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Effect of *Moringa oleifera* on semen quality profiles in endangered Teresa goat under tropical humid island ecosystemP. Perumal¹✉, Jai Sunder¹, A. K. De¹, A. K. Nahak², D. K. Chaurasia², T. C. Balamurugan³, D. Bhattacharya¹¹ICAR–Central Island Agricultural Research Institute, Port Blair–744 105, Andaman and Nicobar Islands, India²OUAT–College of Veterinary Science and Animal Husbandry, Bhubaneswar–751 003, Odisha, India³TANUVAS–Veterinary College and Research Institute, Salem – 636 112, Tamil Nadu, India

ABSTRACT

Objective: To investigate the effect of *Moringa oleifera* leaf extract on semen quality parameters in Teresa goat buck.**Methods:** A total of 25 semen samples from six bucks were selected for the study. Spermatozoa of 150×10^6 were incubated in 0, 300, 500 and 700 µg of leaf extract as group I, II, III and IV, respectively. Liquid stored semen samples were analysed for motility, viability, total sperm abnormalities, plasma membrane, acrosomal and nuclear integrities, seminal plasma intracellular enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH)] and total antioxidant capacity (TAC) and spermatozoa malondialdehyde (MDA) and cholesterol efflux in comparison with the control group (group I) for up to 72 h.**Results:** *Moringa oleifera* leaf extract (500 µg/ 150×10^6 spermatozoa)-treated semen had significantly higher motility, viability, plasma membrane, acrosomal and nuclear integrities, and TAC ($P < 0.05$), and had significantly lower total sperm abnormalities, AST, ALT, LDH, MDA production and cholesterol efflux compared to those in other *Moringa oleifera* leaf extract treated (300 or 700 µg/ 150×10^6 spermatozoa) and control groups at different hours of liquid storage ($P < 0.05$). Semen quality parameters and TAC showed an increasing trend and total sperm abnormalities, MDA production, leakage of intra-cellular enzymes and cholesterol efflux showed a decreasing trend from group I to group III and then an opposite trend from group III to group IV at different hours of liquid storage.**Conclusions:** *Moringa oleifera* leaf extract 500 µg/ 150×10^6 spermatozoa can be used for liquid semen preservation in Teresa goat at Andaman and Nicobar Islands.**KEYWORDS:** *Moringa oleifera*; Semen; Teresa goat; Andaman and Nicobar Islands

1. Introduction

Teresa goat is a unique endangered goat breed in Nicobar group of Islands of Andaman and Nicobar Islands, India and it needs immediately greater attention towards *in-situ/ex-situ* conservation[1]. Reproduction performance and productivity severely decreased during the extreme dry summer months in Teresa goat. Livestock censuses of Government of India revealed that goat population decreased from 2007 to 2019 (4.25%) in Andaman and Nicobar Islands due to various reasons including intensive inbreeding, lack of suitable breeding bucks and breeding management and climate change.

Significance

Teresa goat suffered with poor quality semen and fertility during summer due to thermal, walking, nutritional and oxidative stresses. *Moringa oleifera* contains chemical compounds, acting as potential antioxidants. This study revealed that *Moringa*-treated goat semen had significantly higher sperm quality and seminal antioxidants and lower sperm abnormalities, intracellular enzymatic leakage, stress marker and cholesterol efflux. Therefore, *Moringa oleifera* in extender enhances antioxidants and in turn improves seminal parameters in bucks of Andaman and Nicobar Islands.

✉To whom correspondence may be addressed. E-mail: perumalponraj@gmail.com

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Cold or liquid storage of sperm is used to slow down the metabolism and keep the sperm viable for a longer period of time. Using a Tris-egg yolk-glycerol-based extender, fresh goat semen has reportedly been effectively stored at 5 °C for 7 days[2]. But throughout the lengthy time of storage, the sperm's quality is deteriorated. The superoxide anion radical ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), which are produced by the cellular elements of semen are known as reactive oxygen species (ROS) that play a significant role in this decrease of semen quality parameters and fertility[3]. Lipid peroxidation has a number of negative impacts, including permanent motility loss, sperm DNA damage, and lower fertility rate[3]. In order to increase the semen quality, studies have also been done on caprine semen diluent, which contains different additives/antioxidants[4].

Moringa (M.) oleifera is commonly known as Senjana/Drumstick tree/Horseradish tree. It is a multipurpose tree useful for animals as well as human beings. Its leave contains many nutrients and active compounds such as polyphenols, β -carotene, vitamins A, C and E, protein (27%), calcium, iron and phosphorus, amino acids and several natural anti-oxidant agents. Therefore, it is broadly used in Asian or developing countries[5]. Moreover, it also contains many different phytochemicals such as reducing sugars, crude fibres, resins, flavonoids, alkaloids, organic acids, tannins, sterols, saponins, polyphenols, proteins, and other antioxidants[6] such as quercetin, kaempferol, ellagic acid and apigenin glucoside[7]. Phytochemical estimation reveals that *M. oleifera* leave (w/w) has calcium level equivalent to 1 L of milk, potassium of 3 bananas, vitamin C of 7 oranges, 3 times the iron contents present in spinach, 2 times the protein in milk, and 4 times the quantity of vitamin A in carrots. Different parts of *M. oleifera* such as leaves, seed, roots, bark, flowers, fruit and immature pods are used for different health disorders including cardiac and circulatory systems, antipyretic, antitumor, antiepileptic, antiulcer, anti-inflammatory, antispasmodic, antihypertensive, diuretic, cholesterol lowering, anti-diabetic, antioxidant[8], antibacterial, antifungal[9] and hepatoprotective[10].

