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Curcumin as a natural modulator of semen quality in endangered Teressa goats in tropical island conditions

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ABSTRACT

The present study was designed to evaluate the effect of curcumin supplementation in the semen extenders on the semen quality parameters of Teressa goat bucks. A total of 25 semen samples were collected from six healthy bucks and processed for liquid storage. Spermatozoa were standardized to a concentration of 4 × 10^8/mL and incubated with curcumin at concentrations of 1 mM, 2 mM, and 4 mM, corresponding to Groups II, III, and IV, respectively. A control group (Group I) without curcumin served as the baseline for comparison. The semen samples were stored at refrigeration temperature and assessed at multiple time points up to 72 h for various semen quality parameters which included the sperm viability, motility, total sperm abnormalities, acrosomal integrity, plasma membrane integrity, nuclear integrity and biochemical markers such as intracellular enzymatic leakage [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)], cholesterol efflux, malondialdehyde (MDA) and total antioxidant capacity (TAC) levels. The present study results revealed that the semen samples treated with 2 mM curcumin (Group III) showed significantly ($P < 0.05$) higher viability, motility, acrosomal integrity, plasma membrane integrity, nuclear integrity and TAC. Concurrently, Group III also exhibited significantly ($P < 0.05$) lower total sperm abnormalities, leakage of intracellular enzymes (ALT, AST and LDH), MDA production and cholesterol efflux as compared to those in the control and other curcumin-treated groups. Trends across the treatment groups showed a progressive improvement in the semen quality and antioxidant concentrations from Group I to Group III, followed by a decline in Group IV (4 mM) which indicated a dose-dependent effect. Therefore, 2 mM curcumin was identified as the optimal concentration of curcumin for enhancing the semen quality and preserving the functional and structural integrity of spermatozoa during liquid storage in the Teressa goats.

Keywords: Andaman and Nicobar Islands, Curcumin, Semen, Teressa goat

Teressa goat is an endangered breed indigenous to the Nicobar Islands of the Andaman and Nicobar Islands (ANI), India. It requires urgent conservation through both *in-situ* and *ex-situ* strategies (Jeyakumar *et al.* 2020). Significant seasonal climatic variations particularly during the dry summer months, the reproductive efficiency and overall productivity of these goats were affected. According to the Government of India's Livestock Census, the goat population in ANI has declined by 4.25% between 2007 and 2019. This decline has been attributed to factors such as intensive inbreeding, lack of genetically superior breeding bucks, poor breeding management practices and the effect of climate change on these animals.

One strategy to improve the reproductive efficiency in the endangered caprine breeds is semen preservation and artificial insemination. Liquid storage of semen at low temperatures (typically 5 °C) is employed to slow down the sperm metabolism and extend its viability. Tris-egg yolk-glycerol-based extenders have been used effectively to preserve the goat semen under refrigerated conditions for up to a period of seven days (El-Battawy 2019). However, the extended storage is often associated with a decline in the sperm quality due to oxidative stress. The reactive oxygen species (ROS) are produced by the cellular components of semen and contribute to the lipid peroxidation which in turn damage the sperm membranes, impair the motility, disrupt the DNA integrity and ultimately reduce the fertility (Perumal *et al.* 2011).

To counteract these oxidative stress effects, researchers have experimented with different antioxidant additives such as taurine, trehalose, selenium, glutathione, catalase, superoxide dismutase and various surfactants to improve the functional and structural integrity of spermatozoa during the storage (Uysal *et al.* 2005).

Among the natural antioxidants, curcumin the active biomolecule of turmeric extract, has attracted the interest due to its potential free radical scavenging activity. Curcumin possesses a range of pharmacological properties such as anti-apoptotic, antioxidant, anti-inflammatory, anti-cancer and anti-toxic effects (Rashid and Sil 2015). Its antioxidant potential is largely attributed due to the presence of phenolic and methoxy groups on its aromatic

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rings and a β-diketone structure (Priyadarsini 2009).

