UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
DOI: https://doi.org/10.57038/usjas.v8i01.6955
Uni. Sindh. J. Anim. Sci., vol. 8(1); 7-14, 2024
Email: editors.usjas@usindh.edu.pk
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro.

IMPACT OF ZINC SUPPLEMENTATION ON THARPARKAR BULL SEMEN QUALITY: COMPARATIVE STUDY OF COMMERCIAL AND LAB EXTENDERS

ABDUL HAMEED[1], ABDULLAH CHANNO[2], ASMATULLAH KAKA[1]*, HABIBULLAH JANYARO[3]

[1]Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam.
[2]Pakistan Agricultural Research Council, Arid Zone Research Centre (PARC-AZRC), Umerkot.
[3]Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand.

ARTICLE INFORMATION

Article History:
Received: 14th October 2023
Accepted: 25th February 2024
Published online: 1st June 2024

Author Contributions:
AH conceived the concept, while AC and AK compiled the results. HJ drafted the manuscript.

Key words:
Zinc, Tharparkar bull, Semen quality, BULLXcell®, Tris-based egg yolk extender, Acrosomal Integrity, Cryopreservation.

ABSTRACT

This study was aimed to evaluate the impact of zinc supplementation on chilled and frozen thawed quality parameters of Tharparkar bull semen by comparing the use of BULLXcell® (Commercial) and Tris-based egg yolk (Lab based) extenders impact on semen quality parameters. The research was conducted at the Department of Animal Reproduction, Sindh Agriculture University Tandojam. Total 44 semen ejaculates were collected from four Tharparkar Bulls (A, B, C, and D) aged 4-5 years using an artificial vagina. Macroscopic and Microscopic parameters i.e. color: visual observation, volume: graduated tube, pH: digital pH meter, Progressive Motility (20x magnification), morphology and viability (eosin-nigrosin stain), concentration (haemocytometer method), and membrane integrity (HOST solution), were evaluated. Semen samples with progressive motility, morphology, viability, and membrane integrity exceeding 70% were pooled and processed. The pooled semen samples were further extended by pouring into four groups [A: BullXcell® control, B: BullXcell®+Zinc (0.2 mg/100 ml), C: Tris+Control, D: Tris+Zinc (0.2 mg/100 ml)]. Chilled assessment of progressive motility, morphology, viability, and membrane integrity revealed that Group B (78.65±0.85, 85.23±0.81, 81.22±1.17, 80.51±0.73) ($P<0.05$) demonstrated improved results compared to Group D (73.82±0.91, 81.66±1.14, 76.51±1.22, 76.27±1.07). Similarly, post-thawed assessment of these parameters indicated superior results for Group B (57.25±0.82, 75.17±1.02, 70.22±1.34, 63.55±1.39) ($P<0.05$) compared to Group D (47.35±0.66, 63.24±1.47, 65.05±1.63, 57.14±1.02) followed A and C. Based on the findings, it was concluded that Group B [BullXcell®+Zinc (0.2 mg/100 ml)] significantly improved both Chilled and post thawed quality parameters of Tharparkar cattle bull semen and the inclusion of Zinc in extenders is recommended as it improved viability of spermatozoa.

1. INTRODUCTION

Semen quality is a crucial factor in assessing fertility and reproductive potential of bovine species (Raval and Dhami, 2010). Successful cryopreservation of semen is essential for long-term storage and artificial insemination techniques (Singh *et al.*, 2013). However, the freeze-thawing process can lead to irreversible damage to spermatozoa, resulting in reduced fertility rates (Ardon & Saurez, 2013; Channo *et al.*, 2023).