M. oleifera leaf extract increases the male reproductive potentials by reducing the concentration of reactive oxygen species in the cytoplasm of sperm[11]. In addition, *M. oleifera* leaf extract is used in cattle and buffalo semen extender as an additive/antioxidant to improve the sperm quality[12–14]. On the other hand, dietary supplementation of *M. oleifera* improves libido, sperm concentration and motility in albino rats[15]. However, there is only a limited amount of research on effect of *M. oleifera* in semen extenders on semen quality parameters and semen preservability in caprine species. Therefore, the present study hypothesized that inclusion of *M. oleifera* leaf extract could improve the *in-vitro* semen quality parameters, and seminal antioxidants and reduce the leakage of intracellular enzymes, and cholesterol efflux in semen of Teresa buck of Andaman and Nicobar Islands. With this, the objective of the present study was designed to assess the effect of different

concentrations of *M. oleifera* leaf extract in semen extender on *in-vitro* semen quality parameters, seminal antioxidants, leakage of intracellular enzymes, and cholesterol efflux of liquid preserved semen in Teresa goat buck at Andaman and Nicobar Islands.

2. Materials and methods

2.1. Location of the study

Goat breeding farm at ICAR-Central Island Agricultural Research Institute (ICAR-CIARI), Port Blair, Andaman and Nicobar Islands, India, located between 6°45' to 13°41' North Latitude and 92°12' to 93°57' East Longitude, was the site of the current study. This experiment was conducted in peak of the dry summer season *i.e.* January [rain fall: 125.80 mm, temperature humidity index (THI): 84.16, light hour: 8.54 h] and February (rain fall: 13 mm, THI: 83.73, light hour: 9.37 h) in Andaman and Nicobar Islands, India.

2.2. Experimental animals

Six healthy individuals with body condition score of 2.5 to 3.5 (good condition) were selected for this experiment. Three to four-year-old Teresa goat bucks weighing 34 to 36 kg were selected for the experiment. Teresa goats were kept in a semi-intensive system that allowed them access to the natural pasture from 7:00 a.m. to 12:00 p.m. and kept them in the shed the rest of the day. The bucks were maintained under uniform managerial practices as per the farm schedule. According to the farm schedule, general management procedures for deworming, vaccination, disease prevention, cutting of penile hair, and foot trimming were subjected.

2.3. Preparation of *M. oleifera* leaf alcoholic extract

M. oleifera leaves were collected, identified and authenticated at Division of Horticulture and Crop Improvement, ICAR-CIARI, Port Blair, Andaman and Nicobar Islands. Ethanolic extract of *M. oleifera* leaves was obtained as per the method described by Sinha *et al*[16] with slight modification. In short, fresh *M. oleifera* leaves were collected, rinsed with double distilled water and dried under shade at room temperature for a period of one week and then ground with use of grinder into powder. The grounded powder was mixed with hydro ethanol (ethanol: water, 70: 30 v/v) and intermittent shaking at 3 h interval at room temperature for 48 h. This mixer was centrifuged twice with use of centrifugation machine at 3 000×g for 30 min at 4 °C. The supernatant was removed carefully. Finally, the content was filtered with use of filter paper (Whatmann; size No. 1). The filtrate was dried with air at room temperature and finally preserved at 4 °C–8 °C for the experiment.

2.4. Semen extender preparation

Tris-glucose-citrate-egg yolk (TGCE) semen extender was used in this study which contained Tris 2.4 g, glucose 1 g, citric acid 1.4 g, fresh egg yolk 20 mL, streptomycin 100 mg/mL and penicillin G sodium 100 µg/mL and double distilled water upto 100 mL. The semen extender was divided into four equal aliquots and the different concentrations of *M. oleifera* leaf extract (Group I: 0, Group II: 300, Group III: 500 and Group IV: 700 µg per mL of extender) were added and mixed thoroughly with use of magnetic stirrer for 15 min, filtered and then placed in water bath (37 °C). Semen samples were extended slowly with use of the leaf extract-included TGCE extender (final concentration of 150×10^6 spermatozoa per mL). Thus, these experimental groups had different concentrations of leaf extract in 150×10^6 spermatozoa. The extender for the control group (Group I) contained no leaf extract.

2.5. Semen collection

Semen ejaculates were collected from each buck twice a week between 6.00 a.m. and 7.30 a.m. by utilizing a standardised artificial vagina method. Each buck had two ejaculates collected, with a one-hour break in between. These ejaculates were placed in a water bath (37 °C) immediately after semen collection and tested for routine semen quality characteristics such as volume, colour, pH, sperm concentration, and mass activity. Ejaculates having a wide pH range, strange colour patterns, or a little amount were rejected, while the remaining ejaculates were inspected and processed for future research. The ejaculates were tested for routine seminal parameters and accepted for evaluation after meeting the Minimum Standard Protocol standards such as concentration: $>2.5 \times 10^9$ spermatozoa/mL; mass activity: $>3+$, individual motility: $>70\%$, and overall abnormality not more than 10%. Following the screening methodology outlined above, 50 of 72 ejaculates (6 bucks $\times$ 12 ejaculates) were chosen. Following the preliminary assessments, two successive ejaculates from the same buck (termed "sample" henceforth, $n=25$) were pooled together and treated to a twofold initial dilution with pre-warmed (37 °C) standard caprine specific TGCE extender. Thus, 50 chosen ejaculates were pooled from 72 original collections to provide 25 samples for the experiment. The partly diluted samples were transported to the laboratory in an insulated flask filled with warm (37 °C) water for further processing. Diluted sperm samples were retained in glass tubes and chilled from 37 °C to 5 °C at a rate of 0.2 °C-0.3 °C/min, and then held at 5 °C for the duration of the experiment. The characteristics of sperm quality were tested after 1 h, 12 h, 24 h, 48 h, 60 h, and 72 h.

