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# Impact of season, age and breed on semen microbial load and its association with semen quality in prominent Indian goat breeds

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ABSTRACT

The aim of this study was to estimate the occurrence of bacteriospermia, determine the bacterial load in fresh semen from 2–6-year-old bucks, and assess the association between bacterial load and sperm quality traits in various Indian goat breeds. For the experiment, one hundred forty-four (144) fresh semen ejaculates were obtained from six bucks of various breeds (two bucks from each breed: Jamnapari, Sirohi, and Barbari) using a sterile artificial vagina on a weekly basis, adhering to a standardized collection technique during three distinct seasons (summer, rainy, and winter). After initial evaluation, samples were subjected to bacteriological examination. The total viable bacterial count of fresh semen was measured using the standard plate count method. The results indicated that the bacterial load was 70%, 56.66%, and 63.33% in Sirohi, Jamnapari, and Barbari bucks, respectively. Sirohi bucks exhibited higher bacterial counts (458.36 ± 9.35 CFU/mL) compared to both Barbari (419.28 ± 10.23 CFU/mL) and Jamnapari (413.82 ± 9.77 CFU/mL). An age-wise analysis revealed that older bucks (≥5 years: 456.73±12.56 CFU/mL) had significantly greater microbial loads than younger groups (2.5–3.5 years: 406.90±4.33 CFU/mL; 3.5–5 years: 423.40±9.53 CFU/mL). Microbial loads in rainy season were significant ($p<0.001$) higher than those observed in winter and summer. Moreover, Pearson correlation analysis revealed that a higher microbial load in buck semen was negatively associated with sperm motility, viability, and plasma membrane integrity.

**Keywords:** Bacteriospermia, Barbari, Buck semen, Jamnapari, Microbial load, Sirohi

Bacterial contaminants of semen have been a significant concern for most semen production laboratories, as they adversely affect semen quality and, consequently, fertility (Grieveu *et al.*1995). The production of frozen semen requires a quality control service to maintain both the quality and fertility of the semen. For satisfactory conception, the microbial population in the semen must be below 500 CFU/mL non-pathogenic microorganisms per dose (BIS, 2012). Several microbial species may survive in semen even after cryopreservation (Savvulidi *et al.* 2018). Microorganisms can affect male reproductive function either directly (by reducing sperm motility, decreasing the acrosomal reaction, or increasing sperm deformity) or indirectly (through the production of reactive oxygen species) (Enwuru *et al.* 2016). Additionally, diseases caused by these microorganisms may reduce the immunity of the reproductive system (Noakes *et al.* 2019). Mating with contaminated semen is also considered one of the causes of uterine infections (Pohjanvirta *et al.* 2020). In addition, contaminated semen negatively affects fertilization and can act as a carrier of genital diseases (Zaid and Al-Zubaidy, 2009). The male is responsible for semen contamination during collection, particularly from the glans penis and prepuce, and this can be influenced by seasonal changes (Azawi and Ismaeel, 2012; Sannat *et al.* 2015b). The excessive accumulation of microbes can lead to infertility (Thacker *et al.* 1984). Consequently, contamination of semen with microbes is a significant factor contributing to the failure of AI, which ultimately results in loss of fertility (Thibier and Guerin, 2000; Reda *et al.* 2020). Gangwar *et al.* (2021) reported a non-significant increase in microbial load during the rainy season and found that microbial load negatively correlated with sperm motility, sperm viability, acrosomal integrity, and plasma membrane integrity in Indian goat breeds. High viability and motility of spermatozoa are crucial for successful artificial insemination (AI), as a significant correlation between post-thaw sperm viability and subsequent conception rates has been reported (Larson-Cook *et al.* 2003). Despite this observation, there have been no report on the impact of seasons on bacterial load and semen quality in goat bucks.

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Therefore, this study aimed to investigate the seasonal effect on bacterial load and its relationship with the semen quality of bucks from various Indian goat breeds.

## MATERIALS AND METHODS

*Ethical approval*: Ethical clearance was not required for semen collection.