To overcome this challenge, semen extenders play a vital role in preserving sperm cells and maintaining their quality parameters (Akhter *et al.*, 2010; Rehman *et al.*, 2013). Semen extenders are specialized solutions that provide a suitable environment for sperm survival during the freezing and thawing process. These extenders typically contain cryoprotective agents, buffers to maintain pH, energy sources, and antibiotics to prevent bacterial contamination (Memon *et al.*, 2022; Channo *et al.*, 2023). They play a crucial role in protecting spermatozoa from the detrimental effects of cryopreservation, including osmotic stress, ice crystal formation, and oxidative damage (Channo *et al.*,

*Corresponding Author: asmatullah.kaka@gmail.com

2022). BULLXcell® extender and tris-based egg yolk extender are commonly used semen extenders that have demonstrated effectiveness in preserving semen quality (Pytlík *et al.,* 2022). BULLXcell® extender (IMV France) is a commercially available extender known for its cryoprotective properties and successful preservation of semen in various species (Pytlík *et al.,* 2022). Tris-based egg yolk extender, on the other hand, has been widely utilized as a cryoprotective agent in the preservation of semen in animals (Apu *et al.,* 2012; Emamverdi *et al.,* 2014). Zinc (Zn) is an essential trace element in the body that plays a critical role in reproductive fertility. It is fundamental to membrane stability, sperm tail shape, and sperm Progressive Motility (Fouad *et al.,* 2021). Zinc deficiency can lead to infertility in animals, resulting in problems with spermatogenesis and testicular development (Kumar *et al.,* 2006). Furthermore, insufficient zinc levels may cause more oxidative damage and lower-quality sperm (Kumar *et al.,* 2006; Fouad *et al.,* 2021). Therefore, zinc supplementation has been investigated as a potential approach to improve semen quality and fertility in various species. The Tharparkar cattle breed is an indigenous breed well-adapted to the arid regions of South Asia. However, limited information is available on Tharparkar bull semen and its response to different semen extenders (Gurler *et al.,* 2016). While extensive research has been conducted on semen cryopreservation in other cattle breeds, the specific characteristics and requirements of Tharparkar bull semen remain largely unexplored. The objective of this study was to assess the impact of zinc supplementation on Tharparkar bull semen quality using BULLXcell® and Tris-based egg yolk extender. We hypothesize that zinc supplementation may enhance the chilled and post thawed quality of Tharparkar cattle bull semen. By evaluating the effects of zinc supplementation and comparing the two extenders, this study aims to contribute to the understanding of semen cryopreservation techniques in Tharparkar cattle and potentially improve breeding strategies for this indigenous breed.

2. MATERIALS AND METHODS

Bull Preparation and Semen Collection

Four Tharparker cattle bulls (A, B, C, and D), aged 4-5 years, were housed under a semi-intensive farming system at the Department of Animal Reproduction, Sindh Agriculture University Tandojam, Sindh. All routine farm practices were followed according to recommended farm schedules. Prior to semen collection, the artificial vagina was cleaned, sterilized, and assembled. It was maintained at a temperature of 42°C with an air pressure of 35 mmHg, and the inner surface of the rubber liner was lubricated with petroleum jelly. Semen was collected twice a week using the artificial vagina, immediately transferred to the laboratory, and kept in warm water at 37°C (Kukovics *et al.,* 2011; Channo *et al.,* 2023).

Experimental Design:

After semen collection, the samples were processed for macroscopic (volume, color, pH) and microscopic (wave motion, progressive motility, concentration, morphology, viability, and membrane integrity) evaluations, following the methodology as described by Channo *et al.* (2023) and Memon *et al.,* (2022). Samples with ≥70% progressive motility and normal sperm morphology were pooled and diluted in Group A: BULLXcell®+Control, Group B: BULLXcell®+zinc (0.2 mg/100 ml), Group C: Tris-based egg yolk (Control), and Group D: Tris-based egg yolk+zinc (0.2 mg/100 ml). Semen dilution was performed at 5°C in cooling chamber, adjusting the dilution rate based on the initial sperm concentration to achieve 20 million spermatozoa in a 0.5 ml straw. The semen was extended as described by Kaka *et al.,* (2015) and Channo et al., 2024, and straws were filled and packed using an automatic straw filling machine. The equilibration period was completed within 2 hours at 5°C, after maintaining equilibrium chilled semen parameters were analyzed i.e. Motility, Morphology, Viability and Membrane Integrity. Thereafter, The straws were then stored in liquid nitrogen (-196°C) for at least 24 hours before being assessed for post-thaw quality in which Motility, Morphology, Viability and Membrane Integrity were accessed following the protocols of Kaka *et al.,* (2023); Najafi *et al.,* (2013); Kaka *et al.,* 2012 and Rasul *et al.,* (2002).

