Research Article

Evaluation of Triladyl and a Laboratory-Formulated Tris Extender for Chilled and Frozen Semen of Cholistani Bulls

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Abstract | This study aimed to evaluate and compare the post-chilling and post-thaw quality of Cholistani bull semen diluted with either a commercial extender (Triladyl®) or a laboratory-formulated Tris extender. Sixty-four ejaculates were collected from four fertile 2- to 4-year-old bulls using an artificial vagina. Ejaculates obtained from all bulls were pooled to minimize individual variation, provided they exhibited ≥ 80 % initial motility and normal morphology, then divided and diluted (1: 2) with Triladyl® or Tris. Filled 0.5 mL straws were cooled to 5 °C for 2 h, vapor-frozen 4 cm above liquid nitrogen for 8 min, and plunged into the liquid phase. Post-thaw evaluations included motility, morphology, viability (eosin–nigrosin), and functional membrane integrity (HOST). Data were analyzed using a one-way ANOVA to compare means between extenders. Semen extended with Triladyl® showed significantly higher ($P < 0.05$) post-thaw motility (54.8 ± 0.8 %), normal morphology (74.6 ± 0.9 %), viability (71.1 ± 0.9 %), and functional membrane integrity (51.4 ± 1.0 %) than semen processed with the Tris extender. These findings demonstrate the superior cryoprotective efficacy of Triladyl® over the laboratory Tris extender in maintaining post-thaw semen quality of Cholistani bulls.

Keywords | Cholistani bull, Semen extender, Triladyl, Tris, Cryopreservation, Post-thaw quality

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INTRODUCTION

Semen cryopreservation is a vital technique in animal breeding, enabling the long-term storage and transport of genetic material while preserving valuable traits in domestic livestock (Engdawork *et al.*, 2024). This method has revolutionized artificial insemination (AI), making it a cornerstone of modern reproductive biotechnology. Numerous studies have investigated semen cryopreservation in farm animals such as cattle, sheep, goats,

pigs, horses, and poultry, focusing on extender formulations, cryoprotectants, and post-thaw semen quality (Mukherjee *et al*., 2023). These efforts have led to standardized, efficient cryopreservation protocols, enhancing breed improvement programs globally. AI using cryopreserved semen has played a major role in genetic improvement of dairy and beef herds. In Pakistan, AI was introduced in the 1950s, initially emphasizing crossbreeding with exotic breeds (Philipsson., 2000). More recently, focus has shifted to conserving and improving native breeds through selective breeding and AI (Ullah *et al*., 2025).

Pakistan is home to 15 well-recognized indigenous cattle breeds, including Sahiwal, Red Sindhi, Tharparkar, and Cholistani, among others (Siddiky, 2015). These account for about 30% of the national herd, while the remaining 70% are non-descript. With mechanization reducing the need for draught animals, there is increasing interest in optimizing dairy and meat production through genetic improvement.

Semen extenders are critical for sperm preservation during cryopreservation. These formulations typically contain cryoprotectants (e.g., glycerol, ethylene glycol), buffers (e.g., Tris, phosphate), egg yolk or milk, antibiotics, and antioxidants, all contributing to sperm viability and integrity during freezing and thawing (Bustani and Baeiee, 2021). For example, bovine semen performs better with extenders containing 20% egg yolk and 7.5% glycerol, while goat semen may require different formulations (Raheja *et al*., 2018).

Among extenders, both laboratory-formulated and commercially available types are used. Tris-based extenders are commonly prepared in laboratories using Tris buffer, citric acid, fructose, egg yolk, and glycerol. Triladyl®, a commercial extender, contains a similar base with proprietary additives (Samsudin *et al*., 2019). Several studies report better post-thaw motility and viability with Triladyl® than with other extenders (Naz *et al*., 2018).

The Cholistani breed, developed from Sahiwal crosses in the Cholistan desert, is characterized by a large, hardy body, long ears, and disease resistance (Farooq *et al*., 2010). Its milk and meat are highly valued by local communities. However, there is a lack of published data on cryopreservation outcomes for Cholistani bull semen (Farooq *et al*., 2024). Preserving the breed's genetics is critical given challenges such as habitat loss, climate change, and declining population.

Therefore, this study was designed to evaluate the efficacy of Triladyl® compared to a laboratory-prepared Tris extender in improving the chilled and frozen semen quality of Cholistani bulls.

MATERIALS AND METHODS

Study location and animals

The research was conducted at the Semen Production Unit, Pitafi Livestock Farm, Mirpur Mathelo, District Ghotki, Sindh, Pakistan. The study was carried out over a period of three months and involved four healthy, trained, and fertile Cholistani bulls (IDs: 002, 032, 008, and 028), aged 4 to 5 years. The bulls were maintained on a daily diet of 2 kg concentrate per animal, seasonal green fodder, wheat or paddy straw, and were allowed free access to clean water. Deworming and vaccination were carried out as per farm guidelines. All procedures were approved by the Institutional Animal Ethics Committee of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agricultural University, Tandojam (Director Advanced Study Sindh Agriculture University Tandojam).