2.6. Semen evaluation

Seminal parameters, viz. sperm motility[3], viability and total

sperm morphological abnormalities by eosin–nigrosin staining[17], acrosomal integrity by Giemsa staining[18] and plasma membrane integrity by hypo-osmotic swelling test[19] and nuclear integrity by Feulgen's staining technique[20] were determined with standard procedures.

2.7. Biochemical assays

An aliquot of semen from each sample was centrifuged at $3000 \times g$ for 15 min at 4 °C; sperm pellets were separated and washed by resuspending in phosphate buffer saline (PBS) and centrifuged (three times). A drop of seminal plasma was examined under a high power microscope to establish that it was free of spermatozoa. The spermatozoa were added to 1 mL of deionized water following the final centrifugation, snap-frozen, and kept at -80 °C for further analysis. Spermatozoa concentration was assessed at the moment of estimate and then rediluted to include 150 million cells per mL. Malondialdehyde (MDA) and total cholesterol were measured in spermatozoa, while aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and total antioxidant capacity (TAC) were assessed in seminal plasma.

Lipid peroxidation level of spermatozoa was measured by determining the MDA production using thiobarbituric acid (TBA) as per the method of Suleiman *et al*[21]. Antioxidants in seminal plasma (mM/L) were estimated by TAC colorimetric assay kit (709001; Cayman Chemical Co., USA) as per the manufacturer's guidelines. The cholesterol content in spermatozoa was estimated with use of cholesterol assay kit (Span Diagnostics Ltd., India), and results were expressed as µg cholesterol/ 10^8 spermatozoa. The activities of intracellular enzymes such as AST, ALT and LDH were estimated in the seminal plasma by assay kit (Span Diagnostics Ltd., India).

2.8. Statistical analysis

To determine any possible differences in the observed experimental parameters with respect to treatment and liquid storage periods (control & treatments and different hours of storage), two-way analysis of variance (ANOVA) was applied using Statistical Analysis Software (SAS, Version 9.3.1; SAS Institute, Inc., Cary, NC, 2011) and for multiple comparison, Duncan's multiple range test was applied. Data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). Differences were considered significant if $P < 0.05$.

2.9. Ethics statement

Experimental procedures were approved by the Institutional Animal Ethics Committee of ICAR-CIARI, Port Blair, Andaman and Nicobar Islands, India, with approval No: IXX15703 and project code: HORTCIARISIL202100400228 dated 26/04/2021. All the experiments were performed according to the international guidelines on ethical use of animals.

3. Results

3.1. Fresh semen evaluation

The present study revealed that Teresa goat buck semen was mostly creamy white in colour with an average volume of (0.92 ± 0.23) mL, mass activity of 3.75 ± 0.05 , pH of 6.88 ± 0.04 , concentration of $(3.74 \pm 0.07) \times 10^9$ sperm cells per mL, motility of $(86.16 \pm 1.62)\%$, viability of $(87.32 \pm 0.93)\%$, total sperm abnormality of $(6.26 \pm 0.16)\%$, acrosomal integrity of $(88.79 \pm 1.10)\%$, plasma membrane integrity of $(87.60 \pm 1.36)\%$, nuclear integrity of $(86.22 \pm 0.67)\%$, TAC of (1.34 ± 0.06) mM/L, MDA of (2.19 ± 0.03) nM/ 10^8 sperm, total cholesterol of (26.99 ± 0.88) $\mu\text{g}/10^8$ sperm, AST of (43.26 ± 1.35) U/L,

ALT of (15.34 ± 0.34) U/L and LDH of (215.94 ± 3.10) U/L.

3.2. Liquid semen evaluation

Semen treated with 500 μg leaf extract/ 150×10^6 spermatozoa had significantly ($P < 0.05$) higher motility, viability, plasma membrane, acrosomal and nuclear integrities and TAC, and had significantly ($P < 0.05$) lower total sperm abnormalities, leakage of AST, ALT and LDH, MDA production and cholesterol efflux compared to those in the control, 300 μg and 700 μg treated groups at different hours of liquid storage (Table 1 and Table 2). Further, these semen quality parameters and antioxidant showed an increasing trend and total sperm abnormality, MDA production, leakage of intra-cellular

Table 1. Comparison of quality parameters of liquid stored Teresa goat spermatozoa following preservation with *Moringa oleifera* leaf extract (0, 300, 500 and 700 μg leaf extract/ 150×10^6 spermatozoa) at different hours of liquid storage.

