RESEARCH ARTICLE

Curcumin and L-Cysteine Supplementation in Tris Extender Improves Freezability of Jamnapari Buck Semen

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Abstract

The purpose of the study was to assess how varying concentrations of curcumin and L cysteine HCl affect Jamnapari buck semen's ability to withstand cryopreservation-induced cryodamage. Four Jamnapari bucks, aged 2-3 years, participated in the current investigation. Twenty-four ejaculates (six from each buck) were collected and diluted with Tris-Egg-Yolk-Citric acid-Fructose-Glycerol extender containing no additives T0 (control), and that supplemented with Curcumin T1, T2 (Curcumin 1 & 2 µg/mL), and L-cysteine HCl T3 & T4 (L cysteine 2 & 5 mM/L), respectively. The samples were cryopreserved in accordance with standard procedures. As compared to T0 (control), the sperm motility, liveability and HOS reactivity were significantly ($p<0.05$) improved, whereas the sperm abnormality and leakage of enzyme, transaminases (AST-ALT) and phosphatases (ACP-AKP) were significantly declined in T1, T2, T3 and T4 groups, particularly in treatment groups T2 and T3. Thus, supplementing Jamnapari buck semen with 2 µg/mL curcumin and 2 mM/L L-cysteine HCl in Tris extender appears to be helpful in reducing cryopreservation-induced cryodamage.

Keywords: Cryodamage, Cryopreservation, Curcumin, Extender, Jamnapari buck semen, L-cysteine.

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Introduction

Goats can more easily obtain genetic material for artificial insemination (AI) through cryopreservation of semen (Leboeuf *et al.,* 2000). However, frozen-thawed semen decreases motility and viability because cryopreservation and thawing causes harmful alterations in the sperm's membrane and ultrastructure (Watson, 2000) as a result of excessive free radical production. A variety of substances have been tried and tested as semen additives to reduce cryopreservation-induced cryodamage in domestic animals, with varying degrees of success (Verma *et al.,* 2023; Alam *et al.,* 2025; Kumar *et al*., 2026). Additionally, curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a physiologically active polyphenolic molecule found in turmeric, is a great scavenger of free radicals. *In vitro*, curcumin inhibited activated macrophages' generation of free radicals, such as superoxide anions, H_2O_2, and nitrite radicals, all of which are responsible for inflammation (Mathuria and Verma,2008). Curcumin supplementation reduced cryopreservation-induced cryo-damage in sperm cells of buffalo bulls (Khalil *et al.,* 2025). Additionally, cysteine supplementation reduces the harmful effects caused by cryopreservation in the semen of buffalo bulls (Panwar *et al.,* 2024), Zaraibi bucks (Altyeb *et al.,* 2022), and bulls (Hayanti *et al.,* 2022). Antioxidant properties of cysteine improved the quality of dromedary camel semen that was cooled and preserved (Seyedasgari *et al.,* 2024). The objective of the study was to examine the effects of varying concentrations of curcumin and L cysteine on key characteristics of frozen-thawed semen of Jamnapari buck

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Materials and Methods

Animals and Sampling

Four Jamnapari bucks, 2-3 years old, were included in the experiment. They were kept under similar feeding and management settings at the Deep Frozen Semen Lab of Veterinaery College, ANDUAT, Ayodhya, Uttar Pradesh (India). Using an artificial vagina (at 39 to 41°C), six ejaculates were obtained from each buck, making a total of 24 ejaculates from

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4 bucks. Pre-freeze and post-thawed semen were evaluated for various seminal characteristics.