Semen collection and bull preparation

Donor and teaser animals were bathed prior to semen collection, preputial hair was clipped off, and the region was wiped with an iodine solution to prevent contamination. The hindquarters were also cleaned. Semen was collected using an artificial vagina (AV) prepared following conventional procedures twice per week in the morning for a total of 64 ejaculates (16 per bull) (Nadaf *et al*., 2022). The AV was maintained at 45–48 °C with an internal pressure of 35–44 mmHg and coated with petroleum jelly/liquid paraffin. Ejaculates were immediately transferred to a 37 °C water bath for evaluation.

Semen evaluation

Semen quality was evaluated both macroscopically and microscopically

Macroscopic analysis: The volume of each ejaculate was measured in graduated tubes; color was visually identified as milky, creamy, white, or translucent (Mason *et al*., 2023); and pH was determined using indicator strips (Merck, Germany).

Microscopic analysis: The wave motion was assessed under 4× magnification using a phase-contrast microscope (JG41) and scored on a scale of 0 to +++++ (Kumar, 2015). Motility was evaluated in triplicate following 1:100 dilution with normal saline, counting at least 100 sperm cells per replicate. A hemocytometer was used for concentration determination (Nadaf *et al*., 2022). The eosin–nigrosin stain was employed to assess morphology and viability (Klimowicz-Bodys *et al*., 2012). The hypo-osmotic swelling test (HOST) using tri-sodium citrate and fructose solution was applied to determine functional membrane integrity after incubation at 37 °C for one hour. Swollen spermatozoa were considered membrane-intact.

Design of experiments and preparation of extenders

Only ejaculates exhibiting ≥ 80 % motility, viability, and normal morphology and ≥ 70 % HOST-positive sperm were pooled and divided into two groups:

- Group A: Extended with commercial Triladyl® (Minitube, Germany).
- Group B: Extended with laboratory-formulated Tris-egg-yolk extender.

The two extenders were prepared at 5 °C with sperm concentration adjusted to 10–20 million per 0.5 mL straw. Triladyl® composition: Tris, citric acid, glycerol, spectinomycin, gentamicin, tylosin, lincomycin, egg yolk (20 %), and distilled water (750 mL).

Antibiotic concentrations were standardized to gentamicin 250 µg/mL, tylosin 100 µg/mL, lincomycin 150 µg/mL, and spectinomycin 100 µg/mL.

Laboratory Tris extender: Tris (3.81 g), citric acid (1.97 g), fructose (1.25 g), glycerol (7 mL), egg yolk (20 mL), penicillin 1000 IU/mL, streptomycin 1 mg/mL, and distilled water (73 mL).

Semen processing (cooling, freezing, and thawing)

The diluted semen was cooled to 5 °C for 2 hours, filled into 0.5 mL color-coded straws (red = Triladyl®, blue = Tris), sealed, and equilibrated at 5 °C for another 2 hours. Straws were then frozen 4 cm above liquid-nitrogen vapor for 8 minutes and plunged into liquid nitrogen (–196 °C) for storage overnight (Fox, 2012). Thawing was performed at 30 °C for 15 seconds. Chilled and post-thaw semen samples were analyzed for motility, morphology, viability, and membrane integrity using the same procedures as for fresh semen.

Statistical analysis

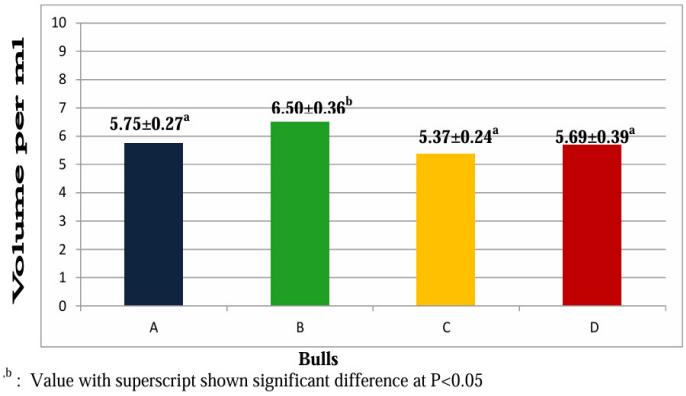
Data were analyzed using one-way ANOVA followed by the least significant difference (LSD) test in Statistix 8.1 Software (2006). All measurements were conducted in triplicate, and results were expressed as mean ± SEM. Statistical significance was considered at P < 0.05.

RESULTS

The current experiment was conducted on four trained Cholistani bulls, and a total of 64 relevant semen ejaculates (n= 16 per bull) were collected twice per week over a period of eight weeks during the early mornings. Only those ejaculates that met the selection criteria of 80% motility, 80% normal morphology, 80% viability, and 70% functional membrane integrity were evaluated and extended using Tris-based and Triladyl extenders.

