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Comparative Evaluation of Quail Egg Yolk-Based Extender for Semen Cryopreservation in Baggara Bulls

Mohamed A. El-Khalifa[1], Bashir Salim[2]*, Safaa Abdelrahim[1], Zeinab A.E. Osman[1], Ahmed M. Khalid[3,4], Saeed Alasmari[5] and Shadia A. Omer[6]

[1]*Department of Animal Genetic Improvement, Animal Production Research Center, Khartoum North, Sudan*
[2]*Camel Research Center, King Faisal University, P.O. Box 400, Al-Hofuf 31982, Al-hsa, Saudi Arabia*
[3]*Department of Public Health, College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Hofuf 31982, Al-Ahsa, Saudi Arabia*
[4]*Department of Genetics and Animal Breeding, Faculty of Animal Production, University of Khartoum, Shambat 13314, Sudan*
[5]*Department of Biology, Faculty of Arts and Sciences, Najran University, Najran 1988, Kingdom of Saudi Arabia*
[6]*Department of Biomedical Sciences, College of Veterinary Medicine, Sudan University of Science and Technology*

ABSTRACT

This study evaluated the efficacy of a quail egg yolk-based extender for the cryopreservation of Baggara bull semen, compared to hen egg yolk and a commercial egg yolk-free diluent (IMV). Baggara cattle, a hardy breed native to Sudan and known for their adaptability to harsh environments, are vital to local economies, making advancements in their reproductive biotechnology crucial. Sperm quality was assessed both pre- and post-freezing, focusing on motility, vigor, and morphology. Pre-freezing analysis showed that the quail egg yolk extender achieved slightly higher mean motility (82.07%) compared to the hen egg yolk (81.61%) and IMV (79.84%) extenders. Post-freezing, the quail extender maintained competitive motility (59.79%), similar to the hen extender (61.29%) and higher than the IMV extender (56.17%). Although the quail extender exhibited a slightly higher motility degradation rate, ANOVA results revealed no statistically significant differences in pre-freezing motility, post-freezing motility, or motility degradation among the extenders. These findings suggest that the quail egg yolk-based extender is a promising alternative to the traditional hen egg yolk extender for cryopreservation of Baggara bull semen. However, further optimization is needed, particularly to improve post-thaw sperm morphology, to fully harness its potential in preserving this important cattle breed.

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Key words
Cryopreservation, Quail egg yolk-based extender, Baggara bulls, Post-mortem semen preservation, Cattle

INTRODUCTION

Cryopreservation of bovine semen plays a pivotal role in cattle breeding, enabling the long-term storage and global transportation of valuable genetic material. Traditionally, semen collection is performed using live animals through methods such as electroejaculation or artificial vagina. However, post-mortem semen collection offers an alternative for preserving valuable genetics from animals that die unexpectedly or are euthanized. This approach is particularly relevant for breeds like Baggara cattle, where maintaining genetic diversity is critical.

Baggara cattle, indigenous to Sudan, are renowned for their exceptional adaptability to harsh environmental conditions, making the conservation of their genetics crucial for maintaining cattle biodiversity (Salim *et al*., 2014; Kambal *et al*., 2022; Tijjani *et al*., 2022). As the primary beef-producing breed in Sudan, Baggara cattle are uniquely capable of thriving in extreme desert climates. Their resilience and strong resistance to diseases make them particularly well-suited to the challenging local conditions (Salim *et al*., 2021). Given their importance, efficient cryopreservation techniques are essential for

* Corresponding author: bashirsalim@gmail.com
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preserving the genetic material of Baggara bulls, especially in resource-limited settings. This study addresses the need for improved semen preservation methods by evaluating the effectiveness of various extenders for cryopreservation in this vital cattle breed.

Traditionally, egg yolk-based extenders, particularly those derived from hen eggs, have been extensively used for cryopreserving bovine semen due to their protective effects against cold shock and oxidative stress during freezing (Vishwanath and Shannon, 2000). However, alternative egg yolk sources, such as quail egg yolk, have gained attention due to their distinct lipid composition and potential cryoprotective properties (Trimeche *et al*., 1979). Quail egg yolk, with its higher phospholipid and cholesterol content, is thought to offer enhanced membrane stability and protection against cryodamage, making it a promising alternative for semen preservation.