Statistical Analysis:

Data collected on semen characteristics were subjected to a two-way analysis of variance (ANOVA) using the Statistics software (2006). LSD was used to determine differences among the means of the different experimental groups.

3. RESULTS AND DISCUSSION

The cryo-preservation process often leads to a significant reduction in spermatozoa motility and viability, mainly due to various stresses experienced

during freezing and thawing. These stresses include chemical, osmotic, temperature-related, and mechanical stress (Leboeuf *et al.*, 2000; Channo *et al.*, 2023). The Zinc supplementation enhanced chilled and post thawed semen quality parameters as shown in figure I and II, including motility, morphology, viability, and membrane integrity, in both Group B and Group D. The addition of zinc at a concentration of 0.02 mm/ml showed positive effects on sperm parameters. The motility of spermatozoa was assessed and highest motility was observed in Group B 79.09±0.87. This finding aligns with a study by Ansari *et al.*, (2012) who reported a motility of 76.11±0.7. However, it was higher than the motility observed by Kaka *et al.*, (2015) and Yadav *et al.*, (2019) (74±1.6 and 74.46±0.53). The differences in results could be attributed to variations in processing techniques, extender compositions, or the use of antioxidants. Previous studies by Ansari *et al.*, (2012); Kaka *et al.*, (2015);Yadav *et al.*, (2019) and Channno *et al.*, (2023) examined motility in BIOXcell® extender with varying concentrations of vitamin E (α-Tocopherol). The highest morphological quality was also observed in Group B (BULLXcell®+zinc), with a mean value of 86.13±0.79. This result agreed with the findings of Kaka *et al.* (2015) and Yadav *et al.* (2019), who reported values of 86.62±1.2 and 88.91±3.2. However, the present study's morphology results were higher than those reported by Ansari *et al.* (2012) (80.52±2.2). Similar to the motility study, previous research by Ansari *et al.* (2012); Kaka *et al.* (2015); Yadav *et al.* (2019); Panhwar *et al.*, (2023) and Channo *et al.* (2023). The hypo-osmotic swelling test (HOST) was used to evaluate spermatozoa membrane functionality, and membrane integrity. The highest membrane integrity was also observed in Group B BULLXcell®+zinc, with a mean value of 81.40±0.77. This result was in agreement with the findings of Yadav *et al.*, (2019), who reported a mean value of 82.12±2.1. However, the present study's membrane integrity value was lower than those reported by Ansari *et al.*, (2012) and Kaka *et al.*, (2015) (90.01±0.7 and 87.87±0.6).