Measuring fresh semen of Cholistani bulls Macroscopic characteristics (Mean ± SEM)

Figure 1 shows the mean ejaculate volume (ml) of semen collected from the four bulls. There was a significant variation (P < 0.05, one-way ANOVA) among individuals. Bull B recorded the highest semen volume (6.50 ± 0.36 ml), followed by Bull A (5.75 ± 0.27 ml) and Bull D (5.69 ± 0.39 ml), while Bull C recorded the lowest volume (5.37 ± 0.24 ml). The overall mean semen volume across all bulls was 5.83 ± 0.32 ml, providing a general indication of semen output in Cholistani bulls. These inter-individual differences can be attributed to physiological variation, testicular size, and reproductive health status.



b : Value with superscript shown significant difference at P<0.05

Figure 1: Semen volume (ml) collected from Cholistani cattle bull.

Color

The color of the semen of Cholistani bulls was determined visually in the collection tube right after collection. Table 1 shows the results. The ejaculates were creamy white in color and thick and clear.

Table 1: Color of semen collected from Cholistani cattle bull.

Bull	Color of semen
A	Creamy White
B	Creamy White
C	Creamy White
D	Creamy White

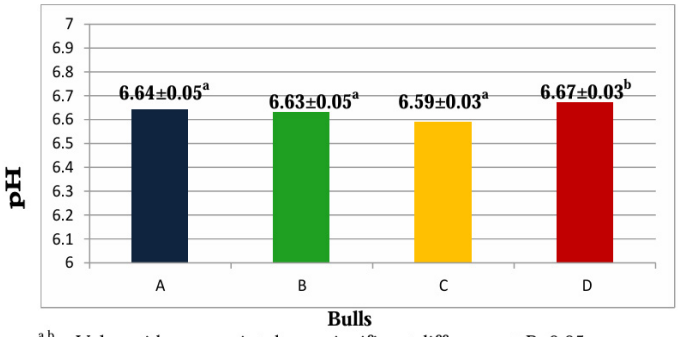
PH

Figure 2 represents the mean semen pH. There was a considerable difference (P < 0.05) between the bulls. Bull D (6.67 ± 0.03) had the highest pH, then Bull A (6.64 ± 0.05) and Bull B (6.63 ± 0.05) followed and Bull C (6.59 ± 0.03) had the lowest.

Wave motion

The fresh semen under the microscope was seen to have

the normal swirling motion called wave motion. In this experiment, the rapid swirling motion which creates eddies was observed in all ejaculates (Table 2).



a,b : Value with superscript shown significant difference at P<0.05

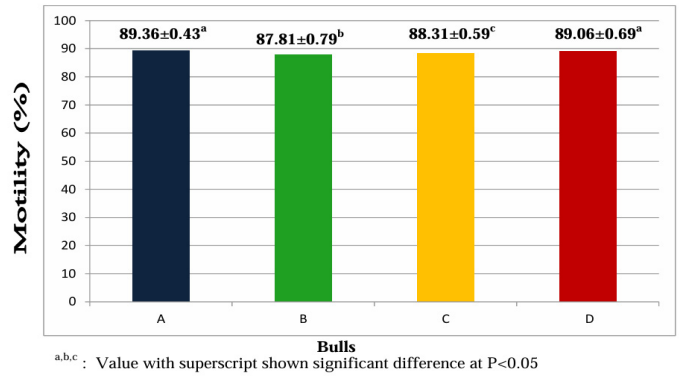
Figure 2: pH of semen collected from Cholistani cattle bull.

Table 2: Wave motion of semen collected from Cholistani cattle bull.

Bull	A	B	C	D
Wave motion	+ + + +	+ + + +	+ + + +	+ + + +

Motility

Figure 3 gives the percentage motility of spermatozoa. There was a considerable difference (P < 0.05) between the bulls. The Bulls A (89.36 + 0.43), D (89.06 + 0.69) and C (88.31 + 0.59) had the highest, middle and lowest motility, respectively.



a,b,c : Value with superscript shown significant difference at P<0.05

Figure 3: Motility of semen collected from Cholistani cattle bull.

Morphology

Figure 4 shows the percentage of morphologically normal spermatozoa. There was a considerable difference (P < 0.05) between the bulls. Bull D (88.19 + 0.57), Bull A (87.12 + 0.76), Bull B (86.31 + 0.96) and Bull C (85.31 + 0.82) had the highest percentage of normal morphology, respectively.

Viability

Figure 5 shows the mean viability percentage of spermatozoa. There was a considerable difference (P < 0.05) between the bulls. Bull A (83.31 ± 1.13), Bull C (83.62 ± 1.43), Bull B (82.25 ± 1.76), and Bull D (lowest value not clearly mentioned in original text--please confirm) gave the highest viability.