Parameters	Group I	Group II	Group III	Group IV
Total motility				
1 h	83.87 $\pm$ 4.65 ^{aA}	85.32 $\pm$ 3.24 ^{aA}	86.87 $\pm$ 3.72 ^{aA}	83.74 $\pm$ 3.58 ^{aA}
12 h	70.54 $\pm$ 3.74 ^{aB}	77.26 $\pm$ 4.67 ^{bB}	82.93 $\pm$ 4.40 ^{cAB}	72.22 $\pm$ 3.64 ^{aB}
24 h	61.65 $\pm$ 2.20 ^{aC}	69.47 $\pm$ 4.65 ^{bC}	78.78 $\pm$ 4.31 ^{cBC}	63.43 $\pm$ 3.74 ^{aC}
48 h	52.27 $\pm$ 2.86 ^{aD}	61.63 $\pm$ 4.26 ^{bD}	74.54 $\pm$ 3.23 ^{cC}	54.39 $\pm$ 3.13 ^{aD}
60 h	43.76 $\pm$ 2.62 ^{aE}	52.81 $\pm$ 3.60 ^{bE}	68.67 $\pm$ 3.96 ^{cD}	45.72 $\pm$ 3.30 ^{aE}
72 h	38.12 $\pm$ 3.11 ^{aF}	47.72 $\pm$ 3.38 ^{bF}	58.83 $\pm$ 3.64 ^{cE}	40.68 $\pm$ 2.64 ^{aF}
Viability				
1 h	85.72 $\pm$ 3.43 ^{aA}	86.68 $\pm$ 2.76 ^{aA}	87.27 $\pm$ 2.68 ^{aA}	85.58 $\pm$ 3.34 ^{aA}
12 h	72.63 $\pm$ 2.22 ^{aB}	80.36 $\pm$ 2.49 ^{bB}	86.40 $\pm$ 3.03 ^{cA}	74.54 $\pm$ 2.74 ^{aB}
24 h	63.44 $\pm$ 1.91 ^{aC}	72.75 $\pm$ 2.20 ^{bC}	80.79 $\pm$ 2.47 ^{cB}	65.85 $\pm$ 2.91 ^{aC}
48 h	53.45 $\pm$ 3.11 ^{aD}	63.94 $\pm$ 1.54 ^{cD}	77.78 $\pm$ 1.22 ^{dC}	56.65 $\pm$ 1.81 ^{bD}
60 h	44.86 $\pm$ 3.20 ^{aE}	54.11 $\pm$ 1.98 ^{bE}	70.21 $\pm$ 1.64 ^{cD}	46.15 $\pm$ 2.22 ^{aE}
72 h	39.40 $\pm$ 2.84 ^{aF}	48.64 $\pm$ 1.81 ^{cF}	59.85 $\pm$ 2.40 ^{dE}	42.72 $\pm$ 2.10 ^{bF}
Total sperm abnormality				
1 h	6.87 $\pm$ 0.46 ^{aA}	6.68 $\pm$ 0.67 ^{aA}	6.42 $\pm$ 0.36 ^{aA}	7.08 $\pm$ 0.45 ^{aA}
12 h	9.75 $\pm$ 0.66 ^{bB}	8.68 $\pm$ 1.81 ^{abB}	7.56 $\pm$ 0.93 ^{aB}	9.80 $\pm$ 1.27 ^{bB}
24 h	12.91 $\pm$ 2.18 ^{bC}	9.53 $\pm$ 0.71 ^{aB}	8.65 $\pm$ 0.45 ^{aC}	11.85 $\pm$ 0.53 ^{bC}
48 h	14.41 $\pm$ 1.17 ^{dD}	12.28 $\pm$ 0.63 ^{bC}	9.57 $\pm$ 0.41 ^{aC}	13.45 $\pm$ 0.46 ^{cD}
60 h	15.92 $\pm$ 1.22 ^{cE}	13.76 $\pm$ 0.56 ^{bD}	10.92 $\pm$ 0.80 ^{aD}	14.88 $\pm$ 0.93 ^{cE}
72 h	17.56 $\pm$ 1.32 ^{cF}	14.84 $\pm$ 0.75 ^{bD}	11.62 $\pm$ 0.66 ^{aD}	15.51 $\pm$ 0.56 ^{bE}