The post-thaw motility of spermatozoa was improved in Group B: BULLXcell® supplemented with zinc, with a range of 58.31±0.86. These results were consistent with findings by Towhidi and Parks, 2012; Kaka 2015 and Yadav *et al.*, (2019) (61.67±0.59, 51.50±0.6, and 60.08±0.64). However, the values were lower than those reported by Motemani *et al.*, (2017) (64.1±1.6). Additionally, the values were higher than those observed by Towhidi and Parks, 2012; Ansari *et al.*, 2012; Kaka *et al.*, 2015; Kaka *et al.*, (2015b); Shah *et al.*, (2023) and Kaka *et al.*, (2016) (41.4±0.4, 48.54±1.6, 45.5±0.2, 48.94±0.83, and 48±1.0). Similar to the motility study, previous research by Towhidi and Parks (2012); Kaka 2015; Kaka *et al.*, (2015b); Kaka *et al.*, 2016; Yadav *et al.*, (2019) and Channo *et al.* (2023). The post-thaw morphology of Tharparkar bull semen was also higher in the group B: BULLXcell®+zinc, with a mean value of 76.22±0.04. These findings agreed with those of Ansari *et al.*, (2012) and Motemani *et al.*, (2017) (75±3.1 and 75±1.7. However, the mean value of the present study was higher than the results reported by Kaka 2015; Kaka *et al.*, (2015a); Kaka *et al.*, (2015b) and Kaka *et al.*, (2016) (71.00±1.7, 72±1.2, 70.63±0.54, and 66.25±3.4,). Likewise, Ansari *et al.*, 2012; Motemani *et al.*, (2017) and Channo *et al.* (2023) studied morphology in BIOXcell® extender with various concentrations of vitamin E (α-Tocopherol). Moreover, the highest viability was also observed in Group B: BULLXcell®+zinc, with a mean value of 71.27±1.44. This result was consistent with the studies conducted by Kaka (2015); Kaka *et al.*, (2015a); Kaka *et al.*, (2015b); Kaka *et al.*, 2016 and Yadav *et al.*, (2019) (71.75±1.5, 74±1.4, 73.42±0.96, 69.75±2.7, and 69.7±0.33). However, the value was higher than those reported by Towhidi and Parks, 2012; Ansari *et al.*, 2012, and Motemani *et al.*, (2017) (45.1±1.4, 61.70±0.25, and 64.1±1.8). Lastly, membrane integrity was also highest in the BULLXcell®+zinc, with a mean value of 64.68±1.43. This finding agreed with the results of Motemani *et al.*, (2017) (67.1±1.6) and was lower than the results reported by Kaka 2015; Kaka *et al.*, (2015a) and Kaka *et al.*, (2015b) (70.00±2.9, 75±1.6, and 74.84±1.8). However, the value was higher than that observed by Ansari *et al.*, (2012) (60.11±2.3). In conclusion, the cryo-preservation process often results in reduced motility and viability of spermatozoa. However, the supplementation of zinc into the semen extender, has shown positive effects on the quality of chilled and frozen-thawed bull spermatozoa. The inclusion of zinc supplementations at a concentration of 0.02 mm/ml may improve semen quality parameters. However, it is important to note that variations in results may arise

from differences in processing techniques, extender compositions, and the use of antioxidants at various concentrations.

4. CONCLUSION

In conclusion, supplementation of Zinc with (0.2 mg/100 ml) concentration within BULLXcell® semen extender improves chilled and post thawed semen quality parameters of Tharparkar Bull semen.

5. ACKNOWLEDGEMENTS

The author would like to express their heartfelt appreciation to the Department of Animal Reproduction, Sindh Agriculture University, Tandojam, 70060, for their invaluable support and provision of resources throughout the course of this research.

Funding

The research was carried out under the project "Optimization Cryopreservation Protocol for Indigenous (Thari/Tharparkar) Cattle Semen" funded by the Sindh Agriculture Growth Project, Government of Sindh.

IRB approval

The work conducted in the study was approved by the departmental board of studies (BOS) of the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University on 24 June 2022, Meeting No: DAR/168/of 2022.

Ethics statement

It states that all procedures conducted in the study were in compliance with approved ethical policies and protocols of Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Data Availability

The data supporting the findings of this study are available upon request from the corresponding author.

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

REFERENCES

Akhter, S., Ansari, M. S., Rakha, B. A., Andrabi, S. M. H., Iqbal, S., & Ullah, N. (2010). Cryopreservation of buffalo (Bubalus bubalis) semen in Bioxcell® extender. *Theriogenology*, *74*(6), 951-955.

Ansari, A., Aman, A., Siddiqui, N. N., & Iqbal, S. (2012). Bacteriocin (BAC-IB17): screening, isolation and production from Bacillus subtilis KIBGE IB-17. *Pakistan Journal of Pharmaceutical Sciences*, *25*(1).

Apu, A. S., Yahia, K. M., Hussain, S. S., Fakruzzaman, M., & Notter, D. R. (2012). a comparative study of fresh and frozen-thawed semen quality in relation to fertility of black bengal goats semen from six adult male black bengal goats. *Iranian Journal of Applied Animal Sciences*, 2:157-161.

Ardon, F., & Suarez, S. S. (2013). Cryopreservation increases coating of bull sperm by seminal plasma binder of sperm proteins BSP1, BSP3, and BSP5. *Reproduction*, *146*(2), 111-117.

