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Preserving the Genetics of Elite Camels: A Comparative Study of Semen Cryopreservation Using Triladyl and OptiXcell

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@Based on research conducted at the Laboratories and Animal Research Centre, Royal Court Affairs, Sultanate of Oman.

Abstract:

The dromedary camel (*Camelus dromedarius*) occupies a unique position in the cultural, economic, and genetic heritage of many arid regions of the world. In Oman and across the Arabian Peninsula, camels are highly valued for racing, beauty competitions, and preservation of elite bloodlines. Modern reproductive technologies offer opportunities to conserve and disseminate superior genetics; however, successful cryopreservation of camel semen remains one of the major challenges in camel reproductive biotechnology.

The present study evaluated two commercially available semen extenders, Triladyl and OptiXcell, in combination with a controlled-rate fast freezing protocol for cryopreservation of dromedary camel spermatozoa. Semen was collected from breeding males during the peak breeding season and assessed for sperm motility, kinematic parameters, viability, and acrosome integrity before and after freezing.

Both extenders facilitated semen liquefaction and processing. However, Triladyl consistently produced superior post-thaw results, recording significantly higher total motility, progressive motility, average path velocity, and viable-acrosome intact sperm percentages. These findings suggest that the egg-yolk-based Triladyl extender provides enhanced protection against cryogenic damage during freezing and thawing.

The study demonstrates the potential of controlled-rate freezing and appropriate extender selection for improving camel semen preservation and supports further fertility trials aimed at developing practical artificial insemination programs for the camel industry.

Introduction:

The dromedary camel has served humanity for centuries as a source of transport, food,

companionship, and cultural pride. Today, the camel industry extends beyond traditional uses and encompasses racing, beauty competitions, selective breeding, and genetic conservation programs.

In the Sultanate of Oman, camels represent an important component of cultural heritage. Considerable efforts have been directed toward preserving elite racing and show camel bloodlines through scientific breeding programs. The success of such programs increasingly depends upon the application of reproductive biotechnologies.

Among these technologies, semen cryopreservation offers enormous potential. It allows long-term storage of valuable genetics, facilitates artificial insemination, reduces the need for transporting breeding males, and contributes to the establishment of genetic resource banks.

Despite its importance, cryopreservation of camel semen remains challenging. The viscous nature of camel semen, delayed liquefaction, and sensitivity of spermatozoa to freezing injury have limited the development of reliable protocols. While several extenders and freezing techniques have been evaluated, no universally accepted cryopreservation protocol currently exists for dromedary camels.

The present study was undertaken to compare the effectiveness of two commercial semen extenders, Triladyl and OptiXcell, when used with a controlled-rate fast freezing protocol and to evaluate their effects on post-thaw sperm quality.

Materials and Methods:

Experimental Animals:

Three healthy breeding dromedary camels aged between nine and twelve years were used during the peak breeding season from December to February. Animals were maintained in individual paddocks and fed green lucerne, oats, dates, and mineral supplements.

Semen Collection:

Semen was collected weekly using a bovine artificial vagina with an oestrous female serving as a mount animal. Twenty-three ejaculates were obtained during the study period.

Figure 1. Semen collection from a breeding dromedary camel using an artificial vagina with a female mount animal. Semen obtained through this procedure was subsequently processed and evaluated for cryopreservation studies.

Immediately after collection, semen volume, colour, and gross motility were recorded. Samples were maintained at 37°C until processing.

Semen Processing:

Each ejaculate was divided equally into two portions and diluted (1:1) with pre-warmed:

- Triladyl supplemented with 20% egg yolk
- OptiXcell

Following complete liquefaction, sperm concentration was measured and samples were further diluted to a final concentration of 100×10^6 spermatozoa/mL.

