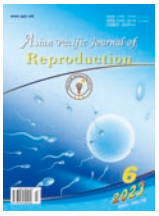

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Seasonal stress on semen quality profiles, seminal biochemical and oxidative stress attributes in endangered Teressa goat of Andaman and Nicobar Islands

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ABSTRACT

Objective: To measure seasonal effects on semen quality profiles, seminal biochemical and oxidative stress attributes in fresh and liquid stored semen in monsoon and dry seasons.

Methods: A total of 10 Teressa bucks (3-4 years) were selected from breeding farm, ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India. Semen samples (n=25 per season) were collected through artificial vagina method and preserved at refrigerated temperature (5 ℃) for 48 h using Tris citrate glucose based extender. We detected semen quality parameters [volume, mass activity, pH, sperm concentration, total motility, viability, total sperm abnormality, and plasma membrane, acrosomal and nuclear integrities], biochemical profiles [aspartate amino transferase (AST), alanine amino transferase (ALT) and total cholesterol], and oxidative stress markers [total antioxidant capacity (TAC) and malondialdehyde (MDA)] during monsoon and dry seasons.

Results: Semen quality parameters significantly differed between seasons (P<0.05) and among storage periods (P<0.05). Volume, pH, mass activity, motility, viability, acrosomal, plasma membrane and nuclear integrities, and TAC were significantly higher (P<0.05). Sperm concentration, sperm abnormalities, MDA, AST, ALT and total cholesterol were significantly lower in fresh semen of monsoon than dry season (P<0.05). Motility, viability, acrosomal, plasma membrane and nuclear integrities, and TAC were significantly decreased (P<0.05) while sperm abnormality, AST, ALT, total cholesterol and MDA were significantly increased as liquid semen storage period advanced (P<0.05).

Conclusions: Monsoon season has higher beneficial effects on semen quality profiles and liquid stored semen remained usable for upto 48 h. Good quality ejaculates with higher TAC and lower MDA can be cryopreserved and will be used for artificial insemination.

KEYWORDS: Andaman and Nicobar Islands; Teressa goat; Semen quality profiles; Season; Liquid storage

1. Introduction

Teressa goat (*Capra hircus*) in Nicobar and Andaman goat (*Capra hircus*) in Andaman Islands are two major meat goat breeds in Andaman and Nicobar Islands. These goat breeds are heat resistant as similar to other tropical goat breeds; however, their reproduction and production performances are affected under the extreme weather conditions. Goat population was decreased (4.25%) from 2007 to 2019 in Andaman and Nicobar Islands as per livestock census of Government of India due to increased intensive inbreeding, lack of suitable breeding management and suitable breeding bucks. Therefore, production and per capita availability of chevon are decreased. Further, 30%-40% does remain without breeding due to lack of suitable breeding buck in the locality. Goats

Significance

Teressa goat population is reducing due to various reasons including climate change in Andaman and Nicobar islands. Monsoon season enhances and summer season decreases the reproduction performance. The study revealed that Teressa buck had significantly higher semen quality profiles and lower sperm abnormalities in semen collected during monsoon than dry season. Thus, monsoon season had significantly higher beneficial effects on fertility improvement in Teressa goat under tropical humid island ecosystem in Andaman and Nicobar Islands.

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are following natural service in Andaman and Nicobar Islands with various limitations and therefore, severe economic losses to goat farmers. These limitations would be solved by artificial insemination and assisted reproductive techniques.

Buck is a half of flock and its selection is a starting point in goat improvement program; therefore, selection of breeding buck, semen collection and artificial breeding are essential for genetic improvement in caprine species. In recent years, artificial breeding with liquid semen has become an important tool in goats throughout the world. Various factors including season influence semen quality profiles in caprine species[1]. Season alters hormone, libido, scrotal and testicular biometrics due to variation in photoperiod or temperature humidity index and rainfall[2]. High ambient temperature and relative humidity trigger the degenerative changes in scrotum and testes resulting in reduction of testicular weight, size and consistency, which in turn affect the semen quality. Testicular temperature should be at least 3 ℃-4 ℃ (34.8 ℃-35.2 ℃) lower compared to rectal temperature to achieve maximum functional efficacy of scrotum and testes in caprine species[3]. Higher testicular and scrotal temperatures minimize the blood flow to the scrotum and testis resulting in hypoxia or anoxia and degeneration of testicular structures, which result lower semen quality and poor fertility[4]. Thus, the season is one such variable influences the semen quality and fertility. Heat tolerance capacity of indigenous goat species is higher than exotic species as characterized by lower sperm abnormalities[5]. In goat, photoperiod is a major environmental variable that regulates the daily rhythm of melatonin production which in turn influences the sexual activity, and reproductive cycle[6] which consequently alters the semen quality; thus, photoperiod determines seasonality in caprine species. Even though season less influences the reproduction of buck than doe, buck exhibits a prominent seasonal variation in spermatogenesis[7].

Teressa goat breeds throughout the year; however, some sort of seasonality has been expressed in this goat as reproductive and metabolic hormones and libido were significantly higher and cortisol and prolactin were significantly lower in monsoon than dry season[8,9]. Retrospective analysis of 20 years (2001-2020) kidding rate revealed that average kidding rate was higher in dry (56.27%) than monsoon (43.72%) season and singles (54.00% *vs.* 45.99%), twines (53.63% *vs.* 46.36%), triplets (72.72% *vs.* 27.27%) and quadruplets (100% *vs.* 0%) were higher in dry than monsoon season in Teressa goat. These data indicated that higher fertile breeding has occurred in July and August (monsoon season) and lower fertile breeding has occurred in January and February (dry season) considering the gestation length of goat varies from 145 to 155 days. Therefore, it is assumed that season has significance influence on semen production in Teressa goat[8]. Seasonal variation in semen quality was reported in bovine[5], and bubaline[10], species and are higher in spring or winter or monsoon than dry or summer season. Higher temperature during summer adversely affects the epididymal function resulting in increase of semen acidity and higher total sperm abnormality.