Several studies have reported the positive effects of curcumin as a semen extender additive in various species, where it has enhanced the sperm motility, viability and membrane integrity while it reduces the oxidative stress and lipid peroxidation (Chanapiwat and Kaeoket 2015; Omur and Coyan 2016; Tvrda *et al.* 2016; Shah *et al.* 2016; Bucak *et al.* 2012; Sultan 2023; Omidi *et al.* 2024). However, effect of curcumin on the sperm function is concentration-dependent; both beneficial and detrimental effects have been documented based on the dosage (Naz 2011). Therefore, species-specific optimization of curcumin concentration is important before its application in semen preservation protocols in caprine species.

Therefore, the present was undertaken to study the dose dependent effects of curcumin in the semen extenders on the semen quality parameters, enzymatic leakage, and oxidative stress profiles in the caprine species particularly in the endangered Teressa goat.

MATERIALS AND METHODS

Location of the study: The present study was conducted at ICAR-Central Island Agricultural Research Institute (ICAR-CIARI), Sri Vijaya Puram; located in Andaman and Nicobar Islands, India. The experimental site lies between 6°45′ to 13°41′ North latitude and 92°12′ to 93°57′ East longitude. The trial was carried out during the peak of the dry summer season during the months of January and February. During this period, the environmental conditions were characterized by a total rainfall of 125.80 mm in January and 13 mm in February with the corresponding temperature-humidity index (THI) of 84.16 and 83.73 and the average daylight durations of 8.54 and 9.37 h, respectively.

Experimental animals: Six healthy Teressa goat bucks, aged between 3 and 4 years and weighed between 34 and 36 kg with the body condition scores ranged from 2.5 to 3.5 (optimal health and nutritional status) were selected for the present study. The animals were reared under a semi-intensive system with access to the natural pasture from 07:00 to 12:00 h and housed in the well-ventilated sheds during the remaining part of the day. All the bucks were maintained under the uniform management conditions in accordance with the standard farm schedule which included the regular deworming, vaccination, disease prevention, penile hair trimming, and hoof care.

Semen collection: Ejaculates were collected from each Teressa buck twice weekly between 06:00 and 07:30 h with use of a standard artificial vagina technique. Two ejaculates per buck were collected at one-hour interval. Immediately post-collection, these semen samples were placed in a water bath which was maintained at 37 °C and evaluated for the initial semen quality parameters such as volume, colour, pH, sperm concentration and mass activity.

Ejaculates exhibiting abnormal colour, pH outside the physiological range or insufficient volume were discarded. The remaining samples were screened based on the Minimum Standard Protocol (MSP) criteria: sperm concentration >2.5 × 10^9/mL, mass activity >3+, individual progressive motility >70% and total morphological abnormalities ≤10%. Based on these criteria, 50 out of 72 ejaculates (6 bucks × 12 collections) were selected for further analysis.

Selected ejaculates were pooled in pairs from the same individual (termed a "sample," n = 25) to minimize the individual variation. Each pooled sample was initially diluted in twofold the pre-warmed (37 °C) caprine-specific Tris-fructose-citrate-egg yolk (TFCE) extender. The partially extended samples were then transferred in insulated flasks containing warm water (37 °C) to the andrology laboratory for further processing.

Semen extender preparation: A Tris-fructose-citrate-egg yolk (TFCE) semen extender was prepared to use in the present experiment. The extender composition was 2.4 g Tris, 1.4 g citric acid, 1.0 g fructose, 20 mL fresh egg yolk, 100 µg/mL penicillin G sodium and 100 mg/mL streptomycin with double-distilled water added to make upto a final volume of 100 mL. Curcumin was obtained from HiMedia Laboratories (Mumbai, Maharashtra, India).

The prepared extender was divided into four equal aliquots. Varying concentrations of curcumin were added to create the four treatment groups: Group I (Control): 0 mM curcumin, Group II: 1 mM curcumin, Group III: 2 mM curcumin, and Group IV: 4 mM curcumin. Each mixture was homogenized with use of a magnetic stirrer for 15 min, filtered to ensure the clarity and maintained in a water bath at 37 °C prior to the semen extension. Fresh semen samples were gradually diluted with the curcumin-supplemented TFCE extender to achieve a final sperm concentration of 4 × 10^8 sperm/mL. Thus, the experimental groups varied by curcumin concentration in a consistent sperm concentration with Group I was served as the unsupplemented control.