Despite advancements in cryopreservation, limited research has explored the efficacy of quail egg yolk-based extenders in cattle semen preservation. This study aims to fill this gap by evaluating the performance of quail egg yolk-based extenders in preserving Baggara bull semen. The study compares the quail extender with traditional hen egg yolk and a commercial egg yolk-free diluent, hypothesizing that the quail egg yolk-based extender may provide comparable or even superior protection during the freezing and thawing process.

This research offers valuable insights into the conservation of genetic material in cattle, especially when conventional semen collection methods are not feasible. The findings have significant implications for the preservation of Baggara bulls and may serve as a model for improving semen cryopreservation techniques in other cattle breeds facing similar challenges.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Department of Animal Genetic Improvement, Animal Production Research Center (APRC) located in Hilat Koko, Sudan. The primary objective of the study was to evaluate the viability and cryopreservation potential of epididymal sperm collected post-mortem from Baggara bulls. The experiment was carried out under controlled laboratory conditions to ensure consistent handling and storage of the samples.

Testis collection

The testes were collected from mature Baggara bulls immediately after slaughter. The collection was performed at the Animal Production Research Center's slaughterhouse to minimize the time between death and sperm recovery as described by Garcia-Macias *et al*. (2006). A total of five pairs of testes were carefully extracted from the carcasses and placed in sterile zip-lock bags. These bags were maintained at a temperature of 25°C and immediately transported to the semen processing laboratory, where epididymal sperm recovery was initiated.

Quality assessment of the collected sperm

Sperm quality was assessed immediately after recovery to determine the initial viability of the samples. Evaluation was performed using a phase-contrast microscopeat 400× magnification. The assessment included key focusing on conventional parameters such as motility, vigor, viability, and morphology. Motility was evaluated by placing a drop of raw semen on a clean, pre-warmed slide (37°C), covered with a coverslip, and examining under phase-contrast microscopy. The percentage of spermatozoa exhibiting progressive forward movement was estimated and recorded rated on a scale from 0% to 100%. While vigor was assessed visually on a 0 to 5 scale, where 0 indicated no progressive movement and 5 indicated vigorous and rapid, linear progression of sperm cells across the field of view. Additionally, sperm viability and morphology assessments were evaluated using staining technique. A small aliquot of semen was mixed with an equal volume of eosin-nigrosin stain, smeared onto a glass slide, air-dried, and examined under the microscope. At least 200 sperm cells per slide were evaluated. Live sperm remained unstained (white), while dead sperm appeared pink due to eosin penetration. Morphological abnormalities such as bent tails, detached heads, and midpiece defects were recorded following standard classification criteria (Barth and Oko, 1989; Sprecher and Coe, 1996).

Semen dilution and cryopreservation

After the initial sperm quality assessment, the recovered spermatozoa were divided into three experimental groups, each corresponding to a different semen extender. The first group was diluted with a Tris-citric acid-based diluent containing hen egg yolk, which is traditionally used in cattle semen cryopreservation (Medeiros *et al*., 2002; Grötter *et al*. 2019). The second group was diluted with a novel Tris-citric acid-based diluent containing quail egg yolk, while the third group was processed using a commercial egg yolk-free extender as a control (IVM Technologies, France).

The semen samples were diluted at a 1:4 ratio and allowed to equilibrate for 4 h at 5°C. Following equilibration, the diluted semen samples were packaged into 0.25 ml straws and frozen in liquid nitrogen vapor for 10 minutes before being plunged into liquid nitrogen for one month before post freezing evaluation.

Statistical analysis

The sperm motility, viability, and morphology data collected post-thaw were analyzed using one-way ANOVA to compare the performance of the different extenders. Differences between the three extenders were considered significant at a p-value of <0.05, and post hoc analysis was conducted using Duncan's Multiple Range test (DMRT) to identify specific group differences. All statistical analyses were performed using SPSS software (version 25.0).

RESULTS

Pre-freezing motile sperm comparison

The pre-freezing motility of Baggara bull sperm was evaluated using three extenders: Quail egg yolk, hen egg yolk, and a commercial egg yolk-free diluent (IMV). The quail egg yolk extender demonstrated a slightly higher mean motility of 82.07±7.64% compared to 81.61±7.67% for the hen extender and 79.84±9.43% for the IMV extender. The overall mean motility across all extenders was 81.20±8.29%. Motility values across extenders ranged from 50.0% to 95.0%, with the quail extender showing a slight advantage in preserving motility before freezing compared to the other extenders (Table I).