Liquid storage of semen at 5 ℃ is used to reduce the sperm metabolism to extend the viability, membrane integrity and motility of spermatozoa for an extended period of time. However, the semen quality is deteriorated during this extended storage period. Moreover, liquid semen can be stored at 5 ℃ for 6 days successfully with Tris based extender with or without cryoprotectant (glycerol). The influence of liquid storage on the semen quality is varied with seasons[11] and were measured by different authors[1] in different goat breeds; lacking a similar information for Teressa goat. Knowledge on seminal parameters in various seasons helps to enhance the goat breeding improvement. Further, this study will help to identify a suitable season for semen collection and liquid storage for higher fertility. Therefore, objective of the present study was to ascertain the seasonal effect in semen production and its quality profiles and to determine the duration of liquid storage for higher fertility in Teressa goat in Andaman and Nicobar Islands to pursuit future semen collection and cryopreservation.

2. Materials and methods

2.1. Area of the study

The present study was carried out at Goat Breeding Farm, ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India and is located in between 6°45' to 13°41' North Latitude and in between 92°12' to 93°57' East Longitude. The seasons in Andaman and Nicobar Islands were classified into monsoon (May to November) and dry summer (December to April) as per the monsoon availability in Andaman and Nicobar Islands. Sun light hours per day were higher (P<0.05) in dry summer (9.20±0.74) compared to the monsoon (4.28±0.89) season. Rainfall (mm) was higher (P<0.05) in the monsoon (444.92±13.62) compared to the dry summer (89.04±8.84) season. Temperature humidity index (THI) was higher in the dry summer (85.59±1.15) compared to monsoon (84.92±1.59) season in the experimental location.

2.2. Experimental animals

Ten healthy (body condition score: 3.0-3.5 out of 5, 1: extremely thin to 5: extremely fat, with 0.5 point-increment; classified as good) bucks of 3-4 years of age with body weight of (32-37) kg were selected for the present study. Selection of the experimental bucks was done based on the previous history and records of semen production and its quality profiles with non-significant variation in their semen quality parameters over a period of the time. Each five experimental bucks were kept in two separate rooms with sufficient spaces and allowed to a natural lighting without physical contact

or vision with female. Teressa goats were maintained in the semi-intensive system where they were allowed for grazing from 7:00 to 12: 00 a.m. The bucks and does were allowed grazing separately in different grazing areas in different times; bucks were allowed in first for grazing and then does were allowed. Experimental bucks were maintained under uniform managemental practices as per the farm schedule. This experiment was conducted in peak of the respective seasons, *i.e.* month of July (rain fall: 583.30 mm, THI: 82.87, light hour: 3.50 h) and August (rain fall: 622.20 mm, THI: 82.05, light hour: 4.17 h) for monsoon season and month of January (rain fall: 125.80 mm, THI: 84.16, light hour: 8.54 h) and February (rain fall: 13 mm, THI: 83.73, light hour: 9.37 h) for dry summer season in the year of 2019 and 2020. Throughout this study, the nutrition of bucks remained uniform and constant. The diet fed to bucks consisted of mixed jungle forages (18.50% dry matter and 10.30% crude protein) and sufficient concentrates (86.40% dry matter and 13.70% crude protein) fortified with mineral mixture and salt and offered *ad libitum* potable drinking water. General management practices for deworming, vaccination, disease prevention, trimming of penile hair and hoof trimming were subjected as per the farm schedule. Teressa goat has 83.08% tupping percentage, 151.19% kidding percentage, 1.54 kidding rate, 6-8 weeks weaning period and 3.84% pre-weaning mortality over a period of five years in Andaman and Nicobar Islands.

2.3. Semen collection and processing

The semen ejaculates were collected only twice per week from any buck between 5:00 and 8:00 h through standardized artificial vagina method. Two ejaculates were collected from each buck with a short period of rest (approximately 1 h) between the two collections. Ejaculates were collected in a graduated transparent sterilized collection tube covered by an insulating jacket and these ejaculates were preserved in a water bath (37 ℃) immediately after semen collection and routine semen quality parameters such as volume, colour, pH, mass activity and sperm concentration were evaluated. Ejaculates with wide variation in pH, abnormal colour patterns or too low volume were discarded; rest of the good quality ejaculates were analysed microscopically and processed for further work. After the preliminary evaluations of these 100 ejaculates, two consecutive ejaculates of a same buck were pooled together (termed "sample" hereafter, n=50); 25 samples in each season were collected for further study. After preliminary analysis, these ejaculates were allowed to twofold initial dilution with pre-warmed (37 ℃) Tris-glucose-citrate extender (Tris: 2.42 g, glucose: 1.00 g, citric acid: 1.30 g, streptomycin: 100 mg/mL and penicillin G sodium: 100 μg/mL). These partially extended semen samples were transferred to the semen processing laboratory within the insulated flask containing warm water with temperature of 37 ℃ for further processing and preservation. Thus, a total of 50 semen samples (n=50; 25 samples×2 seasons) were collected during monsoon and dry summer seasons.

Each semen sample was extended (final concentration of 150 $\times 10^6$ spermatozoa per mL) with use of the Tris-glucose-citrate (TGC) dilutor and diluted semen samples were aliquoted in six reagent bottles for measurement of semen quality parameters at different liquid storage periods. Liquid storage of semen (at 5 ℃) reduces the sperm metabolism which in turn maintains the viability, plasma membrane and acrosomal membrane integrities and motility of spermatozoa at higher level for an extended period of time. Therefore, wide spread of artificial insemination is possible in caprine species. However, semen quality is deteriorated during this extended storage period. Optimum semen quality for artificial insemination was maintained upto 48 h in liquid storage with use of TGC extender. Therefore, the semen quality was checked upto 48 h in the present study. The semen samples in the six reagent bottles were placed in a glass pot containing warm water (37 ℃). The pot was placed in a refrigerator at 5 ℃ for liquid preservation of semen. The temperature decreased from 37 ℃ to 5 ℃ slowly over a period of 120 min. The semen quality parameters were recorded at 0, 6, 12, 24, 36 and 48 h post dilution. The liquid stored semen samples were taken out from refrigerator and placed in water bath (37 ℃) for 30 s and then semen quality parameters were evaluated.