Evaluation of Sperm Quality:

Computer Assisted Sperm Analysis (CASA) was used to assess:

- Total Motility (TM)
- Progressive Motility (PM)
- Average Path Velocity (VAP)
- Straight-Line Velocity (VSL)
- Curvilinear Velocity (VCL)
- Amplitude of Lateral Head Displacement (ALH)
- Beat Cross Frequency (BCF)
- Straightness (STR)
- Linearity (LIN)

Sperm viability and acrosome integrity were evaluated using FITC-PNA/PI staining.

Cryopreservation Protocol:

- Samples were cooled gradually from 35°C to 4°C over 90 minutes and equilibrated at 4°C for three hours.
- Semen was packaged in 0.5 mL French straws and frozen using a programmable freezer according to the following protocol:
  - ✓ +4°C to –12°C at 5°C/minute
  - ✓ –12°C to –140°C at 50°C/minute
- Straws were then plunged into liquid nitrogen (-196°C) for storage.
- After 24 hours, samples were thawed at 37°C for 60 seconds and evaluated.

Statistical Analysis:

Data were analyzed using mixed-model regression procedures and expressed as mean ± SEM.

Results:

The average ejaculate volume was 4.3 ± 0.22 mL, while the average sperm concentration was 465.6 ± 19.9 × 10^6 spermatozoa/mL.

All ejaculates ranged in colour from white to creamy white. Complete liquefaction occurred within 30 minutes in both extenders, allowing efficient processing. This relatively rapid liquefaction facilitated semen processing and minimized delays prior to cryopreservation.

In fresh semen, Triladyl produced significantly higher progressive motility and average path

velocity compared with OptiXcell.

Table 1. Comparison of Fresh Semen Quality in Dromedary Camels

Parameter	Triladyl	OptiXcell	P Value
Total Motility (%)	86.4 ± 0.4	85.0 ± 0.6	0.104
Progressive Motility (%)	25.5 ± 0.3	23.8 ± 0.3	0.006*
Average Path Velocity (µm/s)	120.6 ± 1.8	110.8 ± 2.8	0.015*
Viable–Acrosome Intact Sperm (%)	70.0 ± 1.1	67.6 ± 0.9	0.140

*Significant difference (P < 0.05)

Following cryopreservation, sperm quality declined in both groups, reflecting the physiological stress associated with freezing and thawing. Nevertheless, Triladyl maintained significantly superior post-thaw sperm quality.

Table 2. Comparison of Post-Thaw Semen Quality in Dromedary Camels

Parameter	Triladyl	OptiXcell	P Value
Total Motility (%)	63.1 ± 0.9	55.5 ± 1.5	<0.001***
Progressive Motility (%)	13.5 ± 0.2	11.0 ± 0.3	<0.001***
Average Path Velocity (µm/s)	73.8 ± 1.5	64.5 ± 1.8	<0.001***
Viable–Acrosome Intact Sperm (%)	44.3 ± 0.9	38.4 ± 0.6	<0.001***

***Highly significant difference (P < 0.001)

Key Finding:

Under a controlled-rate fast freezing protocol, Triladyl demonstrated superior cryoprotective performance compared with OptiXcell, resulting in 13.7% higher total motility, 22.7% higher progressive motility, 14.4% higher average path velocity, and 15.4% higher viable-acrosome intact sperm after thawing.

A summary comparison of fresh and post-thaw semen quality is presented in Tables 1 and 2. While both extenders supported acceptable fresh semen characteristics, Triladyl demonstrated superior preservation of sperm function following cryopreservation, maintaining significantly higher motility and viability parameters after thawing.

Discussion:

The successful cryopreservation of camel semen remains a critical requirement for the advancement of assisted reproductive technologies in camel breeding programs.

The present study demonstrated that extender composition significantly influences post-thaw sperm survival. Triladyl consistently outperformed OptiXcell in several key sperm quality parameters, particularly total motility, progressive motility, average path velocity, and acrosome integrity.

The superior performance of Triladyl is likely related to the combined protective effects of glycerol

and egg yolk. Egg yolk contains low-density lipoproteins that stabilize sperm membranes and reduce damage caused by cold shock and ice crystal formation during freezing.