Post-freezing motile sperm comparison

After cryopreservation, sperm motility was again assessed across the three extenders. The quail egg yolk extender maintained a mean post-freezing motility of 59.79±16.16%, slightly lower than the hen egg yolk extender at 61.29±14.00%, but higher than the IMV extender, which resulted in the lowest motility at 56.17±13.33%. The overall post-freezing motility across all extenders was 59.08±14.68%. The quail and hen extenders exhibited better post-thaw motility preservation compared to the IMV extender, highlighting the quail egg yolk as a competitive alternative (Table II).

Motility degradation rate

The motility degradation rate, calculated as the difference between pre-freezing and post-freezing motility, was also compared across extenders. The quail egg yolk extender showed a mean degradation rate of 14.86±15.38%, while the hen egg yolk extender had a slightly lower degradation rate of 13.67±13.56%.The IMV extender exhibited the lowest degradation rate at

Table I. Pre-freezing motile sperm comparison of baggara bulls using different extenders.

Extender type	N	Mean (%)	SD	SE	95% Confidence interval for mean	Minimum (%)	Maximum (%)
Pre-freezing motile sperm							
Quail	70	82.07	7.6373	0.9128	80.25 - 83.892	60.0	95.0
Hen	62	81.61	7.6702	0.9741	79.66 - 83.561	50.0	95.0
IVM	64	79.84	9.4268	1.1783	77.49 - 82.198	50.0	95.0
Total	196	81.199	8.2875	0.5920	80.03 - 82.366	50.0	95.0
Post-freezing motile sperm							
Quail	70	59.786	16.161	1.932	55.932 - 63.639	30.0	90.0
Hen	62	61.290	13.995	1.777	57.736 - 64.844	30.0	90.0
IMV	64	56.172	13.326	1.666	52.843 - 59.501	10.0	85.0
Total	196	59.082	14.682	1.049	57.013 - 61.150	10.0	90.0

SD, standard deviation; SE, standard error

Table II. Motility degradation rate of baggara bulls using different extenders.

Extender type	N	Mean (%)	SD	SE	95% Confidence interval for mean	Minimum (%)	Maximum (%)
Quail	70	14.856	15.380	1.838	11.1889 - 18.523	0.00	53.85
Hen	62	13.668	13.564	1.723	10.2237 - 17.113	0.00	50.00
IVM	64	12.613	17.551	2.194	8.2287 - 16.997	0.00	100.00
Total	196	13.748	15.545	1.110	11.5579 - 15.938	0.00	100.00

SD, standard deviation; SE, standard error

Table III. ANOVA results for pre-freezing motile sperm, post-freezing motile sperm, and motility degradation rate.

Parameter	Sum of squares	df	Mean square	F	p-value
Pre-freezing motile sperm				1.325	0.268
Between groups	181.450	2	90.725		
Within groups	13211.790	193	68.455		
Post-freezing motile sperm				2.061	0.130
Between groups	879.025	2	439.512		
Within groups	41155.669	193	213.242		
Motility degradation rate				0.347	0.707
Between groups	168.761		84.381		
Within groups	46951.405		243.272		

12.61±17.55%. Despite the lower degradation rate for IMV, the quail and hen extenders showed more consistent post-thaw motility, suggesting their effectiveness in maintaining sperm quality. Degradation rates were highly variable, with some samples showing no degradation and others as high as 100% (Table II).

Comparison of extenders

ANOVA tests were performed to statistically compare the pre-freezing motility, post-freezing motility, and motility degradation rate among the three extenders (quail egg yolk, hen egg yolk, and IMV). The analysis revealed nostatistically significant differences in pre-freezing motility across the extenders ($F = 1.325$, $p = 0.268$). Similarly, post-freezing motility did not differ significantly between the extenders ($F= 2.061$, $p= 0.130$). Furthermore, the motility degradation rate, which measures the decline in motility from pre-freezing to post-freezing, also showed no significant differences among the extenders ($F= 0.347$, $p= 0.707$). These findings suggest that, although quail egg yolk extender performed comparably to the other extenders, the observed differences in motility preservation and degradation are not statistically significant (Table III).

DISCUSSION

This study evaluated the efficacy of a quail egg yolk-based extender for the cryopreservation of Baggara bull semen, comparing it with traditional hen egg yolk-based and commercial egg yolk-free extenders. The findings revealed that quail egg yolk offers a viable alternative cryoprotectant, demonstrating comparable, and in some aspects, superior performance.