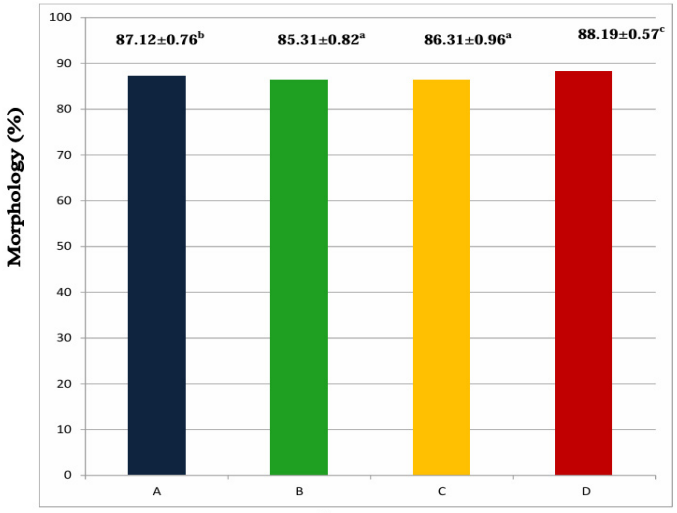


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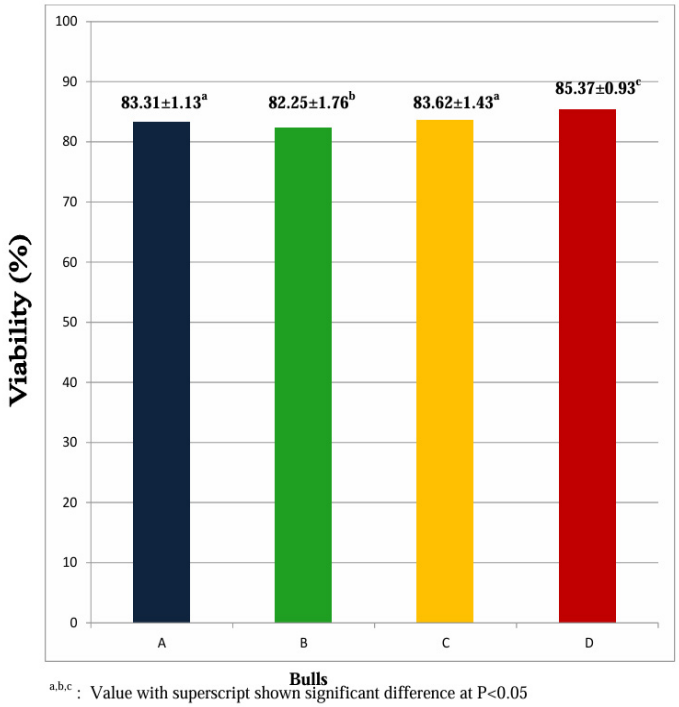
Figure 4: Morphology of spermatozoa collected from Cholistani cattle bull.

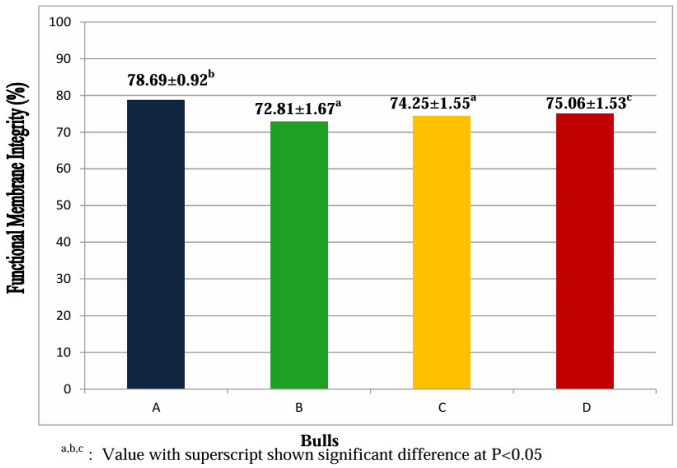


a,b,c : Value with superscript shown significant difference at P<0.05

Figure 5: Viability percentage of spermatozoa collected from Cholistani cattle bull.

Membrane integrity of functions

Figure 6 demonstrates the percentage of spermatozoa that had intact functioning membranes. There was a large difference (P < 0.05) between the bulls. The maximum was recorded in Bull B (78.69 ± 0.92), Bull D (75.06 ± 1.53) and Bull C (74.25 ± 1.55) and the lowest in Bull A (72.81 ± 1.67).

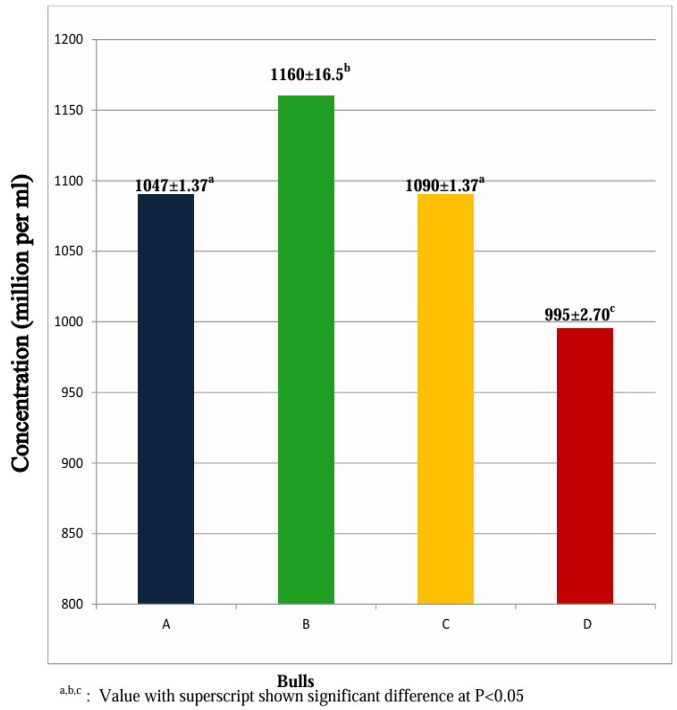


a,b,c : Value with superscript shown significant difference at P<0.05

Figure 6: Functional membrane integrity of spermatozoa collected from Cholistani cattle bull.