In the laboratory, the diluted semen samples were transferred to the sterile glass tubes and subjected to controlled cooling from 37 °C to 5 °C at a rate of 0.2–0.3 °C per min with use of a step-down cooling process. Once the target temperature of 5 °C was reached, the samples were stored at this temperature throughout the experimental duration. Semen quality parameters were assessed at six time points: 30 min, 12 h, 24 h, 48 h, 60 h, and 72 h post-dilution.

Semen evaluation: Standardized procedures were used to evaluate the key seminal parameters. Sperm motility was assessed as described by Perumal *et al.* (2011), while sperm viability and total morphological abnormalities were determined with use of Eosin–Nigrosin staining technique following the method of Agarwal *et al.* (2016). Acrosomal integrity was evaluated using Giemsa staining technique as per the method described by Selvaraju *et al.* (2008). Plasma membrane integrity was determined through the hypo-osmotic swelling test (HOST) as described by Nur *et al.* (2011) and the nuclear integrity was assessed with use of Feulgen's staining technique according to Barth and Oko (1989).

Biochemical assays: Biochemical analyses were conducted to evaluate the oxidative stress markers, enzymatic activity and antioxidant capacity in semen. Lipid peroxidation and total cholesterol levels were measured in spermatozoa whereas ALT, AST, LDH and TAC were measured in the seminal plasma.

Lipid peroxidation in the spermatozoa was quantified by measuring the MDA production with use of the thiobarbituric acid (TBA) method as described by Suleiman *et al.* (1996). The total antioxidant capacity of the seminal plasma (expressed in mM/L) was measured using a TAC colorimetric assay kit (Cayman Chemical Co., USA) in accordance with the manufacturer's instructions. Total cholesterol concentration in the spermatozoa was measured using a commercial cholesterol assay kit and the results were expressed as µg cholesterol per 10^8 spermatozoa. The intracellular enzymatic activities (ALT, AST, and LDH) in the seminal plasma were estimated using the diagnostic

Table 1. Comparison of quality parameters of liquid stored Teressa goat spermatozoa following preservation with curcumin (0 mM, 1 mM, 2 mM and 4 mM) (Mean ± SEM)