The controlled-rate fast freezing protocol employed in this study also appeared beneficial. Similar observations have been reported in camels, horses, sheep, and other domestic species, where rapid freezing rates reduced cryoinjury and improved post-thaw sperm quality.

Although freezing inevitably reduced motility and viability compared with fresh semen, the post-thaw values achieved in this study compare favourably with many earlier reports in dromedary camels. These findings support continued exploration of controlled-rate freezing systems as part of future camel breeding programs.

Practical Significance for the Camel Industry:

Reliable semen cryopreservation has far-reaching implications for the modern camel industry.

Figure 2. Breeding dromedary camel maintained in a genetic improvement and conservation program in the Sultanate of Oman.

Potential applications include:

- Preservation of elite racing camel genetics
- Conservation of rare and valuable bloodlines
- Establishment of national genetic resource banks
- Facilitation of artificial insemination programs
- Reduced transportation of breeding males
- Enhanced genetic improvement through selective breeding

These advantages are particularly important for countries where camels represent both cultural heritage and economic value.

Future Directions:

While the findings are encouraging, additional studies are needed before routine commercial application can be recommended.

Future research should focus on:

- Field fertility trials using frozen-thawed semen
- Optimization of thawing protocols
- Evaluation of novel cryoprotectants and antioxidants
- Development of camel-specific semen extenders
- Assessment of pregnancy rates under practical breeding conditions

Such investigations will contribute to the establishment of standardized cryopreservation protocols for dromedary camels.

Relevance to India:

India is home to one of the world's most significant dromedary camel populations, particularly in the arid and semi-arid regions of Rajasthan and Gujarat. Indigenous breeds such as the Bikaneri, Jaisalmeri, Kachchhi, and Mewari camels have played an important role in transportation, agriculture, milk production, and the livelihoods of pastoral communities for generations.

In recent decades, declining camel populations and changing production systems have highlighted the need for conservation and genetic improvement strategies. Reproductive biotechnologies, including semen cryopreservation and artificial insemination, offer valuable tools for preserving superior germplasm, supporting breed conservation programs, and facilitating the dissemination of desirable genetic traits.

The findings of the present study have relevance beyond the Arabian Peninsula and may contribute to future efforts aimed at strengthening camel breeding and conservation programs in India. As scientific interest in camel production continues to grow, the development of reliable semen preservation protocols will be an important component of safeguarding the genetic resources of this unique species for future generations.

Conclusion:

This study demonstrated that the egg-yolk-based extender Triladyl is superior to the lipoprotein-based extender OptiXcell when used with a controlled-rate fast freezing protocol for cryopreservation of dromedary camel semen.

Triladyl maintained significantly higher post-thaw total motility, progressive motility, average path velocity, and viable-acrosome intact sperm percentages. These findings support its use in future fertility trials and provide valuable information for the development of practical artificial insemination programs in dromedary camels.

As interest in camel breeding, conservation, and genetic improvement continues to grow worldwide, including in countries such as India where indigenous camel breeds represent valuable genetic resources, advances in semen cryopreservation will play an increasingly important role in preserving elite

bloodlines and supporting sustainable breeding programs.

On International Camel Day, such advances underscore the important role of reproductive biotechnology in conserving valuable camel genetics for future generations.

About the Author:

Dr. Narayan Pratap is a veterinarian and specialist in animal reproduction with extensive experience in reproductive biotechnology, semen cryopreservation, artificial insemination, and genetic conservation. During his professional career, he served in veterinary research, teaching, and reproductive biotechnology programs in India and later in the Sultanate of Oman, where he headed the Division of Reproduction at the Laboratories and Animal Research Centre, Directorate General of Veterinary Services, Royal Court Affairs. His work focused on advancing reproductive technologies in camels and stallions, contributing to breeding programs and the preservation of valuable genetic resources.

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