Acrosomal integrity				
1 h	85.32 $\pm$ 2.89 ^{aA}	87.84 $\pm$ 3.16 ^{aA}	88.68 $\pm$ 2.78 ^{aA}	86.45 $\pm$ 3.71 ^{aA}
12 h	73.62 $\pm$ 3.08 ^{aB}	81.53 $\pm$ 1.93 ^{bB}	87.48 $\pm$ 2.18 ^{cA}	75.34 $\pm$ 2.20 ^{aB}
24 h	65.31 $\pm$ 2.84 ^{aC}	74.29 $\pm$ 2.25 ^{bC}	81.62 $\pm$ 2.57 ^{cB}	66.43 $\pm$ 2.71 ^{aC}
48 h	54.08 $\pm$ 2.35 ^{aD}	64.22 $\pm$ 2.00 ^{bD}	77.37 $\pm$ 2.27 ^{cC}	56.56 $\pm$ 2.57 ^{aD}
60 h	47.83 $\pm$ 2.59 ^{aE}	56.34 $\pm$ 3.15 ^{bE}	71.48 $\pm$ 3.18 ^{cD}	49.62 $\pm$ 3.52 ^{aE}
72 h	42.03 $\pm$ 2.74 ^{aF}	49.48 $\pm$ 1.59 ^{bF}	56.35 $\pm$ 1.81 ^{cE}	44.61 $\pm$ 1.83 ^{aF}
Plasma membrane integrity				
1 h	85.74 $\pm$ 3.74 ^{aA}	86.28 $\pm$ 3.46 ^{aA}	88.56 $\pm$ 3.89 ^{aA}	85.37 $\pm$ 2.87 ^{aA}
12 h	71.93 $\pm$ 2.69 ^{aB}	79.68 $\pm$ 2.54 ^{bB}	85.54 $\pm$ 2.00 ^{cA}	73.73 $\pm$ 1.83 ^{aB}
24 h	62.25 $\pm$ 3.33 ^{aC}	71.32 $\pm$ 3.01 ^{bC}	79.43 $\pm$ 3.57 ^{cB}	64.17 $\pm$ 2.79 ^{aC}
48 h	52.89 $\pm$ 1.78 ^{aD}	62.18 $\pm$ 2.59 ^{bD}	75.23 $\pm$ 2.08 ^{cC}	55.31 $\pm$ 2.98 ^{aD}
60 h	45.26 $\pm$ 1.27 ^{aE}	54.89 $\pm$ 2.20 ^{cE}	69.78 $\pm$ 1.69 ^{dD}	47.66 $\pm$ 2.81 ^{bE}
72 h	40.31 $\pm$ 1.54 ^{aF}	49.57 $\pm$ 2.25 ^{cF}	53.43 $\pm$ 2.10 ^{dE}	43.75 $\pm$ 1.83 ^{bF}
Nuclear integrity				
1 h	84.65 $\pm$ 2.25 ^{aA}	86.87 $\pm$ 2.25 ^{aA}	87.73 $\pm$ 1.73 ^{aA}	85.76 $\pm$ 1.85 ^{aA}
12 h	72.07 $\pm$ 3.06 ^{aB}	80.71 $\pm$ 2.30 ^{bB}	84.71 $\pm$ 1.86 ^{cA}	74.71 $\pm$ 2.57 ^{aB}
24 h	66.89 $\pm$ 3.11 ^{aC}	73.29 $\pm$ 1.61 ^{bC}	82.29 $\pm$ 1.37 ^{cB}	67.29 $\pm$ 2.13 ^{aC}
48 h	55.66 $\pm$ 2.03 ^{aD}	64.36 $\pm$ 1.93 ^{bD}	77.36 $\pm$ 2.22 ^{cC}	56.36 $\pm$ 2.35 ^{aD}
60 h	48.99 $\pm$ 2.49 ^{aE}	57.09 $\pm$ 3.03 ^{bE}	73.09 $\pm$ 2.81 ^{cD}	48.09 $\pm$ 2.66 ^{aE}
72 h	41.27 $\pm$ 3.13 ^{aF}	50.27 $\pm$ 1.42 ^{bF}	58.27 $\pm$ 1.69 ^{cE}	42.17 $\pm$ 1.83 ^{aF}