2.4. Semen evaluation

Ejaculated volume was recorded directly from the graduated semen collection tube and recorded in millilitre (mL). Mass activity of semen sample was recorded by placing a small drop of freshly collected neat semen on a clean grease free, pre-warmed glass slide at 37 ℃ and examined without cover-slip under low power magnification (100×) of phase contrast microscope (Nikon, Eclipse 80i). Mass activity was graded from 0 to 5 scale based on the appearance of waves and swirls. Sperm concentration ($\times 10^9$/mL) in neat semen was estimated by haemocytometer method adopting the red blood cell (RBC) counting procedure. Special attention was paid during diluting the sample with 1% formal saline, which was cross checked by diluting neat semen at the ratio of 1:200 with the help of a micro pipette. Homogeneity of the semen samples was assured by palm shaking the tube containing the semen samples and also at the time of charging the haemocytometer.

At the time of evaluation, the liquid stored semen samples were taken out from refrigerator and placed in water bath at 37 ℃ for 30 s. Semen quality parameters, *viz.* total motility, viability and total sperm morphological abnormalities by Eosin–Nigrosin staining, acrosomal integrity by Giemsa staining, plasma membrane integrity by hypo-osmotic swelling test and nuclear integrity by Feulgen's staining technique were determined as per standard procedures in the semen samples during liquid storage of semen at 5 ℃ from 0 to 48 h[12].

2.5. Biochemical assays

An aliquot of semen from each sample was centrifuged at 800×*g* for 10 min and sperm pellets and seminal plasma were separated. Sperm pellet was washed by resuspending in phosphate buffer saline (PBS) and centrifuged (thrice). After final centrifugation, 1 mL of deionized water was added to the spermatozoa, snap-frozen and stored at -80℃ until further analysis of malondialdehyde (MDA). The seminal plasma was aliquoted and stored at -80℃ until further analysis of biochemical and antioxidant profiles. At the time of estimation, concentration of spermatozoa was determined and then re-diluted with deionized water to contain 100×10^6 cells/mL. Aspartate amino transaminase (AST), Alanine amino transaminase (ALT), and total cholesterol (Span Diagnostics Ltd., India), and total antioxidant capacity (TAC) (Bio Vision, USA) were estimated in seminal plasma and MDA (thiobarbituric acid-trichlroacetic acid method) was estimated in sperm pellet with use of Microplate Reader (Alere Medical Pvt Ltd, India, AM 2100).

2.6. Statistical analysis

To determine any possible differences in the observed semen quality parameters with respect to between seasons, paired t-test for fresh semen samples and among the liquid semen storage periods in different seasons, repeated measures of two-way analysis of variance (ANOVA) was applied using PROC GLM Univariate model of Statistical Analysis Software (SAS, Version 9.3.1; SAS Institute, Inc., Cary, NC, 2011) and for multiple comparison, Tukey test was applied for the experimental parameters. The data used in the study were tested for normality before analysis using Shapiro Wilk statistics and the outliers were removed. All the data was normally distributed. The mean values were expressed as mean±standard deviation (mean±SD). The results were analysed statistically after arcsine transformation of percentage data. Differences were considered significant if $P<0.05$. Associations among the semen quality parameters in monsoon and dry summer seasons and between semen quality parameters and meteorological parameters (rain fall, THI and light hour) in monsoon and dry summer seasons were analysed for statistical significance using Pearson's correlation coefficient using SAS software. If the r value was greater than 0.50, the correlation was considered as large, 0.50-0.30 was considered as moderate, 0.30-0.10 was considered as small.

2.7. 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: IXX14497 and project code: HORTCIARISIL201801300199 dated 23/08/2018. All animal experiments were performed according to the International guidelines on ethical use of animals.

3. Results

3.1. Semen quality parameters

Semen quality parameters such as volume, pH, mass activity, total motility, viability, acrosomal, plasma membrane and nuclear integrities were significantly higher ($P<0.05$) and sperm concentration and total sperm abnormality were significantly lower ($P<0.05$) in neat semen of monsoon than dry summer season. Similarly, TAC was significantly higher ($P<0.05$) and MDA was significantly lower ($P<0.05$) in neat semen of monsoon compared to that of dry summer season. Biochemical profiles such as AST, ALT and total cholesterol were significantly higher ($P<0.05$) in neat semen of dry summer compared to that of monsoon season. Microbial load was significantly lower ($P<0.05$) in neat semen of monsoon compared to that of dry summer season (Figure 1).

3.2. Liquid semen storage study

Liquid semen storage study revealed that total motility, viability, acrosomal, plasma membrane and nuclear integrities were significantly decreased ($P<0.05$) and total sperm abnormalities was increased as liquid semen storage periods advanced in monsoon and dry summer seasons. Total motility, viability, acrosomal, plasma membrane and nuclear integrities were increased ($P<0.05$) and total sperm abnormalities was decreased ($P<0.05$) in monsoon compared to that of dry summer season at different liquid storage periods (Figure 2).

Biochemical profiles such as AST, ALT, total cholesterol and MDA were increased ($P<0.05$) and TAC was decreased as liquid semen storage periods advanced in monsoon and dry summer seasons. TAC was increased ($P<0.05$) and AST, ALT, total cholesterol and MDA were decreased ($P<0.05$) in monsoon compared to that of dry summer season at different liquid semen storage periods (Figure 3).

3.3. Correlation analysis

3.3.1. Among the semen quality parameters

Correlation analysis revealed that the semen quality parameters were correlated ($P<0.05$) with each other's in monsoon and dry summer seasons in Teressa goat. Volume, pH, mass activity, total motility, livability, acrosomal, plasma membrane and nuclear integrities and TAC were positively correlated ($P<0.05$) with each other and these semen quality parameters were negatively correlated ($P<0.05$) with sperm concentration, total sperm abnormalities, total cholesterol, MDA, AST and ALT in both monsoon and dry summer seasons (Table 1, Table 2).