Sperm concentration

Figure 7 displays the mean concentration of sperm. There was a large difference (P < 0.05) between the bulls. The maximum concentration was observed with Bull B (1160 ± 16.5 million/ml), Bull C (1090 ± 1.37million/ml) and Bull A (1090± 1.37million/ml), and the lowest was recorded with Bull D.

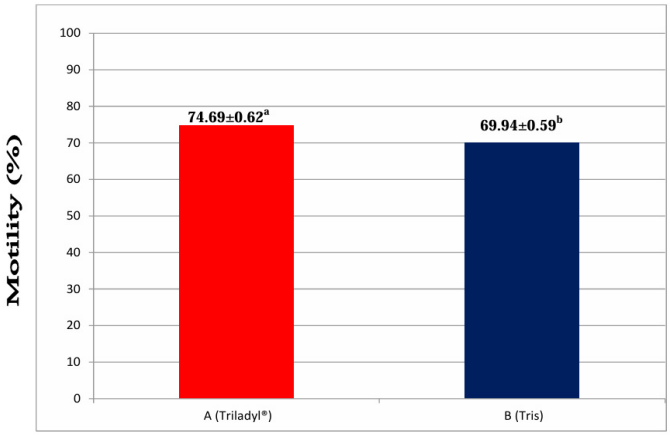


a,b,c : Value with superscript shown significant difference at P<0.05

Figure 7: Sperm concentration (million/ml) of Cholistani bulls.

Evaluation of cooled semen motility

Figure 8 depicts the average motility of cooled semen in two extenders (A: Triladyl 2; B: Tris). The difference between the groups was significantly significant (P < 0.05). Motility was also much greater in Triladyl (74.69 + 0.62) than Tris (69.94 + 0.59).

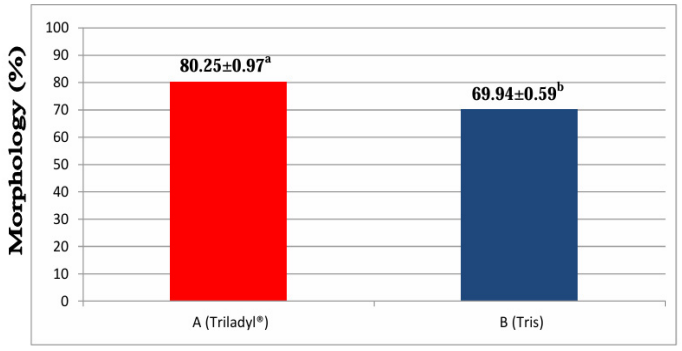


a,b, : Value with superscript shown significant difference at P<0.05

Figure 8: Motility (%) of cooled semen of Cholistani bulls.

Morphology

Figure 9 shows the mean percentage of morphologically normal spermatozoa in cooled semen. There was a large difference (P < 0.05) among extenders. Triladyl 80.25 + 0.97 had higher morphology than Tris 76.44 + 0.89.

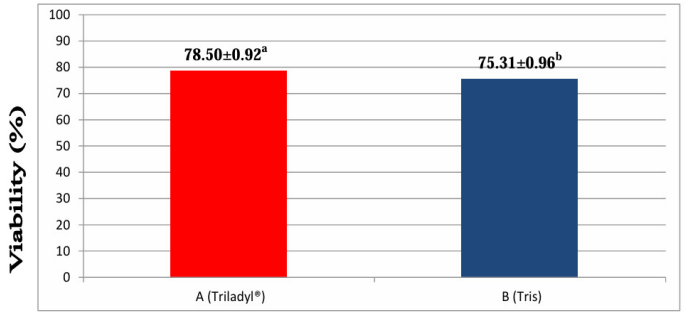


a,b : Value with superscript shown significant difference at P<0.05

Figure 9: Percentage (%) morphology of cooled semen of Cholistani bulls.

Viability

The average percentage of viability is given in Figure 10. There was a large difference (P < 0.05) among extenders. The Triladyl was found to be more viable (78.50 0.92) than Tris (75.31 0.96).