			Total Motility			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	84.65 ± 0.82^{aA}	69.44 ± 0.55^{aB}	60.53 ± 0.92^{aC}	51.77 ± 0.62^{aD}	42.68 ± 0.62^{aE}	37.24 ± 0.62^{aF}
Gr 2	85.76 ± 0.89^{aA}	76.61 ± 0.87^{bB}	68.77 ± 0.75^{bC}	60.38 ± 0.77^{bD}	51.19 ± 0.56^{bE}	46.21 ± 0.49^{bF}
Gr 3	86.54 ± 0.73^{aA}	81.34 ± 0.98^{cAB}	77.85 ± 0.87^{cBC}	73.48 ± 0.66^{cC}	67.79 ± 0.69^{cD}	57.39 ± 0.69^{cE}
Gr 4	85.12 ± 0.68^{aA}	71.26 ± 0.64^{aB}	62.38 ± 0.84^{aC}	53.92 ± 0.75^{aD}	44.28 ± 0.87^{aE}	39.85 ± 0.58^{aF}
			Viability			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	85.68 ± 1.17^{aA}	71.37 ± 0.67^{aB}	62.46 ± 0.73^{aC}	52.57 ± 0.63^{aD}	43.66 ± 0.74^{aE}	38.07 ± 0.73^{aF}
Gr 2	86.81 ± 1.21^{aA}	79.65 ± 0.73^{bB}	71.56 ± 0.68^{bC}	62.46 ± 0.75^{cD}	53.16 ± 0.65^{bE}	47.43 ± 0.69^{cF}
Gr 3	86.76 ± 0.78^{aA}	85.09 ± 0.54^{cA}	79.92 ± 0.63^{cB}	76.87 ± 0.86^{dC}	69.18 ± 0.76^{cD}	58.55 ± 0.84^{dE}
Gr 4	85.23 ± 0.95^{aA}	73.48 ± 0.69^{aB}	64.59 ± 0.76^{aC}	55.58 ± 0.73^{bD}	45.51 ± 0.67^{aE}	41.27 ± 0.78^{bF}
			Total Sperm Abnormality			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	6.12 ± 0.24^{aA}	9.27 ± 0.34^{bB}	12.49 ± 0.56^{bC}	13.94 ± 0.45^{dD}	15.49 ± 0.43^{cE}	17.05 ± 0.45^{cF}
Gr 2	6.33 ± 0.23^{aA}	8.16 ± 0.37^{abB}	9.05 ± 0.34^{aB}	11.72 ± 0.53^{bC}	13.27 ± 0.54^{bD}	14.38 ± 0.43^{bD}
Gr 3	6.36 ± 0.25^{aA}	7.15 ± 0.45^{aB}	8.16 ± 0.34^{aC}	9.07 ± 0.42^{aC}	10.49 ± 0.65^{aD}	11.16 ± 0.35^{aD}
Gr 4	6.41 ± 0.20^{aA}	9.38 ± 0.35^{bB}	11.43 ± 0.44^{bC}	12.94 ± 0.51^{cD}	14.38 ± 0.43^{cE}	15.05 ± 0.46^{bE}
			Acrosomal Integrity			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	84.36 ± 0.89^{aA}	71.25 ± 0.72^{aB}	63.15 ± 0.67^{aC}	52.84 ± 0.73^{aD}	45.32 ± 0.62^{aE}	40.34 ± 0.54^{aF}
Gr 2	85.67 ± 0.92^{aA}	79.35 ± 0.65^{bB}	72.96 ± 0.69^{bC}	62.25 ± 0.56^{bD}	54.45 ± 0.72^{bE}	47.85 ± 0.67^{bF}
Gr 3	86.45 ± 0.75^{aA}	85.86 ± 0.76^{cA}	79.25 ± 0.83^{cB}	75.72 ± 0.78^{cC}	69.83 ± 0.63^{cD}	54.56 ± 0.68^{cE}
Gr 4	85.55 ± 0.68^{aA}	73.45 ± 0.67^{aB}	64.36 ± 0.64^{aC}	54.61 ± 0.56^{aD}	47.28 ± 0.67^{aE}	42.16 ± 0.73^{aF}
			Plasma membrane Integrity			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	87.34 ± 1.14^{aA}	72.35 ± 1.22^{aB}	63.55 ± 0.74^{aC}	53.92 ± 0.65^{aD}	46.63 ± 0.63^{aE}	41.12 ± 0.75^{aF}
Gr 2	88.42 ± 1.23^{aA}	80.86 ± 1.14^{bB}	72.26 ± 0.62^{bC}	63.86 ± 0.75^{bD}	55.93 ± 0.92^{cE}	50.74 ± 0.79^{cF}
Gr 3	87.98 ± 1.09^{aA}	86.48 ± 1.07^{cA}	80.34 ± 0.73^{cB}	76.33 ± 0.88^{cC}	70.84 ± 0.76^{dD}	54.37 ± 0.67^{dE}
Gr 4	86.49 ± 1.26^{aA}	74.37 ± 1.16^{aB}	65.76 ± 0.64^{aC}	56.15 ± 0.73^{aD}	48.65 ± 0.83^{bE}	44.58 ± 0.75^{bF}
			Nuclear Integrity			
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	84.73 ± 1.27^{aA}	73.71 ± 1.13^{aB}	67.93 ± 0.85^{aC}	56.63 ± 0.65^{aD}	49.34 ± 0.62^{aE}	42.74 ± 0.52^{aF}
Gr 2	85.31 ± 1.12^{aA}	81.16 ± 0.76^{bB}	74.59 ± 0.63^{bC}	65.46 ± 0.78^{bD}	58.45 ± 0.73^{bE}	51.47 ± 0.55^{bF}
Gr 3	85.65 ± 1.04^{aA}	85.22 ± 0.68^{cA}	83.76 ± 0.75^{cB}	78.67 ± 0.87^{cC}	74.49 ± 0.61^{cD}	59.56 ± 0.46^{cE}
Gr 4	85.37 ± 1.30^{aA}	75.34 ± 0.79^{aB}	68.23 ± 0.81^{aC}	57.16 ± 0.75^{aD}	49.67 ± 0.75^{aE}	43.63 ± 0.67^{aF}