Preparation of Diluter

On the day of semen collection, the glycerolated egg yolk tris dilutor was prepred as per the standard formula with final osmolarity of 410 mOsm/litre and a pH of 6.8-6.9 (Gangwar *et al.,* 2015). The ejaculated semen samples were diluted with the above dilutor (ocular motility ≥ 70%). Following dilution, the ejaculates were divided into 5 equal aliquots and were categorized as T0 (control, no additive), T1 & T2 with 1.0 and 2.0 µg/mL curcumin (w/v) (Sigma-Aldrich, USA), and T3 & T4 with 2 and 5 mM/L L-Cysteine hydrochloride (w/v) (USB Corporation Cleveland, USA). After evaluating the progressive sperm motility, French mini straws (0.25 mL) were filled, sealed and printed using the automatic filling and sealing equipment (ISEVO, IMV, France). Soon after, the sperm viability, morphology, and HOS reactivity were evaluated in the freshly diluted samples as per Gangwar *et al.* (2015).

Cryopreservation of Semen

The straws (0.25 mL, 80 million sperm per straw) were equilibrated (at 4°C for 4 h) in a cold cabinet before being exposed to liquid nitrogen vapour for 10 min (the straws were kept 2.5 cm above the liquid nitrogen level in a Styrofoam ice box). Lastly, the straws were placed in an LN_2 container for 24 h. The frozen straws were thawed for 30 sec at 37°C for post-thaw assessment.

Evaluation of Sperm Quality Parameters

The progressive sperm motility, viability, morphology, and HOS reactivity (Gangwar *et al.,* 2015) were evaluated in the pre-freeze (freshly diluted) and in frozen-thawed semen using standard laboratory procedures.

The seminal plasma from freshly diluted and frozen-thawed samples was separated by centrifugation at 1100 x g for 20 min in order to test for enzyme leakage of glutamic oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), alkaline phosphatase (AKP), and acid phosphatase (ACP) using standard kits (CliniQuant TM micro) and a Merilizer machine (Meril Diagnostic Pvt. Ltd.).

Statistical Analysis

Graph Pad Prism 5 was used to analyze the data (mean ±SE). The factorial model used pre-freeze and post-thaw seminal characteristics as dependent factors and the effects of curcumin and L cysteine as independent variables. The differences between the treatment groups were evaluated using an ANOVA. To determine whether there was a significant difference between various curcumin and L cysteine concentrations at $p<0.05$, a post hoc Tukey's test was performed.

Results and Discussion

Almost all paramerters studied were improved with both the additives as compared to control group, immediately on dilution of semen, however, the magnitude of change was dose-dependent and most appreciable ($p<0.05$) with 2 µg/mL curcumin and/or 2 mM/L L-cysteine HCl compared to other levels. Furthermore the effect was most pronounced with both the levels of curcumin and lower level of L-cycteine, while higher level of L-cysteine was detrimental/ toxic on some of the sperm parameters at post-thaw stage (Table 1).

Progressive Sperm Motility

The T0 (control) group experienced a significant ($p<0.05$) decrease in sperm progressive motility; however, supplementing tris-extender with L-cysteine and curcumin reduced the fall in sperm motility caused by equilibration and cryopreservation, with the greatest effect observed in the T2 group (2.0 µg/mL curcumin (Table 1). The current mean post-thaw (PT) sperm motility was consistent with the report of Anghel *et al.* (2010). In contrast to the present results, other researchers found higher PT motility in 125 µmol/L curcumin-supplemented semen (Soleimanzadeh and Saberivand, 2013; Ismail *et al.,* 2020) and lower in 250 µmol/L curcumin-supplemented semen (Naz, 2011). Curcumin's capacity to increase antioxidant status, mitochondrial function, and membrane integrity while decreasing apoptosis may be the cause of its positive effects (An *et al.,* 2025). Curcumin appears to have a dose-dependent effect on semen, being advantageous at lower quantities and harmful at greater amounts.

Panwar *et al.* (2024) reported that cysteine supplementation improves mammalian sperm cells. Cysteine, an α-amino acid, is a precursor of intracellular glutathione (GSH) synthesis , which neutralizes the reactive species of oxygen and catalyzes hydrogen or other superoxide detoxification (Memon *et al.,* 2011); can protect the sperm membrane function from the damaging effect of free radicals by directly scavenging free radicals (Bilodeau *et al.,* 2001), thus improving post-thawed seminal quality of buck semen (Adel *et al.,* 2024).