Channo, A., Kaka, A., Kalwar, Q., Jamali, I., Jelani, G., Bakhsh, M., Dahri, G. N., & Goil, J. P. (2022). An overview of bovine cystic ovarian disease. *Pakistan Journal of Zoology*, *54*(5), 2437.

Channo, A., Kaka, A., Memon, A. A., Chand, M., Malhi, M. B., Kalwar, Q., Tunio, S. A., & Panhwar, M. I. (2023). Post Thawed Quality of Tharparkar Bull Semen Extended in Bioxcell TM and Tris-Based Egg Yolk Extenders Supplemented with Vitamin E (α-Tocopherol). *Pakistan Journal of Zoology*, 1-6.https://dx.doi.org/10.17582/journal.pjz/20220523220514

Channo, A., kaka, A., Memon, A., Malhi, M., Bakhsh, M., Kalwar, Q., Kalhoro, D., Sethar, A., & Jariko, R. (2024). Effects of α-Tocopherol on chilled quality parameters of Tharparkar

bull semen extended in Lecithin and Tris based egg yolk extender. Journal of the Hellenic Veterinary Medical Society, 74(4),6511–6516. https://doi.org/10.12681/jhvms.31491

Emamverdi, M., Zhandi, M., Shahneh, A. Z., Sharafi, M., Akhlaghi, A., Motlagh, M. K., Dadkhah, F., & Davachi, N. D. (2014). Flow cytometric and microscopic evaluation of post-thawed ram semen cryopreserved in chemically defined home-made or commercial extenders. *Animal Production Science*, *55*(4), 551-558.

Fouad, W. F., & Ashour, A. F. (2021). Selenium and Zinc as Supplements to Extenders Frozen Semen for Improving Sperm Characteristics during Cryopreservation. *Journal of Animal and Poultry Production*, *12*(2), 71-77.

Gürler, H., Malama, E., Heppelmann, M., Calisici, O., Leiding, C., Kastelic, J. P., & Bollwein, H. (2016). Effects of cryopreservation on sperm viability, synthesis of reactive oxygen species, and DNA damage of bovine sperm. *Theriogenology*, *86*(2), 562-571.

Kaka, A., 2015. http://psasir.upm.edu.my/id/eprint/56730/1/FPV%2012RR,pp,88-118.

Kaka, A., Haron, W., Yimer, N., Channo, A., Jahejo, A. R., Ebrahimi, M., & Kalhoro, D. H. (2023). Enhancement of frozen-thawed quality of bull semen after enrichment of docosahexaenoic acid extended in tris-extender. *Journal of Animal Health and Production*, *11*(1), 7-13.

Kaka, A., M. U. Samo, T. H. Rahoo, Z. U. Rehman, Z. Shah, M. Mushtaq, U. Kaka, and A. A. Behan. (2012). "Study on post thawing quality of kundhi buffalo semen." *Journal of Animal and Plant Sciences*, *22*, 2: 59-62.

Kaka, A., Memon, A. A., Khatri, P., Kumbhar, H. K., Kalhoro, D. H., Tariq, M., Pirzado, S. A., & Tunio, A. N. (2016). Determination of quality characteristics of Khundi buffalo bull semen. *Journal of Basic and Applied Sciences*, *12*, 394-397.

Kaka, A., Wahid, H., Rosnina, Y., Yimer, N., Khumran, A. M., Sarsaifi, K., Behan, A. A., & Ebrahimi, M. (2015b). α-Linolenic acid supplementation in BioXcell® extender can improve the quality of post-cooling and frozen-thawed bovine sperm. *Animal reproduction science*, *153*, 1-7.

Kukovics, S., Gyoker, E., Németh, T., & Gergátz, E. (2011). Artificial insemination of sheep–possibilities, realities and techniques at the farm level. *Artificial insemination in farm animals*.

Kumar, N., Verma, R. P., Singh, L. P., Varshney, V. P., & Dass, R. S. (2006). Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (Bos indicus $\bf\times $ Bos taurus) bulls. *Reproduction Nutrition Development*, *46*(6), 663-675.