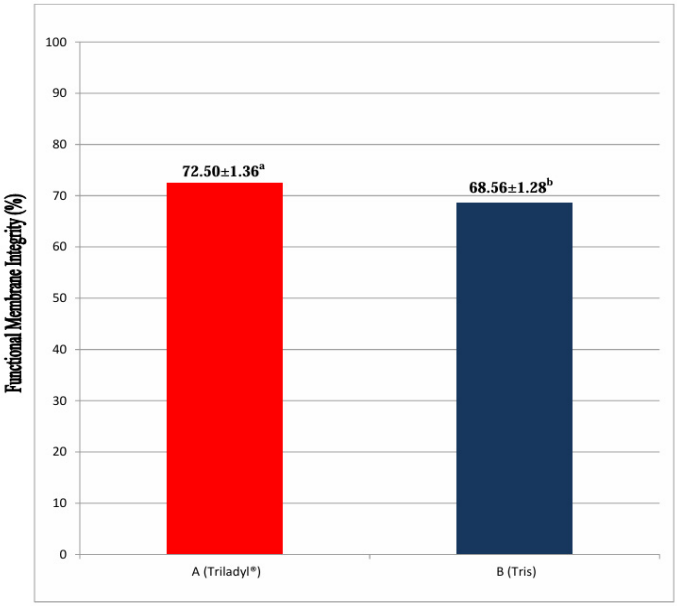


a,b : Value with superscript shown significant difference at P<0.05

Figure 10: Percentage viability of cooled bull semen of Cholistani bulls.

Membrane integrity of functions

The average functional membrane integrity is presented in Figure 11. There was a large difference (P < 0.05) among extenders. There was an increase in values of Triladyl (72.50 ± 1.36) than that of Tris (68.56 ± 1.28).

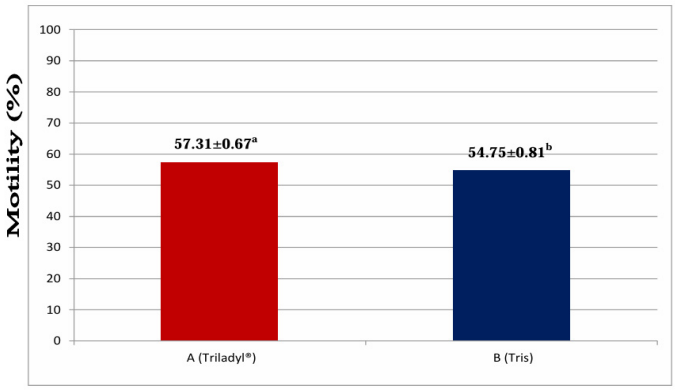


a,b : Value with superscript shown significant difference at P<0.05

Figure 11: The percentage of functional membrane integrity of cooled semen of Cholistani bulls.

Evaluation of frozen semen motility

Figure 12 shows the average motility of frozen semen. There was a large difference (P < 0.05) among extenders. The motility was much more in Triladyl (57.31 0.67) compared to Tris (54.75 0.81).

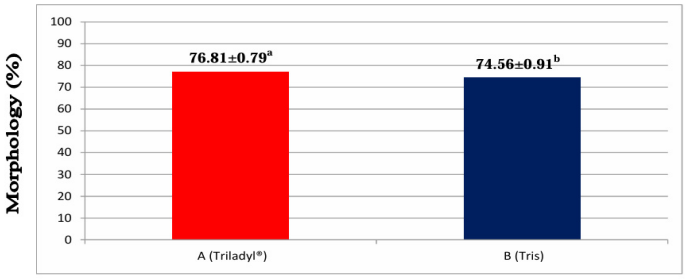


a,b : Value with superscript shown significant difference at P<0.05

Figure 12: Percentage motility of frozen bull semen of Cholistani bulls.

Morphology

The average percentage of the morphology of frozen semen is depicted in Figure 13. There was a large difference (P < 0.05) among extenders. The Triladyl 76.81 + 0.79 was found to be higher morphologically than Tris (74.56 + 0.91).

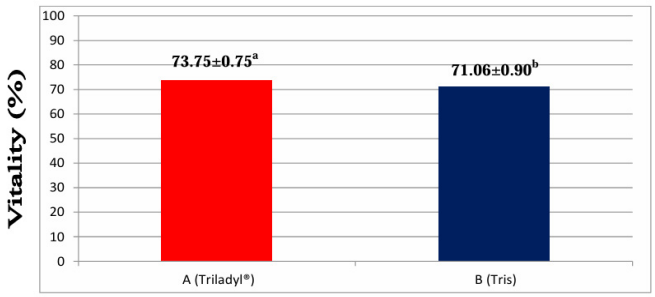


a,b : Value with superscript shown significant difference at P<0.05

Figure 13: The morphology (percentage) of Cholistani bulls frozen semen.

Viability

Figure 14 gives the mean viability of frozen semen. There was a large difference (P < 0.05) among extenders. The triladyl (73.75 +0.75) had a higher viability as compared to the tris (71.06 +0.90).

