in Tris – fructose yolk at 48 hours of storage at 5°C. A significant (P<0.05) difference in the percentage of live sperm existed between the row semen (52.57 ± 4.67) and spermatozoa extended in the Green buffer-yolk (66.29 ± 1.97; Table 2). The effect of extenders on sperm morphology of the chilled camel semen at 24 hours is declared in Table 3. Green buffer-yolk is shown to be more beneficial (P<0.05) to camel spermatozoa than the Tris – fructose yolk extender in the percentage of abnormal acrosome (13.86 ± 2.67 Vs. 16.14 ± 3.46, respectively) and the percentage of live sperm (57.43 ± 3.77 Vs. 41.86 ± 5.78, respectively; Table 3). Similarly, during storage of the camel semen at 48 hours, a significant (P<0.05) difference between the Tris – fructose yolk and Green buffer-yolk extenders was found in the percentage of abnormal acrosome (26.75 ± 4.87 Vs. 19.00 ± 3.81, respectively)

Table 2. Effect of extenders on sperm morphology of the chilled camel semen at 0 hour (mean ± SEM).

Extenders	Spermac® stain	Vital stain	
	% Abnormal acrosome	% Abnormal tails	% Live sperm
Row semen	13.71 ± 3.08	16.86 ± 3.22	52.57[a] ± 4.67
Tris – fructose yolk	12.00 ± 2.19	22.67 ± 8.42	62.17 ± 5.02
Green buffer® yolk	12.29 ± 2.23	20.00 ± 5.72	66.29[b] ± 1.97

Means with dissimilar superscripts in the same column are significantly different at P<0.05

Table 3. Effect of extenders on sperm morphology of the chilled camel semen at 24 hours (mean ± SEM).

Extenders	Spermac® stain	Vital stain	
	% Abnormal acrosome	% Abnormal tails	% Live sperm
Tris – fructose yolk	16.14[a] ± 3.46	17.43 ± 3.99	41.86[a] ± 5.78
Green buffer® yolk	13.86[b] ± 2.67	16.71 ± 4.10	57.43[b] ± 3.77

Means with dissimilar superscripts in the same column are significantly different at P<0.05

Table 4. Effect of extenders on sperm morphology of the chilled camel semen at 48 and 72 hours (mean ± SEM).

Time of incubation	Extenders	Spermac® stain	Vital stain	
		% Abnormal acrosome	% Abnormal tails	% Live sperm
48 hours	Tris – fructose yolk	26.75[a] ± 4.87	10.25 ± 3.57	39.75[a] ± 7.06
	Green buffer® yolk	19.00[b] ± 3.81	16.00 ± 5.93	48.60[b] ± 5.61
72 hours	Tris – fructose yolk	35.16 ± 3.37	11.42 ± 3.64	32.83 ± 3.46
	Green buffer® yolk	22.00 ± 6.43	7.67 ± 2.19	35.33 ± 6.96

Means with dissimilar superscripts in the same column and time are significantly different at P<0.05

and the percentage of live sperm (39.75 ± 7.06 Vs. 48.60 ± 5.61, respectively; Table 4).

Table 5 shows the rate of increase of abnormal acrosome during storage of camel semen at 5°C. There was a highly significant (P<0.01) difference between the Tris – fructose yolk and Green buffer® yolk extenders in the rate of increase of abnormal acrosome (193.00 % Vs. 79.01%, respectively; Table 5). However, there was no significant difference between the Tris – fructose yolk and Green buffer-yolk extenders in the rate of decrease of percent live sperm (47.19% Vs. 46.70%) during storage of camel semen at 5°C (Table 6).

Discussion

A comparison done between two extenders to test the sperm viability in form of % motility, membrane integrity and acrosomal status of dromedary spermatozoa. The viability of camel sperm was significantly superior when stored in the Green buffer-yolk to in the Tris-fructose yolk extenders. Similar results were reported in dromedary camels (Skidmore, 2004; Skidmore, 2005). Moreover, the best results of pregnancy rate (72.7%) were achieved when camel semen diluted in Green buffer-yolk (Morton *et al,* 2010) than the rate (40%) resulted when Tris-fructose yolk extender used (Deen *et al,* 2003).

Table 5. The rate of increase of abnormal acrosome during Storage of camel semen at 5°C.

Extenders	0 hour at 5°C	After 72 hours at 5°C	Rate of increase (%)
Tris – fructose yolk	12.00 ± 2.19	35.16 ± 3.37	193.00[a]
Green buffer® yolk	12.29 ± 2.23	22.00 ± 6.43	79.01[b]

Means with dissimilar superscripts in the same column are significantly different at P<0.01

Table 6. The rate of decrease of percent live sperm during Storage of camel semen at 5°C.

Extenders	0 hour at 5°C	After 72 hours at 5°C	Rate of increase (%)
Tris – fructose yolk	62.17 ± 5.02	32.83 ± 3.46	47.19
Green buffer® yolk	66.29 ± 1.97	35.33 ± 6.96	46.70

Means with dissimilar superscripts in the same column are significantly different at P<0.01

In the present study, there was no sperm motility noticed in the Tris-fructose yolk extender at 48 hours of storage at 5°C. This result was identical to a previous study (Wani *et al,* 2008). The inferiority of the Tris-fructose yolk extender may be attributed to the absence of lactose in this extender (Wani *et al,* 2008).