Data are expressed as mean $\pm$ SD. Means bearing different superscripts within columns (A, B, C, D, E and F) and rows (a, b, c and d) differ significantly ($P < 0.05$), $n=25$ samples. Group I : Control (0 μg leaf extract/ 150×10^6 spermatozoa), Group II : 300 μg leaf extract/ 150×10^6 spermatozoa, Group III : 500 μg leaf extract/ 150×10^6 spermatozoa and Group IV : 700 μg leaf extract/ 150×10^6 spermatozoa.

Table 2. Comparison of biochemical attributes of liquid stored Teresa goat semen following preservation with *Moringa oleifera* leaf extract (0, 300, 500 and 700 µg leaf extract/150×10⁶ spermatozoa) at different hours of liquid storage.

Parameters	Group I	Group II	Group III	Group IV
Total cholesterol, µg/10 ⁸ sperm				
1 h	23.67±2.21 ^{aA}	24.98±2.34 ^{aA}	25.56±2.38 ^{aA}	24.82±2.43 ^{aA}
12 h	18.56±2.42 ^{aB}	21.68±2.59 ^{bcB}	23.99±2.74 ^{cb}	20.54±2.30 ^{abB}
24 h	14.76±2.18 ^{aC}	17.49±2.52 ^{bc}	20.65±1.81 ^{cC}	18.32±2.25 ^{bcB}
48 h	11.68±1.78 ^{aD}	12.29±2.30 ^{aD}	16.31±2.74 ^{bd}	12.55±2.54 ^{aC}
60 h	8.87±1.56 ^{aE}	9.14±2.40 ^{aE}	13.08±1.71 ^{bE}	8.55±2.47 ^{aD}
72 h	5.66±1.61 ^{aF}	6.09±1.93 ^{aF}	10.13±2.32 ^{bF}	7.55±2.27 ^{aD}
Total antioxidant capacity, mM/L				
1 h	1.36±0.17 ^{aA}	1.38±0.21 ^{aA}	1.42±0.18 ^{aA}	1.37±0.16 ^{aA}
12 h	0.80±0.17 ^{aB}	0.88±0.19 ^{aB}	1.12±0.14 ^{bb}	0.82±0.12 ^{aB}
24 h	0.65±0.14 ^{abC}	0.76±0.17 ^{abC}	0.89±0.19 ^{bc}	0.70±0.12 ^{abC}
48 h	0.57±0.17 ^{aCD}	0.63±0.14 ^{abCD}	0.77±0.09 ^{bCD}	0.58±0.12 ^{aCD}
60 h	0.43±0.09 ^{aDE}	0.54±0.17 ^{abDE}	0.65±0.12 ^{bDE}	0.46±0.19 ^{aDE}
72 h	0.34±0.12 ^{aE}	0.41±0.17 ^{abE}	0.52±0.19 ^{bE}	0.37±0.14 ^{aE}
Malondialdehyde, nM/10 ⁸ sperm				
1 h	2.16±0.06 ^{aA}	2.14±0.05 ^{aA}	2.12±0.06 ^{aA}	2.15±0.05 ^{aA}
12 h	2.72±0.04 ^{abB}	2.45±0.07 ^{abB}	2.21±0.09 ^{abB}	2.44±0.07 ^{abB}
24 h	3.35±0.34 ^{aC}	2.92±0.09 ^{bc}	2.47±0.14 ^{aC}	2.93±0.12 ^{bc}
48 h	3.85±0.34 ^{aD}	3.32±0.12 ^{bd}	2.97±0.09 ^{aD}	3.32±0.14 ^{bd}
60 h	4.47±0.39 ^{aE}	3.75±0.07 ^{bE}	3.33±0.12 ^{aE}	3.85±0.09 ^{bE}
72 h	4.98±0.31 ^{aF}	4.01±0.17 ^{bF}	3.56±0.09 ^{aE}	3.97±0.14 ^{bF}
Aspartate amino transferase, U/L				
1 h	46.53±3.51 ^{aA}	44.45±3.73 ^{aA}	43.76±3.47 ^{aA}	45.57±2.76 ^{aA}
12 h	66.26±2.32 ^{aB}	61.52±2.71 ^{abB}	56.64±2.05 ^{aB}	65.48±3.01 ^{aB}
24 h	81.92±2.00 ^{aC}	73.28±1.10 ^{bc}	68.66±1.78 ^{aC}	78.73±1.39 ^{aC}
48 h	90.50±1.81 ^{aD}	78.88±1.32 ^{bd}	71.75±1.15 ^{aD}	83.69±1.61 ^{aD}
60 h	99.97±1.86 ^{aE}	82.25±1.05 ^{bE}	75.43±1.07 ^{aE}	88.28±1.39 ^{aE}
72 h	109.72±2.96 ^{aF}	88.36±1.17 ^{bF}	78.47±1.37 ^{aF}	95.54±1.46 ^{aF}
Alanine amino transferase, U/L				
1 h	17.13±0.76 ^{aA}	16.76±0.56 ^{aA}	15.82±0.67 ^{aA}	16.98±0.85 ^{aA}
12 h	21.58±0.78 ^{aB}	19.29±0.24 ^{abB}	16.36±0.51 ^{aB}	20.42±0.58 ^{aB}
24 h	24.07±0.56 ^{aC}	21.48±0.34 ^{bc}	17.56±0.90 ^{aC}	22.65±1.17 ^{aC}
48 h	28.70±0.95 ^{aD}	23.67±0.85 ^{bd}	18.73±1.17 ^{aD}	25.82±0.80 ^{aD}
60 h	35.98±0.68 ^{aE}	27.28±0.88 ^{bE}	21.44±1.27 ^{aE}	28.25±1.10 ^{bE}
72 h	41.17±1.95 ^{aF}	30.92±1.66 ^{bF}	26.19±0.95 ^{aE}	32.68±1.20 ^{aF}
Lactate dehydrogenase, U/L				
1 h	222.17±6.92 ^{aA}	218.35±8.76 ^{aA}	217.76±6.78 ^{aA}	220.23±8.67 ^{aA}
12 h	265.33±6.36 ^{aB}	255.12±5.75 ^{abB}	234.42±6.66 ^{aB}	250.80±7.15 ^{abB}
24 h	295.24±6.19 ^{aC}	270.36±5.95 ^{bc}	255.45±6.73 ^{aC}	285.05±9.42 ^{aC}
48 h	335.60±6.12 ^{aD}	315.94±8.49 ^{bd}	280.76±6.07 ^{aD}	330.82±6.22 ^{aD}
60 h	370.00±6.76 ^{aE}	350.22±7.86 ^{bE}	305.28±9.79 ^{aE}	368.08±7.61 ^{aE}
72 h	425.23±6.85 ^{aF}	378.71±8.10 ^{bF}	350.66±6.12 ^{aF}	395.00±7.78 ^{aF}

Data are expressed as mean±SD. Means bearing different superscripts within columns (A, B, C, D, E and F) and rows (a, b, c and d) differ significantly ($P<0.05$, $n=25$ samples). Group I : Control (0 µg leaf extract/150×10⁶ spermatozoa), Group II : 300 µg leaf extract/150×10⁶ spermatozoa, Group III : 500 µg leaf extract/150×10⁶ spermatozoa and Group IV : 700 µg leaf extract/150×10⁶ spermatozoa.

enzymes and cholesterol efflux showed a decreasing trend from Group I to Group III and then opposite trend from Group III to Group IV at different hours of liquid storage. Thus, *M. oleifera* leaf extract 500 µg/150×10⁶ spermatozoa was optimum or suitable dosage for liquid semen preservation in Teresa buck. Additionally, leaf extract at 300 µg and 700 µg per mL were inferior compared to 500 µg/mL treatment for these semen quality characteristics, and there was a significant ($P<0.05$) difference between *M. oleifera* leaf extract at 300 µg/mL and 700 µg/mL for these responses. It was obvious from the data of this experiment that addition of *M. oleifera* leaf extract especially at 500 µg/150×10⁶ spermatozoa to the

semen diluent resulted in significant improvement in semen quality, antioxidant activity, and reduction of cholesterol efflux, leakage of intracellular enzymes and MDA production in caprine semen stored in *in-vitro* at 5 °C for 1 h, 12 h, 24 h, 48 h, 60 h and 72 h.