Leboeuf, B., Restall, B., & Salamon, S. (2000). Production and storage of goat semen for artificial insemination. *Animal reproduction science*, *62*(1-3), 113-141.

Memon, A. S., Kaka, A., Channo, A., Bakhsh, M., Kalwar, Q., & Sethar, A. (2022). Effect of Soybean Milk and Tris Egg Yolk Based Extender on Semen Quality Parameters of Tharparkar Bull. *University of Sindh Journal of Animal Sciences (USJAS)*, *6*(03).

Motemani, M., Chamani, M., Sharafi, M., & Masoudi, R. (2017). Alpha-tocopherol improves frozen-thawed sperm quality by reducing hydrogen peroxide during cryopreservation of bull semen. *Spanish journal of agricultural research*, *15*(1), 15.

Najafi, A., Zhandi, M., Towhidi, A., Sharafi, M., Sharif, A. A., Motlagh, M. K., & Martinez-Pastor, F. (2013). Trehalose and glycerol have a dose-dependent synergistic effect on the post-thawing quality of ram semen cryopreserved in a soybean lecithin-based extender. *Cryobiology*, *66*(3), 275-282.

Panhwar, M. I., Kaka, A., Memon, A. A., Channo, A., & Kolachi, H. A. (2023). In-vitro impacts of Triladyl® extender on semen quality parameters of Tharparkar bull. Pure and Applied Biology. *13*(2),134-140.

Pytlík, J., Savvulidi, F. G., Ducháček, J., Codl, R., Vrhel, M., Nagy, S., & Stádník, L. (2022). Effect of extender on the quality and incubation resilience of cryopreserved Holstein bull semen. *Czech Journal of Animal Science*, *67*(3), 75-86.

Rasul, Z., Anzar, M., Jalali, S., & Ahmad, N. (2000). Effect of buffering systems on post-thaw motion characteristics, plasma membrane integrity, and acrosome morphology of buffalo spermatozoa. *Animal reproduction science*, *59*(1-2), 31-41.

Raval, R. J., & Dhami, A. J. (2010). Effect of additives on various spermatozoal attributes of fresh, frozen-thawed and refrigerated semen. *The Indian Journal of Animal Reproduction*, *31*(1), 33-36.

Rehman, F., Zhao, C., Shah, M. A., Qureshi, M. S., & Wang, X. (2013). Semen extenders and artificial insemination in ruminants. *Veterinaria*, *1*(1), 1-8.

Shah, S. H., Memon, A. A., Kaka, A., Tunio, A. N., Channo, A., Kalwar, Q., Panhwar, M. I., Malak, K. A., & Rahoo, B. A. (2023). Evaluation of Ovsynch Estrus Synchronization in Holstein Friesian and Holstein Friesian-Jersey Crossbred Cows at District Thatta, Sindh, Pakistan. *Pakistan Journal of Zoology*. 1-8.

Singh, M. A. H. A. K., RAMTEKE, S. S., GHOSH, S. K., Prasad, J. K., & Rajoriya, J. S. (2013). Efficacy of egg yolk from three avian species on semen freezability of Tharparkar bull. *The Indian Journal of Animal Reproduction*, *34*(2), 25-28.

Towhidi, A., & Parks, J. E. (2012). Effect of n-3 fatty acids and α-tocopherol on post-thaw parameters and fatty acid composition of bovine sperm. *Journal of assisted reproduction and genetics*, *29*, 1051-1056.

Yadav, D., Singh, V., Yadav, S. S., Patel, A., Kumar, A., Sonkar, V., Kumar, A., Yadav, S., Kumar, B., Yadav, B., & Saxena, A. (2019). Effect of glutathione on viability and progressive motility of Hariana bull spermatozoa during cryopreservation in semi-arid region. *Journal of pharmaceutical innovations*, *8*(6), 8-876.

Table 1. Fresh Characteristics of Tharparkar bull semen (mean%±SEM).