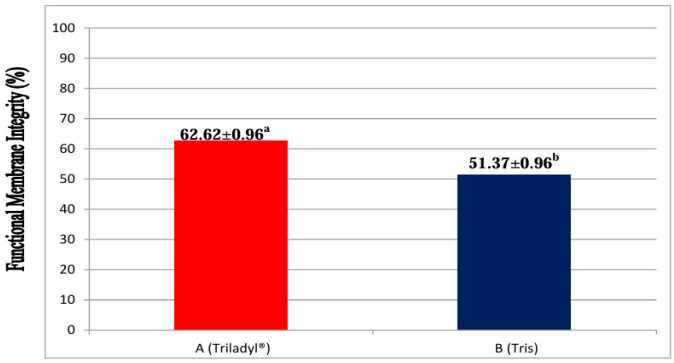


a,b : Value with superscript shown significant difference at P<0.05

Figure 14: The percentage of viability of frozen semen of Cholistani bulls.

Membrane integrity of functions

Figure 15 indicates the average semen functional membrane integrity of frozen semen. There was a large difference (P < 0.05) among extenders. The values were higher in Triladyl (58.62 ± 0.96) than Tris (53.37± 0.96).



a,b : Value with superscript shown significant difference at P<0.05

Figure 15: Membrane integrity (%) of frozen bull semen of Cholistani bulls.

DISCUSSION

This study was conducted to evaluate the semen quality

of four fertile Cholistani cattle bulls aged 4 to 5 years. A total of 64 ejaculates (n=16 per bull) were collected over 8 weeks using the artificial vagina (AV) method. The ejaculates that met minimum quality standards (motility ≥80%, morphology ≥80%, viability ≥80%, and functional membrane integrity ≥70%) were processed further. The semen samples were extended using two extenders: A laboratory-prepared Tris-based extender (Group A) and a commercially available Triladyl® extender (Group B). Two-colored straws (red and blue) were used to distinguish between the groups during cooling and freezing evaluations. The semen quality was assessed at three stages: fresh, cooled, and frozen.

The ejaculated semen volume from Cholistani bulls in the current study ranged from 5.37 ± 0.24 to 6.50 ± 0.36 ml. These findings are consistent with those of Farooq *et al.* (2013), who reported a semen volume of 6.24 ± 0.16 to 6.50 ± 0.19 ml from Tharparkar bulls. Similarly, Farooq *et al.* (2013) reported an average semen volume of 7.20 ± 0.38 ml from Cholistani bulls. In contrast, other studies reported lower volumes, including Mahmood *et al.* (2014), and Farooq *et al.* (2015), who observed volumes of 2 to 4.5 ml, 4.5 ± 0.22 to 5.1 ± 0.07 ml, and 3.53 ± 0.08 to 3.36 ± 0.12 ml in Tharparkar, Cholistani, and Gir bulls respectively. Rehman *et al.* (2014) reported a higher range (9 to 12 ml) in Holstein and Sahiwal cattle. The normal semen volume in bulls typically ranges from 1 to 10 ml (Kumar, 2015). These variations can be attributed to differences in breed, age, testicle size, frequency of semen collection, body weight, and semen collector proficiency (Shaheen *et al.*, 2023).

All four bulls produced semen with a creamy white appearance, indicating good concentration and quality. This observation aligns with the reports of Bremer (2023), Statham *et al.* (2019). Who observed similar color in Cholistani, Tharparkar, and Sahiwal bulls. However, Kumar (2021) observed a wider range of colors (creamy to watery) in Gir bulls. Variations in semen color may be influenced by breed, age, nutrition, and overall animal health.

The pH of fresh semen ranged from 6.59 ± 0.03 to 6.67 ± 0.03, within the normal physiological range of 6.1 to 7.4 for bull semen (Dina, 2013). These findings are similar to those reported by Elmi (2020), who recorded pH values ranging from 6.6 ± 0.08 to 6.9 ± 0.09, 6.7 ± 0.40 to 6.9 ± 0.43, and 6.56 ± 0.04 to 6.61 ± 0.04, respectively. Conversely, Dina (2013) reported lower pH values (5.15 to 6.53). Variations in semen pH may be due to breed, season, age, and semen handling practices.

Wave motion, an important parameter to assess semen quality, was observed as ++++ in all four bulls, indicating strong and vigorous motility. These findings are consistent with Dina (2013), and Elmi (2020), who reported similar wave motion scores in Cholistani, crossbred, and Tharparkar bulls.

Motility in fresh semen ranged from 87.81 ± 0.79% to 89.36 ± 0.43%, surpassing the 60% threshold recommended for cryopreservation (Arif *et al.*, 2025). This is comparable to values reported by Elmi (2020), who observed motility between 85.1±0.68% and 90.63±0.56%. However, lower motility values were noted by Dina (2013), likely due to differences in bull age, breed, and semen collection practices.

Morphologically normal spermatozoa were observed in the range of 85.31 ± 0.82% to 88.19 ± 0.57%, which meets the minimum standard (70%) for breeding soundness (Palmer, 2021). Who reported 80.3 ± 0.83% to 88.7 ± 0.83% in Tharparkar bulls. They are slightly lower than Patel *et al.* (2024) but higher than reports by (Singh, 2015).

Sperm viability ranged from 82.25±1.76% to 85.37±0.93%. These findings agree with Naik (2019), though Patoo (2013), reported slightly higher values (91.00 ± 1.16%) in Tharparkar bulls. Differences may stem from breed, nutritional status, or semen processing techniques.

