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Research Article

Effect of OPTIXcell and Triladyl Extender on Post Thaw Quality of Cryopreserved Dromedary Camel Spermatozoa

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Abstract

This study compared the effect of OPTIXcell and Triladyl extenders on post thaw quality of cryopreserved dromedary camel spermatozoa. Semen collection by artificial vagina was carried out at weekly intervals from three dromedary bulls, using an estrous female as a mount animal. Ejaculates (n = 23) were divided into two portions and diluted 1:1 in OPTIXcell (IMV Technologies, France) and Triladyl plus 20% egg yolk (Minitube, Germany) extenders and incubated in a water bath (37°C) for liquefaction. Immediately after complete liquefaction, semen samples were further diluted to 100 × 10^6 spermatozoa/mL with the same diluent as used earlier. Diluted semen samples were incubated at 4°C for 3h in a cold handling cabinet (IMV, France) for equilibration.

Equilibrated semen samples were filled in 0.5mL French straws and frozen at 5°C/min from +4 to -12°C and at 50°C/min from -12 to -140°C using programmable freezer (Mini DigitCool, IMV, France), and then straws were plunged in LN 2 for storage. Sperm motion characteristics (CASA, CEROS, Version12, Hamilton Thorne Biosciences, USA), viability and acrosome integrity (FITC-PNA/PI; Kershaw-Young., *et al*, Anim Reprod Sci, 138:3-4, 2013) were assessed in semen samples before equilibration (fresh semen) and after thawing. Statistical analysis was performed using mixed model regression in GENSTAT (version 17, VSN Int.). The average semen volume and sperm concentration was 4.3 ± 0.22 mL and 465.6 ± 19.9 x 10^6 spermatozoa/mL, respectively. There was no difference between extenders in total motility (TM), progressive velocity (VSL), track speed (VCL), lateral head amplitude (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN) and percentage of viable-intact acrosome spermatozoa between extenders in fresh semen, but Triladyl recorded higher progressive motility (PM) and path velocity (VAP) than OPTIXcell (25.6 ± 0.4% vs 23.8 ± 0.3% and 120.7 ± 1.8 µm/s vs 110.9 ± 2.8 µm/s, respectively; P and lt; 0.05). Triladyl showed higher TM, PM, VAP and percentage of viable-intact acrosome spermatozoa than OPTIXcell in post thawed semen (63.0 ± 0.9% vs 55.6 ± 1.5%, 13.6 ± 0.3% vs 11.0 ± 0.3%, 73.8 ± 1.5 µm/s vs 64.5 ± 0.80 µm/s, and 44.4 ± 4.4% vs 38.5 ± 0.6%, respectively; P and lt; 0.001). In conclusion, the frozen-thawed sperm quality of semen diluted with Triladyl was superior to OPTIXcell in dromedary camels.

Keywords: *Dromedary Camel; Spermatozoa; Programmable Freezer; OPTIXcell; Triladyl*

Introduction

Artificial insemination (AI) with fresh, chilled or frozen semen is not a standard, routine procedure in camels as it is in other animal species. Freezing of dromedary camel spermatozoa has been challenging due to a variety of difficulties including complications associated

with handling camel semen, because of the gelatinous nature of the semen. There is an urgent need to develop an extender and standardize a reliable protocol of cryopreservation not only to preserve the genetics of elite racing and show camels but also to propagate the traits over a vast population.

Many chemically defined and egg-yolk based extenders have used to provide camelid sperm the tolerance of freezing and thawing procedures, however poor post-thaw semen quality was reported [1-3].

Semen cryopreservation protocols are yet to be established in dromedary camels to the same extent as they are in the major domestic and farm animal species and no universally accepted protocol has resulted so far [4]. Most cryopreservation attempts in camel semen so far have been carried out over static liquid nitrogen (LN_2) vapour [2,3,5,6] or pellet freezing [7] for periods ranging from 10 to 15 minutes, followed by plunging into LN_2 (-196°C) for storage. There is a dearth in literature regarding using controlled rate of freezing by a programmable freezing device in dromedary camels, hence the present study was carried out to evaluate the effect of fast freezing protocol (50°C/min) in two commercial semen extenders OPTIXcell and Triladyl on the post thaw quality of camel spermatozoa.

Materials and Methods

Breeding male dromedary camels (n = 3), aged 9 to 12 years, maintained in individual paddocks and fed with green lucern, oats, dates, and mineral supplementation were used in this study. Semen was collected at weekly intervals using a bovine artificial vagina (30 cm length and 5 cm internal diameter) during the peak breeding season (December to February) using an estrous female as a mount animal.