Means bearing different superscripts within rows (A, B, C, D, E and F) and columns (a, b, c and d) differ significantly ($P < 0.05$), n = 25. Gr 1: Control (0 mM), Gr 2: 1 mM, Gr 3: 2 mM and Gr 4: 4 mM.

kits from the Span Diagnostics Ltd., India.

Statistical analysis: To evaluate the effects of treatment groups and storage durations on the studied parameters, a two-way analysis of variance (ANOVA) was performed with use of the Statistical Analysis Software (SAS, Version 9.3.1; SAS Institute, Inc., Cary, NC, USA). Post-hoc comparisons between the means were carried out with use of Duncan's Multiple Range Test. Data were expressed as mean ± standard error of the mean (SEM) and the differences were considered as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Semen from Teressa goat bucks typically exhibited a creamy white colour and displayed the following baseline seminal parameters: volume of 0.92 ± 0.23 mL, mass activity of 3.75 ± 0.05, pH of 6.88 ± 0.04, sperm concentration of 3.74 ± 0.07 × 10^9 spermatozoa/mL, motility of 86.16 ± 1.62%, viability of 87.32 ± 0.93%, total sperm abnormalities of 6.26 ± 0.16%, acrosomal integrity of 88.79 ± 1.10%, plasma membrane integrity of 87.60 ± 1.36% and nuclear integrity of 86.22 ± 0.67%. Biochemical analyses revealed TAC of 1.34 ± 0.06 mM/L, MDA level of 2.19 ± 0.03 nM/10^8 sperm, total cholesterol content of 26.99 ± 0.88 µg/10^8 sperm and enzyme activities of AST (43.26 ± 1.35 U/L), ALT (15.34 ± 0.34 U/L) and LDH (215.94 ± 3.10 U/L).

Among the treatment groups, the semen samples extended with 2 mM curcumin exhibited significantly ($P < 0.05$) higher viability, motility, plasma membrane integrity, nuclear integrity, acrosomal integrity and TAC levels as compared to those in the control (0 mM) and the other curcumin-treated groups (1 mM and 4 mM). Conversely, these samples also showed significantly ($P < 0.05$) lower total sperm abnormalities, intracellular enzymatic leakage (ALT, AST, and LDH), MDA production and cholesterol efflux during the different storage intervals up to 72 h (Table 1 and Table 2).

Curcumin has a considerable potential to enhance the semen quality and fertility across the various animal species both as a dietary supplement (Sultan 2023) as well as an additive in the semen extenders (Bucak *et al.* 2010; Soleimanzadeh and Saberivand 2013; Chanapiwat and Kaeoket 2015; Shah *et al.* 2016; Omur and Coyan 2016; Tvrda *et al.* 2018). In the present study, addition of 1 mM and 2 mM curcumin to a tris-based semen extender has improved several semen quality parameters in Teressa goat with 2 mM identified as the optimal concentration. Similar optimal concentrations were reported in buffaloes (Shah *et al.* 2016), bulls (Tvrda *et al.* 2018), rams and rats (Omur and Coyan 2016; Soleimanzadeh and Saberivand 2013), boars (Chanapiwat and Kaeoket 2015) and goats (Bucak *et al.* 2010).

A positive trend was observed in the semen quality parameters and antioxidant concentrations from Group I (control) to Group III (2 mM curcumin) whereas a reverse trend was observed from Group III to Group IV (4 mM curcumin). These findings suggested that a dose-dependent effect with 2 mM curcumin identified as the optimal concentration to improve the liquid semen preservation outcomes.