In the current study, the significant difference in the % live sperm between the row semen and the Green buffer-yolk extender may be explained by the beneficial effect of Green buffer-yolk in maintaining the membrane integrity of spermatozoa.

As depicted from the present work, Green buffer-yolk is more useful to chilled camel spermatozoa after 24 hours in % abnormal acrosome and % live sperm when compared to Tris-fructose yolk extender. The same pattern was described by Wani *et al* (2008) on using Tris-based extenders for storage dromedary spermatozoa at 4°C. The decrease in acrosome intact spermatozoa stored in the Tris-fructose yolk extender might be explained by the damage caused to acrosomal membrane due to cold shock (De-Leeuw *et al,* 1990). However, still 68 – 78% of spermatozoa were with intact acrosomes after storage for 48 hours at 5°C. The acrosome integrity in liquid semen is stable, also in sheep (Paulenz *et al,* 2002) and pigs (Zou and Yang, 2000).

In both extenders used in the current study, the proportion of viable spermatozoa was higher than that of motile spermatozoa. This is suggested as some viable spermatozoa were immotile in dromedary (Wani *et al,* 2008) and boar (Zou and Yang, 2000). The possible reasons are that the membranes associated with the tail and mitochondria – axonemal system, responsible for motility, are more susceptible to damage during storage than the plasma membrane around the head (Wani *et al,* 2008). Differences in the plasma membrane between different regions of spermatozoa have been well documented (Ladha, 1998) and differences in the damage caused, due to cold shock between these

different regions, have been reported in bulls and boars spermatozoa (De-Leeuw *et al,* 1990).

In the present study, the difference in the rate of decrease of % live sperm of chilled camel semen was not significant between Green buffer-yolk and Tris-fructose yolk extenders. On the same bases, Vyas *et al* (1998) found similar results with both Tris – based and lactose extenders.

Green buffer-yolk is superior to Tris-fructose yolk extender during storage of dromedary semen at 5°C for 72 hours.

References

Amann RP (1989). Can fertility potential of a seminal sample be predicted accurately? Journal of Andrology 10:89-98.

Chen BX, Zhao XX and Huang YM (1990). Freezing semen and AI in the bactrian camel (*Camelus bactrianus*). In Proc. Of the workshop "Is it possible to improve the reproductive performance of the camel"? Paris, Sept 10-12. pp 13.

Deen A, Sumant V and Sahani MS (2003). Semen collection, cryopreservation and artificial insemination in the dromedary camel. Animal Reproduction Science 15(77):223-233.

De-Leeuw FE, Chen HC, Colenbrander B and Verkleij AJ (1990). Cold induced ultrastructural changes in bull and boar sperm plasma membranes. Cryobiology 27:171-183.

Ladha S (1998). Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. Journal of Membrane Biology 165:1-10.

Milovanov VK, Trubkin GD, ChubenkoNS, Tsvetkov IV, Erzin ZK and Meschankin AB (1964). Artificial insemination of livestock in the U.S.S.R. Israel Program for Scientific Translations, Jerusalem. pp 102-104.

Morton KM, Billah M and Skidmore JA (2010). Effect of green buffer storage on the fertility of fresh camel semen after artificial insemination. Reproduction in Domestic Animals. Short Communication, Blackwell Verlag GmbH.

Paulenz H, Soderquist L, Perez-Pe R and Berg KA (2002). Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. Theriogenology 57:823-836.

Skidmore JA (2004). Artificial Insemination. In: Lecture notes for the short course in reproduction in the dromedary camel. L. Skidmore (Ed.). Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Skidmore JA (2005). Reproduction in dromedary camels: an update. Animal Reproduction 2(3):161-171.

Skidmore JA and Billah M (2006). Comparison of pregnancy rates in dromedary camels (*Camelus dromedarius*) after deep intra-uterine versus cervical insemination. Theriogenology 66:292-296.

Statistica for Windows (1993). Release 4.5 A, Statsoft, Inc.

Vishwanath R and Shannon P (2000). Storage of bovine semen in liquid and frozen state. Animal Reproduction Science 62:23-53.

Vyas S, Goswami P, Rai AK and Khanna ND (1998). Use of Tris and lactose extenders in preservation of camel semen at refrigerated temperature. Indian Veterinary Journal 75:810-812.

Wani NA, Billah M. and Skidmore JA (2008). Studies on liquefaction and storage of ejaculated dromedary camel (*Camelus dromedarius*) semen. Animal Reproduction Science 109(1-4):309-318.

Zeidan AEB, Abou-Aiana RM, Ahamadi EAA, Abd El-Aziz NA, Sayah MS, Ammar AH and Abd El-Salaam AM (2008). Viability and enzymatic leakage of cooled camel spermatozoa in relation to different extenders. American Eurasian Journal of Agriculture and Environmental Science 4(2):142-149.

Zhao XX (2000). Semen characteristics and artificial insemination in the bactrian camel. In: Recent advances in camelid reproduction. J.A. Skidmore and G.P. Adams (Eds.). Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

Zou CX and Yang ZM (2000). Evaluation on sperm quality of freshly ejaculated boar semen during *in vitro* storage under different temperatures. Theriogenology 53:1477-1488.