Sperm Viability

The treatment groups' sperm viability was significantly ($p<0.05$) higher than the control group (Table 1). Supplementing extender with 2 µg/mL Curcumin (T2) and 2 mM/L L-cysteine (T3) appeared beneficial in reducing the loss of membrane function caused by cryopreservation. Although not as much as the other doses of curcumin and L-cysteine, the addition of 1 µg/mL curcumin (T1) and 5 mM/L L-cysteine (T4) also considerably increased PT sperm viability. The PT viability mean values in the untreated control group matched the findings of Kulaksiz *et al.* (2020). Memon *et al.* (2011) found that semen supplemented with cysteine had decreased PT-sperm viability, while Ismail *et al.* (2020) found that semen supplemented with curcumin had increased PT sperm viability.

Curcumin may help to reduce the damage caused by H_2O_2 to sperm (Zhang *et al.,* 2017). A derivative of the amino acid

Table 1: Effect of different concentrations of Curcumin (T1 and T2) and L-Cysteine (T3 and T4) on seminal attributes and enzyme leagae of Jamnapari buck semen at pre-freeze (on dilution) and post-thaw stage (Mean ± SE, n=24)

Parameters	Pre-freeze					Post-thaw				
	T0 (Control)	T1 (1 µg/mL)	T2 (2 µg/mL)	T3 (2 mM/L)	T4 (5 mM/L)	T0 (Control)	T1 (1 µg/mL)	T2 (2 µg/mL)	T3 (2 mM/L)	T4 (5 mM/L)
Sperm motility (%)	78.67 ±0.88^{a}	79.83 ±0.94^{b}	80.50 ±0.76^{b}	79.67 ±0.80^{b}	79.33 ±0.84^{ab}	48.33 ±0.66^{a}	50.33 ±0.66^{b}	52.67 ±0.66^{c}	51.67 ±0.66^{d}	50.67 ±0.71^{b}
Liveability (%)	86.33 ±0.66^{a}	86.83 ±0.01^{ab}	89.17 ±0.83^{b}	88.50 ±0.84^{ab}	87.50 ±0.61^{ab}	75.00 ±0.96^{a}	77.50 ±0.99^{b}	80.33 ±1.14^{c}	79.50 ±1.17^{c}	77.67 ±0.95^{b}
Abnormality (%)	8.33 ±0.76^{a}	6.66 ±0.49^{b}	5.50 ±0.42^{b}	6.50 ±0.61^{b}	7.33 ±0.66^{ab}	13.17 ±1.40^{a}	10.00 ±1.18^{b}	7.16 ±0.60^{c}	8.00 ±0.63^{bc}	9.00 ±0.57^{bc}
HOS (%)	49.00 ±1.06^{a}	50.83 ±0.94^{ab}	52.50 ±0.76^{b}	51.83 ±0.94^{b}	51.00 ±1.33^{b}	34.80 ±1.62^{a}	41.00 ±1.82^{b}	45.00 ±1.26^{c}	45.67 ±1.08^{c}	42.33 ±1.56^{b}
GOT(µmole/L)	60.33 ±2.72^{a}	58.17 ±2.82^{b}	54.00 ±2.44^{b}	55.00 ±2.78^{b}	56.33 ±3.45^{ab}	137.00 ±4.72^{a}	133.50 ±4.82^{b}	127.70 ±3.80^{c}	130.70 ±4.75^{c}	132.30 ±4.71^{b}
GPT (µmole/L)	119.00 ±3.20^{a}	115.70 ±2.74^{b}	110.50 ±2.23^{c}	112.00 ±2.25^{cd}	114.50 ±2.29^{bd}	263.00 ±4.49^{a}	250.00 ±5.53^{b}	244.30 ±4.33^{b}	246.30 ±4.40^{b}	254.20 ±4.23^{b}
ACP (KAU/100 mL)	27.17 ±1.85^{a}	24.33 ±1.43^{b}	20.83 ±1.47^{c}	22.17 ±1.47^{bc}	25.17 ±1.24^{ab}	59.10 ±1.35^{a}	56.67 ±1.99^{b}	54.67 ±0.76^{b}	56.33 ±1.30^{b}	57.17 ±1.24^{b}
AKP (KAU/100 mL)	43.10 ±2.60^{a}	41.00 ±2.56^{ab}	37.83 ±2.33^{bc}	37.33 ±1.83^{c}	42.33 ±2.39^{ab}	151.00 ±5.09^{a}	141.50 ±4.75^{ab}	132.70 ±4.44^{bc}	135.00 ±3.72^{bc}	140.00 ±4.86^{bc}