Ejaculate volume and colour were recorded immediately after collection and transferred to a 35°C water bath. Gross activity (oscillatory activity of spermatozoa, Scale 1 - 4) was examined by placing a drop of neat semen on a pre-warmed slide and examined under a phase contrast microscopy (magnification: x100, Olympus BX20, Tokyo, Japan) [8]. Ejaculates(n = 23) were divided into two equal portions and diluted 1:1 in pre-warmed OPTIXcell (IMV Technologies, France) and Triladyl(Minitube, Germany) plus 20% egg yolk extender kept in a water bath (35°C) for liquefaction. Immediately after complete liquefaction, sperm concentration was recorded using a Makler Counting Chamber (Sefi-Medical Instruments, Israel). Semen samples were further diluted to a final concentration of 100 × 10^6 spermatozoa/mL with the same diluent as used earlier. Motion characteristics of spermatozoa of fresh semen was evaluated using CASA (CEROS, Version 12, Hamilton Thorne biosciences, USA) pre-adjusted for camel sperm analysis [8]. Three microliters of semen (50 × 10^6 spermatozoa/ml) were placed in a 20 µm standard count analysis chamber (Leja, Nieuw-Vennep, The Netherlands). Five randomly selected microscopic fields were scanned five times each and approximately 500 spermatozoa counted. The total motility (TM), progressive motility (PM), average path velocity (VAP), average progressive velocity (VSL), track speed (VCL), lateral head amplitude (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) of spermatozoa were analyzed. Diluted semen samples were kept in a water jacket filled with 200 mL of 35°C water and cooled to 4°C over 90 minutes, equilibrated at 4°C for 3h in a cold handling cabinet (IMV, France), filled and sealed (MPP Uno, Minitube, Germany) in 0.5mL French straws. Semen straws were transferred into a programmable freezer (Mini Digitcool, IMV, France) at 4°C and frozen initially at a rate of 5°C/min from + 4 to -12°C and then at 50°C/min from -12 to -140°C (6 minutes), straws were then plunged in LN_2 (-196°C) for storage. After 24 hours two straws from each group were thawed over 60 seconds at 37°C (Cito Thaw, Minitube, Germany) and evaluated for post thaw motility. Sperm viability and acrosome integrity (FITC-PNA/PI; Kershaw-Young., *et al.* [9] was assessed in semen samples (fresh and post thawed). Statistical analysis was performed using a mixed model regression in GENSTAT (version 17, VSN Int.) and all data presented as mean ± SEM.

Results

The average semen volume and sperm concentration was 4.3 ± 0.22 mL (range 3 to 6.5 mL) and 465.6 ± 19.9 x 10^6 spermatozoa/mL (range 350 to 671 x 10^6 spermatozoa/mL), respectively. The colour of the ejaculates was observed to range from white to creamy white. It was observed that complete liquefaction of semen occurred within 30 minutes in both extenders (OPTIX cell and Triladyl). The mean values observed in the present study of sperm motion characteristics, viability and acrosome integrity in fresh and post thawed samples

for OPTIXcell and Triladyl are presented in table 1. In fresh semen, Triladyl recorded a significant ($P < 0.05$) higher PM and VAP than in OPTIXcell (25.5 ± 0.3 *vs* 23.8 ± 0.3 and 120.6 ± 1.8 *vs* 110.8 ± 2.8, respectively); however, no significant difference was observed between extenders in TM, VSL, VCL, ALH, BCF, STR, LIN and percentage of sperm with viable-intact acrosome. In post thawed semen, Triladyl showed a higher TM, PM, VAP and percentage of sperm with viable-intact acrosome than OPTIXcell ($P < 0.001$).

Variables CASA	Fresh		P value	Post thawed		P value
	Triladyl	Optixcell		Triladyl	Optixcell	
Total Motility (T.Mot) %	86.4 ± 0.4	85.0 ± 0.6	0.104	63.1 ± 0.9	55.5 ± 1.5	< 0.001**
Progressive Motility (P.Mot) %	25.5 ± 0.3	23.8 ± 0.3	0.006**	13.5 ± 0.2	11.0 ± 0.3	< 0.001**
Path Velocity (VAP)µm/s	120.6 ± 1.8	110.8 ± 2.8	0.015*	73.8 ± 1.5	64.5 ± 1.8	< 0.001**
Progressive Velocity (VSL)µm/s	71.4 ± 2.5	69.3 ± 2.0	0.284	36.9 ± 0.6	35.4 ± 1.5	0.387
Track Speed (VCL) µm/s	240.3 ± 5.8	245.0 ± 7.6	0.624	138.6 ± 2.4	135.8 ± 4.8	0.645
Lateral Amplitude Head (ALH)µm	11.6 ± 0.2	11.6 ± 0.4	0.962	9.0 ± 0.1	8.6 ± 0.2	0.193
Beat Cross Frequency (BCF) Hz	26.2 ± 0.3	26.4 ± 0.3	0.523	28.9 ± 0.2	29.9 ± 0.4	0.079
Straightness (STR) %	51.4 ± 0.5	52.2 ± 0.5	0.224	49.7 ± 0.4	50.7 ± 0.5	0.142
Linearity (LIN) %	28.5 ± 0.3	28.3 ± 0.4	0.768	27.0 ± 0.2	27.3 ± 0.5	0.592
Viable-Acrosome Intact %	70.0 ± 1.1	67.6 ± 0.9	0.140	44.3 ± 0.9	38.4 ± 0.6	< 0.001**