3.3.2. Between the semen quality parameters and meteorological parameters

Correlation was obtained between the semen quality parameters and meteorological parameters for Teressa goat in Andaman and

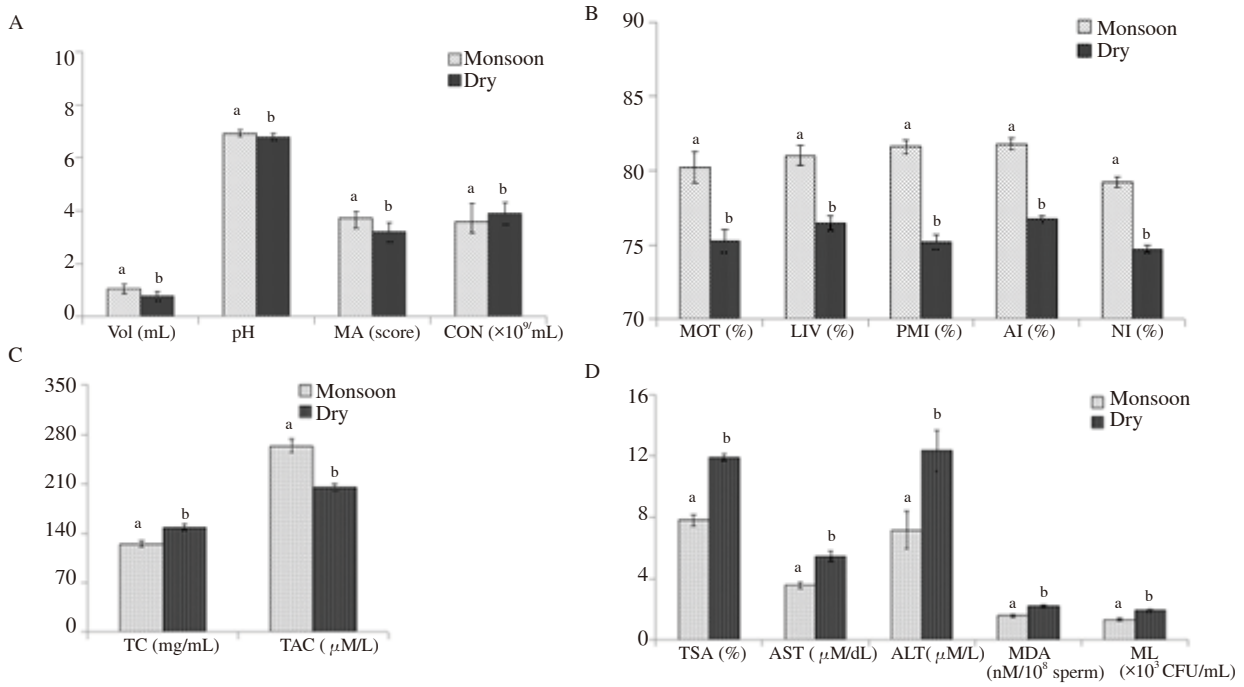

Figure 1. Effect of season on semen quality profiles of Teressa goat buck in Andaman and Nicobar Islands. Plot A: Vol: volume, pH: hydrogen ion concentration, MA: mass activity and CON: sperm concentration. Plot B: MOT: total motility, LIV: livability, PMI: plasma membrane integrity, AI: acrosomal integrity, and NI: nuclear integrity. Plot C: TC: total cholesterol, and TAC: total antioxidant capacity. Plot D: TSA: total sperm abnormality, AST: aspartate amino transferase, ALT: alanine amino transferase, MDA: malondialdehyde, and ML: microbial load. Monsoon: April to November; Dry: December to March. Vertical bar on each point represents standard deviation of mean. Vertical bar with small letters (a, b) indicates significant difference between monsoon and dry seasons (P<0.05). Each 25 (n=25) semen samples in monsoon and dry summer seasons.

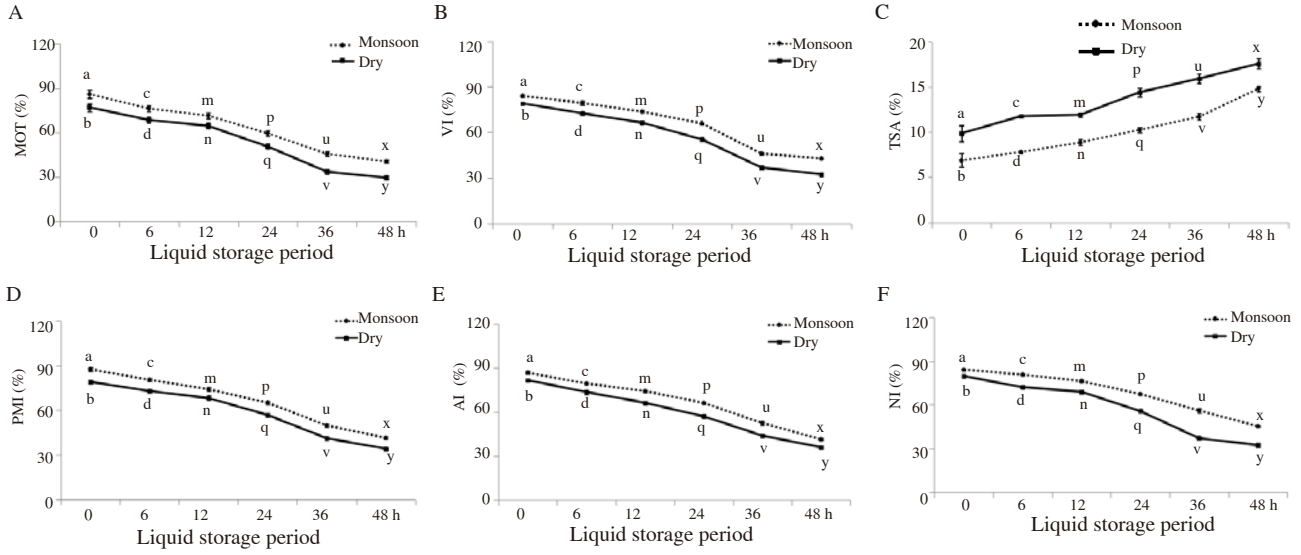

Figure 2. Effect of season and different storage periods (at 5℃) on semen quality parameters in Teressa goat bucks in Andaman and Nicobar Islands. A: total motility (MOT), B: viability (VI), C: total sperm abnormality (TSA), D: plasma membrane integrity (PMI), E: acrosomal integrity (AI), and F: nuclear integrity (NI). Monsoon: April to November; Dry: December to March. Vertical bar on each point represents standard deviation of mean. Semen quality parameters differed significantly between seasons (P<0.05) and among the storage periods (P<0.05). Vertical bar with small letters (a, b at 0 h; c, d at 6 h; m, n at 12 h; p, q at 24 h; u, v at 36 h; x, y at 48 h) indicates significant (P<0.05) difference between monsoon and dry summer seasons. Each 25 (n=25) semen samples in monsoon and dry summer seasons.