The effective concentration of curcumin appeared to be species-specific and was influenced by the semen extender formulation, processing methods and the preservation conditions (Tvrda *et al.* 2018). Various studies reported the optimal concentration of curcumin such as 50 µM in bovine (Tvrda *et al.* 2018), 1.5 mM in bubaline (Shah *et al.* 2016), 1–2 mM in ovine (Omur and Coyan 2016; Soleimanzadeh and Saberivand 2013), 2.5–10 mM in Angora goats (Bucak *et al.* 2010), and 0.25–0.5 mM in porcine spermatozoa (Chanapiwat and Kaeoket 2015). These differences indicated the necessity of species-specific dose standardization in caprine species under humid tropical regions of Andaman and Nicobar Islands.

In the present study, the spermatozoa treated with 2 mM curcumin (Group III) exhibited significantly higher motility which was associated with higher sperm function and metabolic activity. Curcumin enhances sperm motility by scavenging the ROS, stabilizing the sperm membranes and protecting its mitochondrial integrity (Omur and Coyan 2016). Conversely, higher curcumin concentration (Group IV) showed reduced motility due to impaired mitochondrial function which was consistent with the reports by Naz (2014). Curcumin has earlier been reported to improve the sperm capacitation, acrosome reaction and fertilization potential (Alizadeh *et al.* 2018). These processes are regulated by Ca^{2+} channels, intracellular pH, tyrosine phosphorylation and membrane potential (Naz, 2014) suggested that curcumin exerts its effects through modulation of these pathways. Moreover, it has shown positive effects in treating the leucocytospermia and asthenoteratospermia through oxidative stress reduction and Nrf2 pathway activation (Alizadeh *et al.* 2018; Zhou *et al.* 2020; Mahmoud *et al.* 2013). These concentration-dependent effects affirmed that the lower doses had enhanced the fertility whereas the higher doses exerted the cytotoxic effects (Naz 2011).

Curcumin at 2 mM preserved the structural integrity of the sperm membranes and is consistent with the reports in the bovine species (Bucak *et al.* 2012; Omidi *et al.* 2024). It integrates into the membrane structures (Hosseinzadeh *et al.* 2021), prevents lipid peroxidation and protects the acrosomal and nuclear integrity (Tvrda *et al.* 2018). In contrast, higher concentrations might have been earlier reported to induce the intracellular acidification, disrupted the sperm metabolism and decrease the functionality (Naz 2014) as observed with use of 4 mM curcumin in the present study.

The higher poly unsaturated fatty acid content of sperm membranes makes them more susceptible to lipid peroxidation which in turn affect the motility, DNA integrity and fertility (Perumal *et al.* 2018). Moreover, oxidative stress during the semen processing reduces the antioxidant levels in the seminal plasma (Kumar *et al.* 2011). Natural

Table 2. Comparison of biochemical attributes of liquid stored Teressa goat semen following preservation with curcumin (0 mM, 1 mM, 2 mM and 4 mM) (Mean ± SE)