Means bearing different superscripts (a-c) in a row differed significantly ($p<0.05$).

L-cysteine, N-acetyl-cysteine (NAC) aids in the synthesis of glutathione (GSH) and may help replenish the GSH pool that has been depleted due to oxidative stress and inflammation (Wang *et al.,*2014).

Sperm Abnormalities

Curcumin and L-cysteine HCl considerably ($p<0.05$) reduced PT sperm abnormalities compared to control (T0) (Table 1). Treatment T2 showed largest drop, followed by T3, T4, and T1. The current findings were in good agreement with earlier research (Khalil *et al.,* 2025). Through intracellular antioxidants that guard against the harmful effects of ROS, cysteine acts as an antioxidant either directly or indirectly (Perumal *et al.,* 2011).

Hypo-Osmotic SwellingTest

When cucumin and L-cysteine HCl were added, the post-thaw HOS reactive sperm in all treatment groups were significantly ($p<0.05$) higher than the control (Table 1). In comparison to the control, the PT HOS positive sperm were considerably greater in T2 and T3, followed by T1 and T4. These results were consistent with numerous earlier investigations (Elsheshtawy, 2020; Khalil *et al.,* 2025). The HOS test indicates a favorable relationship between mitochondrial activity and plasma membrane integrity. The results of this study show that plasma membrane integrity (viability) and functionality (HOS test) can be effectively improved by supplementing with L-cysteine and curcumin in the semen extender.

Effects on Enzymatic Activity of semen Before and After Freezing

Table 1 shows the various enzymatic activities during the pre-freeze and post-thaw phases. When compared to treatment groups, the PT GOT value was substantially greater in T0. Among the treatment groups, the GOT activity significantly decreased in T2 and T3, than T1 and T4. Additionally, while the values in all treatment gropus were apparently lower than the untreated control (T0), the GPT and ACP values did not differ significantly among treatment groups T1 to T4. The T0 group's AKP value was much lower than that of T2, T3, and T4, but it did not vary significantly from T1. Chaudhary *et al.* (2022) recorded considerably lower GOT activity in curcumin (25 µM) supplemented bull semen compared to control, which is consistent with current findings. Transaminase is highly concentrated in the extracellular fluid as a result of sperm membrane breakage and the ease of enzyme leakage from spermatozoa (Gundogan, 2006). According to Uysal and Bucak (2007), cysteine is an intracellular glutathione precursor that readily penetrates cell membranes, increasing intracellular reduced glutathione (GSH) production, both *in vivo* and *in vitro,* protecting membrane lipids and proteins by indirect radical scavenging.

Conclusion

The supplementing Jamnapari buck semen with 2 µg/mL curcumin and 2 mM/L L-cysteine HCl through Tris extender appears to be helpful in reducing cryopreservation-induced cryodamage improving post-thaw sperm quality, antioxidant status and membrane intrgrity conserving enzymes transaminases and phosphatases in the sperm cell, which in tern expected to improve the *in vivo* fertility of that semen.

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