4. Discussion

M. oleifera has positive effects on *in-vitro* semen quality indices and fertility in many animal species as a dietary source[15] and as an ingredient in semen extenders in livestock species[22–

24]. *M. oleifera* is an adaptogen since it boosts the body's resilience to biological, chemical, and physical stressors and also boosts vitality and energy levels. *M. oleifera* improves the reproductive parameters in male animals[15]. *M. oleifera* has significant effect on semen production and its quality profiles[23,24]. In the current study, semen extender containing 500 µg of *M. oleifera* leaf extract per 150×10^6 spermatozoa significantly improved the semen quality parameters in Teressa buck. Presence of various bioactive ingredients contributes to *M. oleifera*'s protective properties[5–7]. These substances protect the sperm through variety of methods, including an antioxidative action. *M. oleifera* also raises antioxidant level and lowers the level of free radicals[24], which in turn enhances the spermatozoa's ability to fertilise eggs and promote fertility. Additionally, these chemical elements in *M. oleifera* safeguard the cytoskeleton structure of sperm flagella, preserve the integrity of the sperm plasma membranes and mitochondrial membrane. When combined, the vitamins, minerals, phenolic compounds, tannins, flavonoids, saponins, terpenoids and glycosides in *M. oleifera* have a synergistic antioxidant action that lessens the ROS-induced stress during liquid preservation. *M. oleifera* leaf extract contains a sizable quantity of beta-sitosterol, zeatin, quercetin, beta-carotene, caffeoylquinic acid, vitamin C, kaempferol and alpha-tocopherol, which are effective lipid peroxidation chain-breaking agents and directly scavenge a wide range of free radicals[13]. By controlling the lipid peroxidation process, *M. oleifera* leaf extract affects the many enzymatic systems that in turn regulate the formation of ROS and maintain the oxidative load[25]. The leaf extract inhibits the oxidative molecules to display its free radical scavenging action[26]. Through putative antioxidant properties and a synergistic interaction with other antioxidants, *M. oleifera* leaf extract lowers the production of ROS. As a result, antioxidant level was higher and ROS level was lower in the present research. Additionally, *M. oleifera* leaf extract obtained through use of ethanolic extraction has a higher solubility[27] and this leaf extract contains a higher concentration of kaempferol malonyl glucoside, kaempferol, kaempferol hexoside, kaempferol-3-o-acetyl-glucoside, kaempferol-3-o-glucoside, cyanidin-3-o-hexoside, quercetin, quercetin-3-o-glucoside ellagic acid and apigenin glucoside[7]; these compounds are acting as potential antioxidants and have a higher solubility. The extender quickly dissolved the ethanolic leaf extract, which then released its antioxidant power and improved the antioxidants' ability to do their jobs. As a result, the antioxidant content in the research was greater at the beginning of the storage period. *M. oleifera* leaf extract improved the antioxidant abilities and lowered the impact of ROS on sperm plasma membranes; these might be the causes of the higher sperm vitality[22,28]. According to Shokry *et al*[28], the higher antioxidant activities of *M. oleifera* leaf extract might be the cause of this improvement in viability by reducing the impact of ROS on sperm plasma membranes. Oxidative stress is a key cause of male infertility and causes DNA damage and fragmentation in spermatozoa. In Teressa goat bucks, *M. oleifera* leaf extract supplementation reduced DNA fragmentation or nuclear damage[22]. Thus, it prevents the premature capacitation and acrosomal reaction.

Therefore, higher semen quality was observed in *M. oleifera* leaf extract treated semen.

Mammalian sperm plasma membranes are highly polyunsaturated, which renders them susceptible to lipid peroxides which ultimately cause sperm function degradation due to lower motility, higher sperm DNA damage and reduced fertility[29]. The antioxidant systems of seminal plasma and spermatozoa are harmed throughout the semen processing and storage stages[29]. Due to dilution of semen with extender and higher formation of ROS molecules during the liquid preservation process, the concentration of antioxidants is decreased[30]. As a defence mechanism against lipid peroxidation in the sperm and semen, natural and artificial antioxidant systems have been described[31]. Exogenous antioxidants and endogenous antioxidants working together may thereby reduce the effects of oxidative stress during sperm preservation while also enhancing the quality of liquid and cryopreserved semen. According to the current study, *M. oleifera* enhanced the bioavailability of antioxidants, protecting and enhancing their availability[32]. By potentially acting as an antioxidant and working in concert with other antioxidants already in use, *M. oleifera* lowers the production of free radicals and ROS. As a result, in the study of buck's semen treated with *M. oleifera*, the level of antioxidants was increased and MDA production was reduced. According to the results of the current study, *M. oleifera* leaf extract increased the amount of antioxidants and lowered the amount of free radicals in Teressa goat buck semen[22,28].