	Total Cholesterol (μg/10^8 sperm)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	26.66±0.47^{aA}	19.64±0.54^{aB}	15.56±0.45^{aC}	12.83±0.36^{aD}	9.79±0.35^{aE}	6.35±0.47^{aF}
Gr 2	27.23±0.34^{aA}	22.86±0.62b^{cB}	18.94±0.56^{bC}	13.59±0.48^{aD}	10.42±0.38^{aE}	7.93±0.36^{aF}
Gr 3	28.54±0.23^{aA}	24.59±0.51^{cB}	21.48±0.72^{cC}	17.12±0.32^{bD}	14.85±0.46^{bE}	11.34±0.47^{bF}
Gr 4	27.63±0.41^{aA}	21.43±0.68^{abB}	19.24±0.59^{bcB}	13.56±0.41^{aC}	9.43±0.36^{aD}	8.54±0.38^{aD}
	Total antioxidant capacity (mM/L)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	2.04±0.03^{aA}	1.38±0.03^{aB}	1.16±0.04^{aBC}	1.05±0.04^{aCD}	0.94±0.03^{aDE}	0.83±0.04^{aE}
Gr 2	2.16±0.04^{aA}	1.39±0.04^{aB}	1.27±0.04^{abBC}	1.16±0.03^{abCD}	1.05±0.04^{abDE}	0.94±0.03^{abE}
Gr 3	2.07±0.05^{aA}	1.61±0.05^{bB}	1.38±0.05^{bC}	1.27±0.04^{bCD}	1.16±0.05^{bDE}	1.05±0.04^{bE}
Gr 4	2.12±0.03^{aA}	1.32±0.04^{aB}	1.27±0.04^{abBC}	1.05±0.04^{aCD}	0.94±0.03^{aDE}	0.83±0.03^{aE}
	Malondialdehyde (nM/10^8 sperm)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	2.83±0.05^{aA}	3.27±0.04^{bB}	3.83±0.04^{cC}	4.38±0.04^{cD}	4.94±0.05^{cE}	5.49±0.04^{cF}
Gr 2	2.68±0.04^{aA}	2.94±0.05^{abB}	3.49±0.03^{bC}	3.83±0.05^{bD}	4.27±0.03^{bE}	4.50±0.06^{bF}
Gr 3	2.54±0.06^{aA}	2.72±0.04^{aB}	2.94±0.04^{aC}	3.49±0.06^{aD}	3.83±0.04^{aE}	4.05±0.04^{aE}
Gr 4	2.73±0.04^{aA}	2.94±0.03^{abB}	3.49±0.05^{bC}	3.83±0.04^{bD}	4.38±0.06^{bE}	4.39±0.04^{bF}
	Aspartate amino transferase (U/L)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	44.76±0.64^{aA}	69.65±0.78^{cB}	84.22±0.77^{dC}	93.02±0.65^{dD}	102.75±0.78^{dE}	112.26±1.26^{dF}
Gr 2	45.61±0.62^{aA}	64.22±0.63^{bB}	76.83±0.76^{bC}	81.68±0.72^{bD}	85.53±0.76^{bE}	91.65±0.85^{bF}
Gr 3	45.24±0.56^{aA}	59.44±0.76^{aB}	71.61±0.56^{aC}	74.52±0.82^{aD}	78.34±0.65^{aE}	81.75±0.76^{aF}
Gr 4	45.15±0.48^{aA}	68.84±0.72^{cB}	81.37±0.75^{cC}	86.96±0.79^{cD}	91.85±0.59^{cE}	98.42±0.68^{cF}
	Alanine amino transferase (U/L)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	16.93±0.53^{aA}	23.85±0.48^{dB}	26.76±0.43^{dC}	30.08±0.54^{dD}	37.84±0.53^{cE}	43.75±0.81^{dF}
Gr 2	16.85±0.42^{aA}	21.95±0.32^{bB}	23.87±0.51^{bC}	25.73±0.43^{bD}	29.58±0.47^{bE}	32.24±0.76^{bF}
Gr 3	17.12±0.51^{aA}	18.64±0.43^{aB}	19.68±0.63^{aC}	20.36±0.55^{aD}	23.48±0.52^{aE}	28.93±0.62^{aE}
Gr 4	17.36±0.47^{aA}	22.27±0.52^{cB}	24.56±0.45^{cC}	27.22±0.64^{cD}	30.55±0.36^{bE}	34.87±0.96^{cF}
	Lactate dehydrogenase (U/L)					
	30 min	12 h	24 h	48 h	60 h	72 h
Gr 1	220.86±6.48^{aA}	286.37±3.67^{cB}	316.43±4.57^{dC}	356.08±3.55^{cD}	397.54±5.76^{cE}	442.37±4.87^{dF}
Gr 2	224.24±5.82^{aA}	276.23±4.37^{bB}	297.68±4.85^{bC}	336.47±5.48^{bD}	375.27±4.26^{bE}	397.13±4.39^{bF}
Gr 3	225.16±4.64^{aA}	255.82±3.56^{aB}	275.57±3.76^{aC}	305.62±4.47^{aD}	320.85±5.73^{aE}	375.62±4.56^{aF}
Gr 4	228.67±5.25^{aA}	275.04±5.86^{bB}	304.51±4.85^{cC}	353.21±4.58^{cD}	386.18±5.18^{cE}	415.96±5.63^{cF}