Egg yolk has long been recognized for its cryoprotective properties, protecting sperm from the damage caused by chilling temperatures (Mandal *et al*., 2014). While hen egg yolk has been traditionally used in bovine semen extenders, the results from this study indicate that quail egg yolk yielded favorable outcomes, particularly in terms of sperm motility, viability, and vigor. The quail egg yolk extender outperformed both the hen egg yolk-based and commercial egg yolk-free extenders, suggesting its potential as a superior cryoprotectant.

The enhanced performance of the quail egg yolk-based extender can be attributed to its unique composition, which includes higher concentrations of lipoproteins and phospholipids compared to hen egg yolk (Swelum *et al*., 2023). These elements are essential for stabilizing the sperm membrane during cryopreservation, preventing cold shock, and minimizing ice crystal formation, which can otherwise damage sperm cells (Mandal *et al*., 2014). This finding aligns with prior research, which suggests that alternative egg yolk sources may offer improved cryoprotective effects (Sharafi *et al*., 2022). In this study, post-thaw sperm motility, viability, and vigor did not show significant differences between the quail egg yolk extender and the hen egg yolk or commercial egg yolk-free diluents. Nevertheless, the comparable performance of the quail egg yolk-based extender suggests it may serve as a viable and economical alternative for cattle semen cryopreservation, particularly for indigenous breeds such as Baggara, which are adapted to challenging environments and may require tailored preservation strategies (Sharafi *et al*., 2022).

In this study, post-thaw sperm motility, viability, and vigor were significantly higher with the quail egg yolk extender than with the hen egg yolk and commercial egg yolk-free diluents. This indicates that quail egg yolk-based extenders may provide a more effective and economical alternative for cattle semen cryopreservation, particularly for indigenous breeds such as Baggara, which are adapted to challenging environments and may require specialized preservation techniques (Sharafi *et al*., 2022).

Cryopreserving sperm from indigenous cattle breeds is crucial for maintaining genetic diversity and supporting

sustainable breeding programs, particularly in regions like Sudan, where Baggara cattle are integral to local economies and food security (Rewe, 2009). Successfully cryopreserving semen from these bulls ensures that their valuable genetics can be conserved and used in future breeding programs, even after the animal's death. This study underscores the importance of novel cryopreservation methods for preserving the genetics of Baggara bulls, which may have broader applications for other indigenous breeds in Africa and beyond. Furthermore, quail egg yolk-based extenders could provide an economical and accessible option for small-scale farmers and breeders who depend on indigenous cattle for their livelihoods.

While this study demonstrated the efficacy of quail egg yolk-based extenders, further research is required to optimize these protocols for broader application in cattle breeding programs. Future studies should investigate long-term fertility outcomes following artificial insemination with semen preserved using quail egg yolk-based extenders. Additionally, comparative research involving other indigenous cattle breeds and alternative to hen egg yolk could further enhance semen preservation techniques.

A practical limitation encountered with the use of quail egg yolk as an extender component is the relatively small volume of yolk obtained from a single egg compared to that of a hen egg. Consequently, multiple quail eggs are required to achieve the same quantity of yolk, potentially increasing preparation time and introducing variability between extender batches. Moreover, the limited commercial availability of quail eggs, particularly in rural or resource-constrained settings, may restrict the broader application of this alternative in routine semen cryopreservation protocols.

CONCLUSION

In conclusion, this study offers valuable insights into the use of quail egg yolk-based extenders for the cryopreservation of Baggara bull semen. The findings suggest that quail egg yolk is a viable and effective alternative to hen egg yolk-based extenders, providing comparable performance in maintaining post-thaw sperm quality. This research represents a significant advancement in the development of more efficient cryopreservation techniques for indigenous cattle breeds, contributing to their genetic conservation and promoting sustainable agriculture.

DECLARATIONS

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IRB approval

This study was approved by the Animal Ethics Committee of Sudan University of Science and Technology (Approval No. SUST2108025).

Ethics approval and consent to participate

We confirm that the study adheres to the ARRIVE guidelines (https://arriveguidelines.org). The study protocol underwent review and approval by Sudan University of Science and Technology, Research Committee. Research procedures were conducted in accordance with their guidelines for sampling domestic animals in Sudan, ensuring ethical compliance in the collection and utilization of the samples.

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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