Parameter	Bull A	Bull B	Bull C	Bull D
Volume	6.38±0.21^{ab}	6.22±0.17^{b}	6.16±0.14^{c}	6.43±0.16^{a}
Colour	Creamy White	Creamy White	Creamy White	Creamy White
pH	6.51±0.03^{cd}	6.53±0.02b^{c}	6.56±0.03^{ab}	6.59±0.03^{a}
Wave Motion	++++	++++	++++	++++
Motility	88.65±0.65^{b}	85.37±1.03^{cd}	87.03±1.45^{bc}	90.24±0.87^{a}
Concentration	1180.5±9.33^{b}	1170.1±16.32^{d}	1175.4±14.24^{c}	1210.5±19.15^{a}
Morphology	89.47±0.51^{abc}	88.94±0.55^{bc}	90.35±0.61^{ab}	90.73±0.73^{ab}
Viability	88.22±0.32^{ab}	87.15±0.66^{b}	86.29±0.71^{c}	88.32±0.38^{a}

^{a,b,c,d} superscripts within columns shows significant difference (P<0.05)

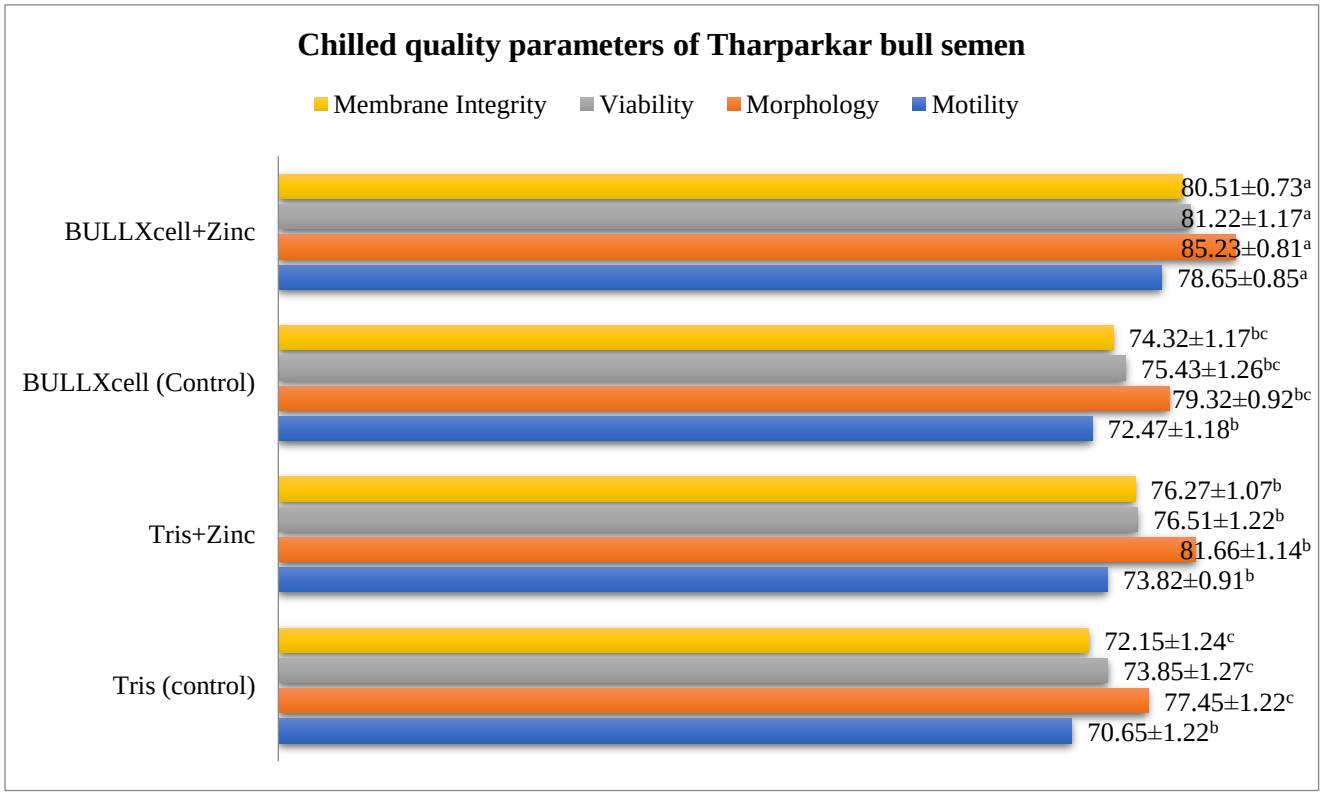

Figure 1. Chilled quality parameters of Tharparkar bull semen extended in BULLXcell® and Tris based egg yolk extender supplemented with and without Zinc

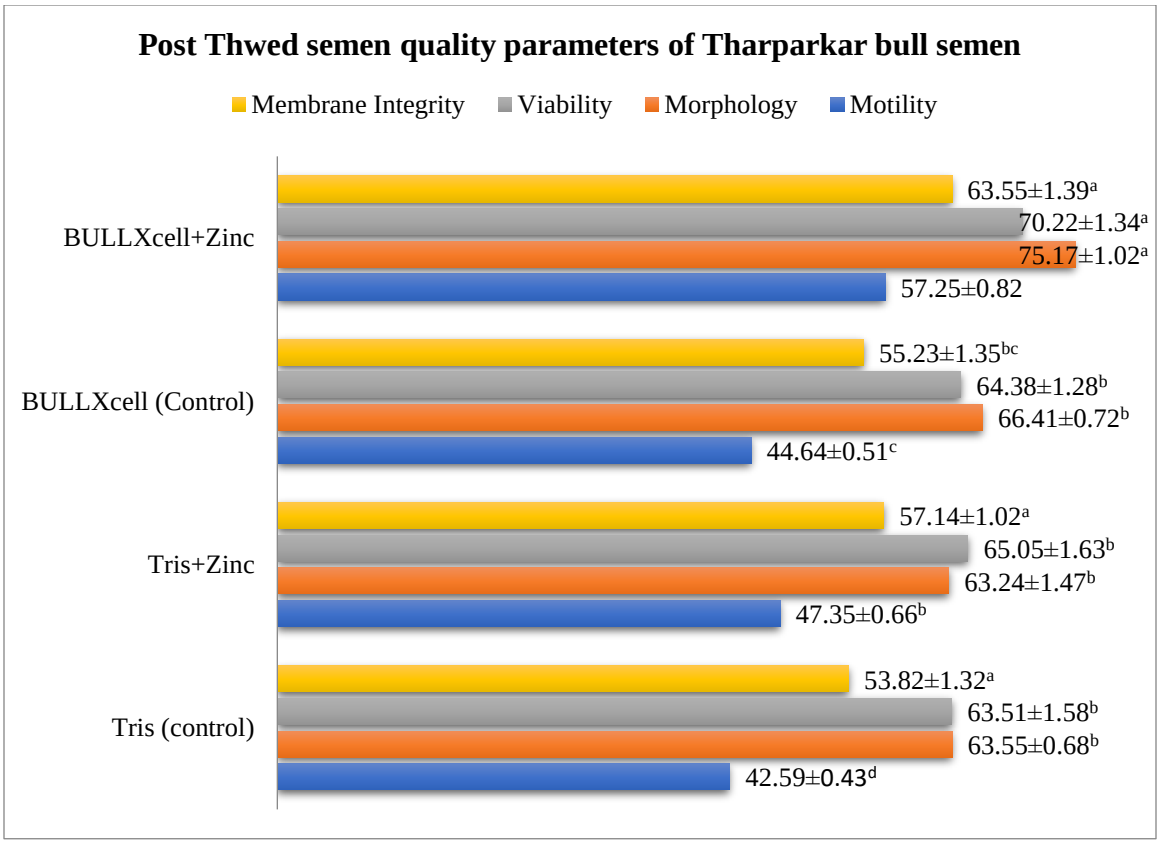

Figure 2. Post Thawed quality parameters of Tharparkar bull semen extended in BULLXcell® and Tris based egg yolk extender supplemented with and without Zinc.