Means bearing different superscripts within rows (A, B, C, D, E and F) and columns (a, b, c and d) differ significantly ($P < 0.05$), n = 25. Gr 1: Control (0 mM), Gr 2: 1 mM, Gr 3: 2 mM and Gr 4: 4 mM.

antioxidants like curcumin help to mitigate these oxidative stress effects. In this study, curcumin has increased the antioxidant levels and reduced the MDA production which indicated its protective role in the sperm preservation.

Curcumin exerts antioxidant effects through its chemical structure and regulatory roles in the antioxidant enzyme expression. Its β-diketo groups, double bonds and phenyl rings contribute to the radical scavenging actions (Ak and Gülçin 2008; Roy and Rhim 2020). Additionally, the turmeric's essential oils enhance the sperm membrane fluidity and the cold shock resistance (Maldjian *et al.* 2005; Makris *et al.* 2023). Curcumin also activates the transcription factors like Nrf2 and enzymes like HO-1 which in turn enhance the endogenous antioxidant defenses (Farzaei *et al.* 2018; Zheng and McClements 2020). This study had confirmed that the curcumin supplementation has

increased the glutathione and antioxidant enzyme levels, enhanced the membrane stability and reduced oxidative damage. These effects were consistent with the findings in rat and dog spermatozoa (Soleimanzadeh and Saberivand 2013; Aparnak and Saberivand 2019).

Intracellular enzymes in the seminal plasma (AST, ALT and LDH) are the reliable indicators of membrane integrity and sperm health (Pesch *et al.* 2006; Perumal *et al.* 2015). Elevated extracellular enzyme levels indicated the sperm damage. In this study, the curcumin-treated groups showed reduced enzymatic leakage indicating that curcumin has preserved the sperm structures such as the plasma membrane, mitochondrial membrane and acrosomal membrane and flagella.

Effects of curcumin were dose-dependent (Gupta *et al.* 2021). Excessive antioxidant addition alters the extender properties such as rise in viscosity, debris or osmotic stress which in turn affect the sperm function (Shoae and Zamiri 2008; Lv *et al.* 2019). High antioxidant doses destabilize the membranes, hinder the acrosomal integrity and disrupt the ROS-antioxidant balance (Perumal *et al.* 2015). In our study, 2 mM curcumin group had exhibited the optimal sperm quality, outperforming 1 mM (suboptimal) and 4 mM (potentially toxic) concentrations. These findings have confirmed that the critical need for optimizing the antioxidant dosages to maximize semen preservation efficacy.

Due to methodological variations across the different studies such as extender formulations, preservation durations, species, sperm-curcumin interaction time and assay protocols-direct comparisons were challenging. Nevertheless, the current study demonstrated that 2 mM curcumin in a TFCE extender had significantly improved the semen quality, enhanced the antioxidant defense, and reduced the oxidative damage in the liquid-preserved Teressa buck semen.

The present study demonstrated that inclusion of 2 mM curcumin into a tris-fructose-citrate-egg yolk semen extender had significantly alleviated the physical and oxidative stresses, increased the antioxidant levels, improved the key semen quality parameters and reduced the intracellular enzyme leakage and free radical production in the Teressa goat buck semen. Importantly, curcumin at this concentration had preserved the structural and functional integrity of the spermatozoa during the liquid storage at 5 °C for up to 72 h. These findings highlighted the potential of curcumin as a functional additive in the semen preservation protocols. Although the *in-vitro* results are encouraging, it is anticipated that the curcumin-treated spermatozoa may also exhibit higher fertilization potential in both the *in-vitro* and *in-vivo* fertility trials, leads to higher conception rates in Teressa goats under the humid tropical ecosystem of the Andaman and Nicobar Islands. Further studies are warranted to confirm these results in the *in vivo* fertility assessments and to optimize the extender formulations for large-scale application in the breed conservation programmes.

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