Table 1: *The mean (± SEM) motion characteristics, viability and acrosome integrity of spermatozoa from ejaculates (n = 23; collected once per week) of dromedary camels.*

Discussion

Cryopreservation of camel semen would be useful in the breeding strategy for this species. However, as stated earlier attempts to cryopreserve camel semen are complicated for a number of reasons and the post thaw survival of the spermatozoa is low. The protocols used for cryopreservation of camel semen are modified protocols from other species; therefore, the aim of this study was to see whether other freezing rates and thawing temperatures would be more optimal for camel spermatozoa.

The present study evaluated the effect of fast freezing protocol (50°C/min) in two commercial semen extenders OPTIXcell and Triladyl on the post thaw quality of male dromedary camel spermatozoa. The results of the present study indicated a significant drop in the motion characteristics, viability and acrosome integrity on the post thawed semen.

The average volume of ejaculates in this study was comparable with that reported in previous studies [2,10,11]. However, sperm concentration in this study was higher when compared to previous studies [12-14]. Semen viscosity is one of the main factors that delays semen processing and the time required for complete liquefaction of semen. In the present study, complete liquefaction of semen occurred within 30 minutes after diluting (1:1) the semen by using a gentle pipetting technique. However, wide variation in liquefaction time reported by several authors ranging from 7 minutes to 8h [12,15-17], which could be due to the manipulation technique of the ejaculate during incubation, extender type, amount of mucin in the ejaculate and general inter-male variation.

The results showed that Triladyl was superior to OPTIXcell in both fresh and frozen-thawed semen, recording the highest percentages of sperm PM and VAP during the fresh form, and the highest percentage of sperm TM, PM, VAP and sperm with viable-intact acrosome. The better sperm-preserving ability of Triladyl over OPTIXcell in the present study could be due to the combined presence of both glycerol and egg yolk. Egg yolk is also thought to have a synergistic effect with other penetrating cryoprotectants like glycerol [18]. The low-density

lipids (LDL) of egg yolk could interact with lipid-binding proteins of seminal plasma [19]. that could induce cholesterol and phospholipid removal from the sperm membrane, resulting in enhancement of sperm viability during storage in liquid or frozen states [20].

Similar to our observations, a study in rams showed that a combination of an egg yolk based media with glycerol significantly decreased the damage to spermatozoa caused by 120h of refrigeration by preserving total motility, plasma and acrosomal integrity [21].

Physiological and functional changes namely, irreversible reduction in sperm motility, viability and acrosome integrity is a sequel of damage to sperm membranes that occur primarily during the freezing and thawing process at temperature between -15 and -60°C and not during storage in liquid nitrogen [22]. The fast freezing protocol, as compared to the conventional one, efficiently preserved the viability and fertilizing capacity of spermatozoa, indicating a new method to improve the fertility of frozen equine semen [23]. Recently, Elwing [24] compared four different freezing protocols (slow to rapid rates) and showed that male dromedary camel sperm have a high tolerance for freezing rates and better values for sperm characteristics were obtained from the fastest freezing rate compared to a slower rate for total and progressive motility and some kinematic parameters. Similarly, many studies in different species showed that fast freezing protocol obtained a better sperm motility [25,26]. The post thaw total motility during the present study was higher than those reported by earlier authors [3,5,27] and could be attributed to the higher motility of fresh semen along with the fast rate of controlled freezing employed. Up-to-date there is contradiction in the results of studies on AI with frozen thawed semen in camel. Zhao., *et al*. [28] claimed that AI with frozen thawed semen in Bactrian camel resulted in 95% pregnancy rate, later a study in dromedary camel reported only 7.1% pregnancy rate [2]. Similarly, the disappointing results of AI with frozen-thawed semen is also a problem in South American Camelids, Vaughan., *et al*. [29] failed to obtain any pregnancy by using different cryodiluents (Triladyl, Green/clear camel buffer and Biladyl A/B). However, a recent study in dromedary camel obtained about 62% pregnancy rate excluding 28.5% of early embryonic death and 9.5% abortion cases at mid gestation period.

Conclusion

The study indicated that egg-yolk based extender (Triladyl) is more suitable than the lipo-protein based extender (OPTIXcell) when used with fast rate freezing protocol for cryopreservation in dromedary camel. Triladyl maintained the semen quality during the cryopreservation process and can be used for *in vivo* trials. While these results are encouraging for the camel industry, the *in vivo* fertility of frozen thawed camel spermatozoa extended in Triladyl must be assessed before its use is recommended in commercial artificial insemination programs.

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