Functional membrane integrity, a critical parameter for sperm cryosurvival, was observed between 72.81 ± 1.67% and 78.69 ± 0.92%. These values align with results from Dina (2013) but are slightly lower than those reported by Elmi (2020). Such differences could be attributed to semen extender quality, individual bull variability, and semen handling.

Sperm concentration ranged from 995 × 10^6 to 1160 × 10^6/ml, similar to findings by Ambar *et al.* (2024). Slightly higher values were reported by Dina (2013). These variations can result from genetic potential, collection frequency, and environmental factors.

During the cooling phase, semen extended in Triladyl® consistently outperformed semen extended in Tris across all measured parameters. Cooled motility in Triladyl® (74.69 ± 0.62%) was significantly higher than in Tris (69.94 ± 0.59%), consistent with Shah *et al.* (2023), who reported motility values of 71.1 ± 1.26% to 76.3 ± 0.65% and 75.5 ± 0.61% in Tharparkar bulls. However, these values were slightly lower than Sukirman *et al.* (2020), who reported motility of 81.50 ± 1.98%, likely due to differences in breed or extender composition.

Post-cooled morphology was also better preserved in Triladyl® (80.25 ± 0.97%) than in Tris (76.44 ± 0.89%),

in agreement with findings of (Elmi, 2020). These results are slightly lower than those reported by Singh *et al.* (2014), who observed 88.00 ± 1.07% in Tharparkar bulls, potentially due to extender formulation or handling procedures.

Cooled viability was higher in Triladyl® (78.50 ± 0.92%) compared to Tris (75.31 ± 0.96%), aligning with Shah (2023), who noted 76.8 ± 0.82% to 81.3 ± 0.21%. Again, Singh *et al.* (2014) reported a higher value (88.00 ± 1.07%), possibly due to genetic or environmental differences.

Functional membrane integrity post-cooling was also superior in Triladyl® (72.50 ± 1.36%) versus Tris (68.56 ± 1.28%). These findings are consistent with Shah (2023) and Singh *et al.* (2014), although the values were lower than the latter's (78.00 ± 1.94%). These observations confirm that Triladyl® offers better protection during the cooling phase, possibly due to its proprietary buffer and cryoprotectant composition. Triladyl® again demonstrated superiority in cryopreservation. Frozen motility in Triladyl® (57.31 ± 0.67%) was higher than in Tris (54.75 ± 0.81%). This finding is supported by Rauf *et al.* (2022), and Elmi (2020).

Frozen morphology was also higher in Triladyl® (76.81 ± 0.79%) than in Tris (74.56 ± 0.91%), consistent with Naz (2018). These results exceed values reported by Singh *et al.* (2014) and who recorded 55.70 ± 3.15% and 61.88 ± 1.22% in Tharparkar and buffalo bulls, respectively.Frozen viability followed the same trend: Triladyl® yielded 73.75 ± 0.75%, outperforming Tris at 71.063 ± 0.90%. These values align with Elmi (2020) but exceed those reported by Singh *et al.* (2014), confirming Triladyl®'s superior protective capabilities during freezing.

Functional membrane integrity was also highest in Triladyl® (62.74 ± 0.96%) versus Tris (51.37 ± 0.96%). These findings match closely with Elmi (2020) and Singh *et al.* (2014). Collectively, these results clearly demonstrate that Triladyl® is a more effective extender than laboratory-formulated Tris for preserving semen quality during cooling and freezing in Cholistani cattle bulls. Factors such as optimized composition, cryoprotectant efficacy, and extender stability likely contribute to these improved outcomes.

CONCLUSION

This study demonstrated that Cholistani bull semen shows high quality in fresh state and maintains superior post-thaw characteristics when extended with Triladyl® compared to Tris. Triladyl® significantly outperformed Tris in preserving motility, morphology, viability, and membrane integrity during both cooling and freezing. These findings emphasize the critical role of extender choice in optimizing semen preservation and artificial insemination outcomes for indigenous cattle breeds. Use of commercial extenders like Triladyl® is recommended for enhancing the reproductive efficiency of Cholistani bulls.

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NOVELTY STATEMENT

This study is the first to provide a comparative assessment of commercial Triladyl® and laboratory-formulated Tris extenders on post-chilling and post-thaw semen quality of Cholistani bulls, a genetically distinct zebu breed native to Pakistan. The findings establish evidence-based insight into optimal cryoprotective extender selection for improved semen preservation in indigenous breeds.

AUTHOR'S CONTRUBATION

AK: Led the study design, supervision, data interpretation, and manuscript preparation. AAM and NAS: Contributed equally to the experimental work, data analysis, and manuscript drafting. AK and AAL: Assisted in laboratory procedures. SNS: Supported data organization and literature review. IUR: Managed data curation. FURS: Facilitated logistics. MU: Assisted in manuscript review. All authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the design, execution, or inter pretation of this research study. AI tools were not used to create, analyze, or modify any part of the manuscript. All content and intellectual input are solely the work of the authors

Conflict of interest

The authors have declared no conflict of interest

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