Nicobar Islands. Volume, pH, mass activity, total motility, livability, acrosomal, plasma membrane and nuclear integrities and TAC were positively correlated (P<0.05) with rain-fall and negatively correlated (P<0.05) with THI and light-hours in monsoon and dry summer seasons. Similarly, sperm concentration, total sperm abnormality, total cholesterol, MDA, AST and ALT were negatively correlated (P<0.05) with rain-fall and positively correlated (P<0.05) with THI and light-hours in monsoon and dry summer seasons in Teressa goat (Table 3).

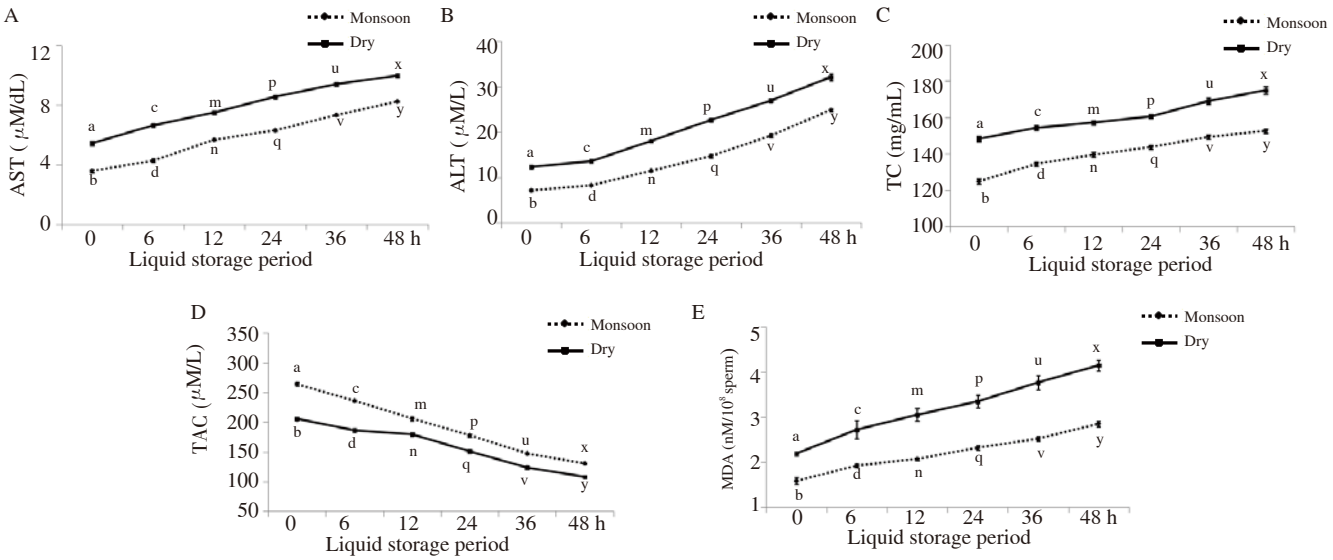

Figure 3. Effect of season and different storage periods (at 5 ℃) on seminal biochemical parameters in Teressa goat bucks in Andaman and Nicobar Islands. A: aspartate amino transferase (AST), B: alanine amino transferase (ALT), C: total cholesterol (TC), D: total antioxidant capacity (TAC) and E: malondialdehyde (MDA). Monsoon: April to November; Dry: December to March. Vertical bar on each point represents standard deviation of mean. Semen quality parameters differed significantly between seasons (P<0.05) and among the storage periods (P<0.05). Vertical bar with small letters (a, b at 0 h; c, d at 6 h; m, n at 12 h; p, q at 24 h; u, v at 36 h; x, y at 48 h) indicates significant difference between monsoon and dry summer seasons (P<0.05). Each 25 (n=25) semen samples in monsoon and dry summer seasons.

Table 1. Correlation among the semen quality parameters of Teressa goat of Andaman and Nicobar Islands in monsoon season.

Parameters	Vol	pH	MA	CON	MOT	LIV	PMI	TSA	AI	NI	TC	TAC	MDA	AST	ALT
Vol	1.00	0.83	0.80	0.87	0.85	0.86	0.84	-0.87	0.85	0.82	-0.85	0.86	-0.84	-0.75	-0.81
pH		1.00	0.76	0.86	0.85	0.81	0.79	-0.80	0.82	0.74	-0.83	0.73	-0.82	-0.84	-0.82
MA			1.00	0.80	0.79	0.78	0.76	-0.76	0.76	0.77	-0.76	0.82	-0.76	-0.78	-0.74
CON				1.00	0.78	0.85	0.73	-0.85	0.79	0.79	-0.77	0.77	-0.77	-0.77	-0.79
MOT					1.00	0.84	0.82	-0.85	0.85	0.86	-0.79	0.79	-0.79	-0.78	-0.82
LIV						1.00	0.84	-0.79	0.82	0.87	-0.82	0.84	-0.76	-0.73	-0.81
PMI							1.00	-0.82	0.79	0.84	-0.80	0.82	-0.71	-0.74	-0.79
TSA								1.00	-0.75	-0.87	0.71	-0.75	0.74	0.83	0.77
AI									1.00	0.79	-0.73	0.73	-0.85	-0.85	-0.83
NI										1.00	-0.75	0.75	-0.77	-0.86	-0.84
TC											1.00	-0.75	0.86	0.78	0.79
TAC												1.00	-0.78	-0.88	-0.84
MDA													1.00	0.76	0.76
AST														1.00	0.82
ALT															1.00

All these parameters are significantly correlated (P<0.05) with each other. Vol: volume, pH: hydrogen ion concentration, MA: mass activity, CON: sperm concentration, MOT: total motility, LIV: livability, PMI: plasma membrane integrity, TSA: total sperm abnormality, AI: acrosomal integrity, NI: nuclear Integrity, TC: total cholesterol, TAC: total antioxidant capacity, MDA: malondialdehyde, AST: aspartate amino transferase and ALT: alanine amino transferase. Monsoon season: April to November.