Seminal plasma enzymes (AST, ALT and LDH) are essential for sperm metabolism and function[33]. Intracellular enzyme in the seminal plasma is a trustworthy indicator of sperm quality because of its connection with the integrity of the sperm plasma membranes[34]. A greater quantity of intracellular enzymes in the extracellular fluid are as a result of sperm membrane damage and enzymatic leakage from the damaged spermatozoa[33]. The link between intracellular enzymes and total motility, progressive motility and live sperm was described by Pesch *et al*[34]. The acrosomal membrane, plasma membrane, mitochondrial membrane and flagella of sperm were preserved by *M. oleifera* leaf extract in the current study. As a result, compared to the semen in the untreated control group, the leaf extract-treated semen had lower leakage of intracellular enzymes.

Semen quality is impacted by *M. oleifera* leaf extract in a dose-dependent manner[28]. A surplus of antioxidant in the semen extender induced plasma membrane fluidity above the desired level according to the report of Shoaie and Zamiri[31], which made the sperm more vulnerable to acrosomal destruction and plasma membrane damage. Additionally, the amount of antioxidant supplied to the extender needs to be assessed since higher antioxidant doses may harm the spermatozoa owing to changes in the extender's physiological state. Higher viscosity of the diluent, higher debris in the diluent, lower osmotic pressure of the diluent, reduced functional integrity of the acrosome and plasma membranes, higher sperm damage and imbalance between the free radicals and antioxidants are some potential changes in the physiological state of the extender[35,36]. Thus, a greater than ideal or threshold concentration of additives, antioxidants, or herbal extracts can change the

extender's physiological state and can cause sperm destruction, negative effects on semen quality parameters and fertility. In goat, spermatozoa survival rate rises as the amount of antioxidant given to the extender increases from control to optimum and then decreases as antioxidant level increases. However, a higher-than-required antioxidant dosage proved as harmful to the spermatozoa[33]. In the present study, spermatozoa treated with leaf extracts (300, 500 and 700 µg/150×10⁶ spermatozoa) had shown a substantial favourable effect as compared to those in the untreated control group. However, 500 µg leaf extract/150×10⁶ spermatozoa in semen extender had shown significantly higher beneficial effect as compared to 300 µg and 700 µg; this implied that 700 µg were overdosed and toxic to spermatozoa and 300 µg was insufficient dose as compared to 500 µg. Thus, 500 µg was optimum or suitable dosage for liquid preservation of Teressa goat semen. The higher semen quality in the bubaline[22,23], bovine[12] and ovine[24] species due to inclusion of *M. oleifera* leaf extract was previously documented in the form of motility and intact acrosomal membrane. Higher percentage of intact plasma membrane and acrosomal membrane of sperm were obtained in the current study that treated with 500 µg leaf extract/150×10⁶ spermatozoa; therefore, this leaf extract treated semen sample had higher motility. It was challenging to compare the findings of the current study with those of earlier studies due to differences in preservation protocols, extender formulations between laboratories, the time of addition/exposure of sperm with leaf extract, the concentration of leaf extracts, between species, different assay methods, experimental species, and individual animal variation.

However, the current study has some limitations. In this investigation, we studied the effect of *M. oleifera* leaf extract on semen quality parameters, intracellular enzymatic leakage, antioxidant profile, MAD production and cholesterol efflux in Teressa goat buck to select a suitable or optimum concentration to enhance the fertility rate. In this study, we prepared limited experimental groups (300 µg, 500 µg and 700 µg of leaf extract in 150×10⁶ spermatozoa) and revealed that 500 µg/150×10⁶ spermatozoa were optimum and suitable dosage for caprine species. Therefore, further studies need to be conducted on effect of *M. oleifera* on the *in-vitro* and *in-vivo* fertility rate to confirm the present findings of the study. Further, studies need to be conducted on different concentrations with minimal range between the experimental dosages to optimize the more accurate concentration to get maximum benefits in Teressa goat semen preservation.

In conclusion, the current study concluded that adding 500 µg of *M. oleifera* leaf extract per 150×10⁶ spermatozoa to the semen extender reduced physical and oxidative stresses, increased antioxidant level, improved semen quality parameters, and decreased enzyme leakage and free radical formation in the semen of Teressa goat buck. Despite the positive results, it was assumed that the sperm cells treated with *M. oleifera* leaf extract will show a better level of fertilising potential in *in-vitro* or *in-vivo* fertility studies with a higher rate of field pregnancy.

Conflict of interest statement

Authors declare that there is no conflict of interest involved in the present work.

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Authors' contributions

P. Perumal contributed to conceptualization; P. Perumal, Jai Sunder and A. K. De performed data curation; P. Perumal, A. K. Nahak and D. K. Chaurasia carried out formal analysis; P. Perumal, A. K. De and D. Bhattacharya contributed to investigation; P. Perumal, A. K. De and T. C. Balamurugan contributed to methodology; Jai Sunder, P. Perumal and D. Bhattacharya contributed to project administration; P. Perumal and A. K. De contributed to writing the original draft; P. Perumal, A. K. De, A. K. Nahak, D. K. Chaurasia contributed to writing, review and editing.

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