4. Discussion

Season has significant effect on semen quality parameters and seminal antioxidant and biochemical profiles in Teressa goat. Goats are seasonal breeders in temperate regions where sexual activities and semen production and its quality profiles are higher in breeding season compared to the non-breeding season whereas in tropics, goats breed throughout the year. Teressa goat is a perennial breeder; however, available literature revealed some sort of seasonality is speculated in it as similar to the other tropical breeds[13]. Short dry summer and longer monsoon seasons are prevailed in Andaman and Nicobar Islands. Livestock suffer severe stress in Andaman and Nicobar Islands due to combined effect of longer photoperiod (9.20 *vs.* 4.28 h/day), higher THI (85.59 *vs.* 83.28/day), reduced rainfall (89.04 *vs.* 444.92 mm/month), higher sea surface temperature (29.94 *vs.* 27.97 ℃/day) and higher solar direct irradiance (6.24 *vs.* 3.47 kWh/m^2/day) in dry summer compared to the monsoon season. With the short width of Andaman group of Islands (average: 24 km and maximum: 52 km), severe stress triggers adverse effects on semen production and its quality profiles in Teressa goat.

In our previous study, follicle stimulating hormone, luteinizing hormone, testosterone, thyroid stimulating hormone,

Table 2. Correlation among the semen quality parameters of Teressa goat of Andaman and Nicobar Islands in dry summer season.

Parameters	Vol	pH	MA	CON	MOT	LIV	PMI	TSA	AI	NI	TC	TAC	MDA	AST	ALT
Vol	1.00	0.83	0.82	0.85	0.85	0.87	0.83	-0.85	0.87	0.78	-0.86	0.83	-0.81	-0.85	-0.85
pH		1.00	0.84	0.81	0.78	0.77	0.78	-0.79	0.83	0.76	-0.78	0.76	-0.77	-0.74	-0.78
MA			1.00	0.82	0.82	0.78	0.71	-0.78	0.80	0.77	-0.80	0.70	-0.74	-0.76	-0.77
CON				1.00	0.83	0.84	0.88	-0.77	0.78	0.84	-0.78	0.76	-0.69	-0.83	-0.67
MOT					1.00	0.85	0.87	-0.82	0.77	0.78	-0.74	0.79	-0.68	-0.84	-0.73
LIV						1.00	0.81	-0.85	0.79	0.79	-0.73	0.69	-0.71	-0.86	-0.71
PMI							1.00	-0.88	0.75	0.77	-0.77	0.78	-0.72	-0.77	-0.83
TSA								1.00	-0.85	-0.76	0.76	-0.73	0.69	0.76	0.81
AI									1.00	0.80	-0.87	0.75	-0.85	-0.85	-0.87
NI										1.00	-0.81	0.74	-0.79	-0.82	-0.75
TC											1.00	-0.87	0.75	0.78	0.77
TAC												1.00	-0.76	-0.79	-0.79
MDA													1.00	0.75	0.73
AST														1.00	0.81
ALT															1.00

All these parameters are significantly correlated (P<0.05) with each other. Vol: volume, pH: hydrogen ion concentration, MA: mass activity, CON: sperm concentration, MOT: total motility, LIV: livability, PMI: plasma membrane integrity, TSA: total sperm abnormality, AI: acrosomal integrity, NI: nuclear Integrity, TC: total cholesterol, TAC: total antioxidant capacity, MDA: malondialdehyde, AST: aspartate amino transferase and ALT: alanine amino transferase. Dry summer season: December to March.

Table 3. Correlation (r) between the semen quality as well as seminal biochemical profiles and meteorological parameters of Teressa goat of Andaman and Nicobar Islands in monsoon and dry summer season.

Parameters	Monsoon season			Dry summer season		
	RF	THI	LHr	RF	THI	LHr
VOL	0.82	-0.84	-0.78	0.79	-0.85	-0.81
pH	0.79	-0.78	-0.73	0.75	-0.77	-0.82
MA	0.82	-0.83	-0.79	0.78	-0.82	-0.80
CON	-0.85	0.84	0.75	-0.83	0.86	0.83
MOT	0.83	-0.81	-0.74	0.79	-0.83	-0.81
LIV	0.79	-0.82	-0.77	0.76	-0.79	-0.78
PMI	0.83	-0.80	-0.75	0.90	-0.79	-0.77
TSA	-0.86	0.82	0.73	-0.78	0.80	0.78
AI	0.85	-0.81	-0.78	0.81	-0.75	-0.74
NI	0.84	-0.78	-0.74	0.76	-0.81	-0.76
TC	-0.83	0.77	0.76	-0.79	0.86	0.83
TAC	0.78	-0.82	-0.75	0.76	-0.79	-0.79
MDA	-0.74	0.80	0.73	-0.78	0.78	0.78
AST	-0.81	0.78	0.79	-0.77	0.84	0.76
ALT	-0.85	0.83	0.76	-0.76	0.79	0.77

All these parameters are significantly correlated (P<0.05) with each other. Vol: volume, pH: hydrogen ion concentration, MA: mass activity, CON: sperm concentration, MOT: total motility, LIV: livability, PMI: plasma membrane integrity, TSA: total sperm abnormality, AI: acrosomal integrity, NI: nuclear Integrity, TC: total cholesterol, TAC: total antioxidant capacity, MDA: malondialdehyde, AST: aspartate amino transferase, ALT: alanine amino transferase, RF: rainfall, THI: temperature humidity index and LHr: light hours. Monsoon season: April to November and dry summer season: December to March.

triiodothyronine, thyroxine and libido were higher and cortisol and prolactin were lower in monsoon compared to dry season[8], which in turn affects the testicular development and semen production profiles in Teressa goat. A similar observation was reported in Arbia bucks that shorter photoperiod enhanced the semen quality parameters[14]. Even though, higher temperature in scrotum (33.75 ℃ *vs.* 30.79 ℃), rectum (39.78 ℃ *vs.* 37.64 ℃) and skin (37.86 ℃ *vs.* 35.74 ℃) during dry summer compared to monsoon season; Teressa goat reproduces successfully throughout the year[3]. Further, these temperatures were higher compared to the temperature of thermal comfort range (for small ruminants: 20 ℃-30 ℃) for Teressa goat in Andaman and Nicobar Islands[15]; but still this goat breeds normally suggest that the testicles and scrotum of Teressa goat have higher thermoregulatory capacity and adapted to the existing adverse climatic variables in Andaman and Nicobar Islands. Here, it is also informed that goat breeds other than Teressa and Andaman goat such as Malabari and its crossbreeds or Boer crossbreds were neither survived nor reproduced successfully in Andaman and Nicobar Islands.

Longer photoperiod, higher THI and shorter rainfall severely affect the secretion and activity of the reproductive and metabolic hormones which in turn adversely affect the sperm production and its quality profiles. Seasonal effect in semen production and its quality profiles are usually parallel for many goat breeds; alteration in its quantity and quality is due to variation in the latitude and longitude[16]. On the contrary, negative energy balance is due to less availability of green fodder with poor minerals and vitamins during dry season results in poor semen production and its quality profiles[17]. Higher daily ambient temperature and longer photoperiod in dry summer were

negatively associated with reproductive and metabolic hormones[18] which in turn causes poor semen quality profiles and loss of fertility. Similar results were reported by Barkawi *et al*[1] in caprine species.

In our study, semen quality parameters were quite satisfactory in Teressa goat, indicating that reproductive system, gonads and accessory glands are functioning properly in monsoon and dry summer seasons. Semen volume was higher in monsoon compared to the dry summer season in Teressa goat. Similar observation was reported that semen volume and sperm count per ejaculate were higher in natural breeding season in caprine species[13]. Sperm concentration followed an opposite direction to the semen volume and seminal hydrogen ion concentration[13], suggesting the seasonal variations in the production and release of seminal plasma by the accessory sex glands. Seminal pH was higher in monsoon compared to dry summer season; similar observations were reported by several authors[19]. Mass motility was higher in monsoon compared to dry summer; similar results were reported in different breeds[20]. Sperm motility is an important parameter to determine the fertilizing efficacy of a male animal; high ambient temperature during dry summer season has negative effect on sperm motility[20]. Sperm motility and livability of goats are positively associated with the semen volume and are affected by season as their levels are higher in monsoon compared to dry summer season. Similar observations were reported in Nubian and its cross-bred bucks[20]. Dead sperm percentage was higher during dry summer compared to monsoon season in Teressa goat. Similar observation was reported in different goat breeds[20].

Sperm motility was declined in dry summer compared to monsoon season in Teressa goat. Similar observation was obtained in bovine species[5] that summer had declined motility compared to winter/monsoon season. Thermal stress triggers derangement in epididymal functions and spermatogenesis, resulting in declining of motility as similar features of cryptorchidism or testicular hypoplasia and testicular degeneration[21]. Temperature could give rise to secondary abnormalities as well with increase in sperm tail and mid-piece abnormalities as in testicular degeneration or partial hypoplasia of testes[22].

Thermal stress during summer season induces lower testosterone production from the Leydig cells and reduces the function of cauda epididymis and seminal vesicles resulting in lower concentration of antioxidants in semen as cauda epididymis and seminal vesicle are source of antioxidants in semen and are thermo sensitive and androgen dependent[23]. Therefore, the production of antioxidants was lower and lipid peroxide (LPO) production was higher in dry summer compared to monsoon season in Teressa goat.

Plasma membrane integrity reflects the biochemical integrity of sperm plasma membrane, and it is involved in the process of capacitation, acrosome reaction and binding of spermatozoa to the oocyte. Summer stress rises the testicular temperature which in turn decreases plasma membrane integrity as similar to scrotal insulation in goat[24]. Similarly, summer season has significant adverse effect on sperm acrosomal integrity in Teressa goat as similar reported in cattle[5], buffaloes[10], and goat[25].

Sperm morphological abnormalities usually return to normal level within 2 months of the scrotal thermal insulation[26]; however, speed of recovery rate is depending upon the severity and duration of the increased testicular temperature exposure and the exposure is prolonged during summer season.

Mammalian sperm membranes are made up of polyunsatured fatty acids (PUFAs); it renders the sperm for lipid peroxidation by partially reduced oxygen molecules such as hydrogen peroxide, superoxide and hydroxyl radicals[27]. LPO in the sperm membrane impairs the sperm functional activities because reactive oxygen species (ROS) reduces the sperm motility, membrane integrity, induces the sperm DNA damage and reduces the fertility through oxidative stress and production of cytotoxic aldehydes. Oxidative stress was used to measure the semen quality parameters as they were positively correlated with fertility[28]. Further, antioxidant system of spermatozoa and seminal plasma is compromised during the semen processing and the antioxidant level was reduced[29]. Oxidative stress also increased as liquid semen storage time advanced, assuming that the sperm stored for more than 48 h would have a lower fertilization potential. However, no information was found in the literature. Natural antioxidant system is a potent defense mechanism against lipid peroxide in semen[30]. Therefore, inclusion of exogenous antioxidants in the semen extender could reduce the adverse effect of oxidative stress during the sperm processing and storage; thus, the quality of chilled semen can be improved.

Liquid storage of semen is one strategy to extend the shelf life of spermatozoa in a reversible reduction of metabolic activity to maintain the viability, membrane integrity and motility of spermatozoa for an extended period of time. Hypothermia is able to reduce metabolic activity of cells by decelerating enzymatic reactions[31] and thus prolong their survivability and fertility; as a result, the storage potential is increased. However, the semen quality parameters are deteriorated during this extended storage period. Sperm motility was decreased as liquid storage period advanced and remained higher than 50% for up to 30 h of storage. The semen sample is remained suitable for artificial insemination for up to 48 h of storage as judged by semen quality parameters and fertility rate. Quality of all the spermatological parameters was influenced by the storage time and motility, morphology and osmotic resistance parameters of sperm were declined with the days of storage in ram[32]. Semen storage for longer period in liquid form triggers physical, biochemical, ultrastructural, and functional damages in the spermatozoa resulting in reduction of semen quality parameters and fertility[33]. Reduced sperm motility as storage period advanced indicates the spermatozoon undergoes a gradual energy loss over a period of time. Similarly, Perumal *et al*[34] reported that the individual motility was decreased from 0 h to 30 h storage. The spermatozoal cell water exchange during the early stages of the liquid preservation causes cell swellings

and shrinkages which may be intolerable for the majority of organelles and might predispose to spermatozoal morphological abnormality; thus, the sperm morphological abnormalities were increased as liquid storage period advanced. This storage-related semen quality reduction was also further subjected to seasonal variations. Spermatozoa lose their capacity to produce adenosine triphosphate (ATP) through mitochondrial respiration mechanism during liquid storage due to mitochondrial ageing or degeneration of mitochondria which results in adverse effects on semen quality parameters and on fertility. Drastic reduction of semen quality parameters was occurred during the first 24 h after storage[35]. The liquid semen may be used with acceptable fertility for period of not exceeding 72 h provided the temperature is maintained consistently. However, the fertilizing capacity starts to decrease after 48 h of preservation. Also, the sperm motility dropped significantly at day 2 of preservation. Similar result was obtained in ram that semen can be preserved at 5℃ for up to 48 h with no injurious effects on motility, membrane integrity or fertilizing potential[36]. Cholesterol along with phospholipids is essential for sperm membrane physical stability and fluidity[37]. Cholesterol efflux from the plasma membrane triggers the capacitation and acrosome reaction; both are crucial for fertilization. Therefore, cholesterol inclusion in extender enhances the capacity of sperm resistance against stresses. In the present study, cholesterol efflux and MDA production were reduced in semen samples of monsoon compared to that of dry summer season in Teressa goat. Therefore, the ejaculates collected during monsoon season had higher semen quality parameters compared to that of dry summer season. Moreover, sperm mitochondria are highly susceptible to oxidative damage and damage is significantly increased as liquid semen storage advanced. The seminal plasma antioxidants are important for sperm function and its metabolism as they protect the sperm from oxidative stress[38] and they are involved in control of cold shock. The production and functional capacity of antioxidants varied with seasons. Higher antioxidant concentration and lower free radical production was reported in winter compared to summer season in semen[5]. Season has significant influence in the biochemical composition of seminal plasma of the goat[39]. Intra cellular enzymes (AST and ALT) in the seminal plasma are essential for sperm metabolism as they are responsible for energy production for survival, viability, motility, and fertility of spermatozoa. Transaminase activities in the seminal plasma are indicators of semen quality as they can help to measure the sperm membrane stability. Higher concentration of abnormal spermatozoa with damaged plasma membrane causes easy leakage of enzymes from the spermatozoa in seminal plasma in liquid stored semen[40]. Higher concentration of AST and ALT in the seminal plasma during liquid storage is due to plasma membrane structural instability or fragile nature of sperm membrane[41]. In the present study, reduced concentration of AST and ALT in semen ejaculates of monsoon compared to dry summer season at different storage periods indicated that the season had significant membrane stabilizing effect in the membranes of acrosome, plasma membrane, mitochondria and flagella of the sperm. The summer thermal stress decreases the androgen production which in turn affects the function of accessory glands, seminal vesicle and epididymis resulting in loss of stability of plasma membrane and acrosomal membrane leads to more leakage of intra cellular enzymes during dry summer season. Thus, the leakage of intra-cellular enzymes was higher in the semen collected during dry summer compared to monsoon season in Teressa goat. Similar report was observed in bovine species[5] that semen collected during summer season had lower sperm acrosomal and plasma membrane integrities and higher extra cellular enzymatic profiles compared to semen from monsoon season as observed in the present study.

In short-day breeder like caprine species, melatonin secretion modulates endocrinological profiles which in turn enhance the semen quality parameters. Further, melatonin is a potent antioxidant and protects the reproductive system from free radicals. In Andaman and Nicobar Islands, the monsoon season has short day light (3.50 h-4.17 h) compared to long day light in dry summer (8.54 h-9.37 h) season. This indicated that during the short day monsoon season, there was higher secretion of melatonin in Teressa goat and protected the seminal, biochemical and antioxidant parameters along with stimulation of hypothalamus to secrete more gonadotropin releasing hormone (GnRH) followed by higher semen production. Moreover, there may be high variation in melatonin secretion throughout the year in the seminal plasma of Teressa goat that could partly explain the differences in sperm quality and fertility observed between the monsoon and dry summer seasons in this caprine species. Similarly, in Teressa goat does, short day may induce secretion of melatonin, stimulation of GnRH followed by expression of heat, breeding, conception and kidding. The goat is poly-oestrus animal and adult female shows repeated estrus cycle after every 19-24-days interval without prominent heat behaviour in summer season. However, melatonin was not measured in male and female goats in the present study. The result of semen quality was positively correlated with result of kidding rate of retrospective study in goat. Therefore, semen samples collected during monsoon season had higher semen quality parameters, antioxidants and lower leakage of enzymes and free radicals in the Teressa goat.

However, the current study has some limitations. In this investigation, we studied the effect of season on semen quality profiles, seminal antioxidants, leakage of intracellular enzymes and seminal biochemical profiles to select a suitable or optimum season to enhance the reproductive performances. We used limited parameters such as semen quality profiles and the results indicated that monsoon season is suitable for breeding related programmes for Teressa goat. Therefore, we need further studies that effect of seasons on cryopreservability and *in-vitro* and *in-vivo* fertility profiles in goat bucks are warranted to confirm the present findings.

In conclusion, monsoon season has higher beneficial effects compared to dry summer season in semen collection and liquid semen preservation and liquid stored semen remain usable for upto 48 h with respect to its semen quality parameters. Good quality ejaculates with higher antioxidants and lower free radicals of monsoon season can be cryopreserved and that will be used for artificial insemination during dry summer or monsoon season for higher fertility and to eliminate the inbreeding in Teressa goat of Andaman and Nicobar Islands. However, the present study has limitations that the sperm was preserved in liquid form for upto 48 h and studied limited number of semen quality profiles. Therefore, further studies are needed on liquid semen preservation for more than 48 h, cryopreservation of semen and *in–vitro* and *in–vivo* fertility trials need to be conducted along with *in–vitro* semen quality parameters in Teressa goat to confirm the present findings.

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; Jai Sunder and A. K. De contributed to data curation; D. Bhattacharya, A. K. Nahak and R. Vikram contributed to formal analysis; P. Perumal and Jai Sunder contributed to investigation; P. Perumal, Jai Sunder, A. K. De and D. Bhattacharya contributed to methodology; P. Perumal and E. B. Chakurkar contributed to project administration; P. Perumal and A. K. Nahak contributed to original draft writing, review and editing.

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