*Study animals*: The study involved six goat bucks, with two from each of the following breeds: Barbari, Sirohi, and Jamnapari from the Deep-Frozen Semen Laboratory, Department of Veterinary Gynaecology and Obstetrics, at the College of Veterinary Science and Animal Husbandry, ANDUAT, located in Kumarganj, Ayodhya, Uttar Pradesh, India. All the bucks were maintained under uniform management, feeding, and watering conditions, supplemented with an additional 5 to 6 hours of daily grazing. Each buck was individually housed in pens designed for adequate cross ventilation and protection from summer heat, as well as access to an open area for sunbathing during the winter months.

*Semen collection*: The collection of semen ejaculates was performed using a sterile artificial vagina on a weekly basis, following a standardized collection technique (Rather *et al.* 2016). The samples were processed for bacteriological examination within one hour of collection. A separate artificial vagina was utilized for each buck during every collection. In total, 144 ejaculates were collected from six bucks, resulting in eight ejaculates from each buck per season (winter: Nov-Jan, summer: April-June, rainy: July-Sep) throughout the year, spanning from November 2024 to September 2025.

*Semen evaluation*: Immediately after collection, the collecting tube was detached from the artificial vagina (AV) and transported to the laboratory, where it was placed in a water bath at 35°C until the semen could be evaluated and processed. The samples were assessed for physical characteristics such as color, consistency, and volume, as well as other seminal parameters including mass motility, individual motility, sperm viability, and plasma membrane integrity.

*Sperm motility*: In brief, 10 µl of diluted semen was placed on a warm slide, covered with a coverslip, and observed under a phase contrast microscope at a magnification of 200X. Multiple areas were analyzed to determine the percentage of individual sperm motility. The final motility score for each sample was calculated as the average of two consecutive evaluations.

*Sperm viability assessment*: The sperm viability assay was performed following the method described by Bloom (1950). A small drop of diluted semen was mixed with 2–3 drops of eosin-nigrosine stain. After one minute, a smear was prepared on a clean slide. The dried smear was then examined under the 40X objective of a phase contrast microscope. Approximately 200 sperm were counted. Dead sperm appeared pink against dark background of the Nigrosin stain, while live sperm were colorless.

*Plasma membrane integrity assessment*: HOST was performed to evaluate the functional integrity of the sperm plasma membrane, following the methodology described by Jayendran *et al.* (1984). Briefly, 100 µl of diluted semen was incubated with 1 ml of hypo-osmotic solution (150 mOsm) at 37°C for one hour. After incubation, a small drop of the diluted semen was examined under a phase-contrast microscope to observe various forms of tail curling. A minimum of 200 spermatozoa were counted across different microscopic fields.

*Bacterial load*: The bacterial load assessment was performed on aseptically collected semen samples using the standard plate count (SPC) technique. The SPC was carried out with the spread plate method and serial dilution techniques. The bacterial load in semen was expressed as the number of colony-forming units (CFU) per milliliter of semen and was calculated using the formula: number of colonies multiplied by the dilution factor of the plate, divided by the milliliters of the diluted semen (Shukla, 2011).

*Statistical analysis*: The statistical analysis was conducted using GraphPad Prism software (Version 5.00). The data generated from this study were expressed as mean ± SEM for sperm motility, viability, plasma membrane integrity, and bacterial load (CFU/ml) of semen. Analysis of variance (ANOVA) was performed to compare the means of semen characteristics and microbial load across different breeds, age groups, and seasons, utilizing the post hoc Tukey test. A p-value of less than 0.05 was considered statistically significant. The chi-square test was employed to assess the effect of breed on the occurrence of bacteriospermia in buck semen. Additionally, Pearson's correlation matrix analysis was conducted to explore the interrelationships among various sperm quality parameters and microbial load.

## RESULTS AND DISCUSSION

In the present study, the seminal of bacterial load was found to be 70.83%, 64.58% and 66.66% in Sirohi, Jamnapari and Barbari bucks, respectively. Significant

Table 1. Occurrence of bacteriospermia in different semen ejaculates of Sirohi, Jamnapari and Barbari breeds of goat

| Risk factor | Total semen sample examined | Number of positive samples for bacteriospermia | Prevalence (%) | $\chi^2$ | *p-value* |
|---|---|---|---|---|---|
| Breeds | | | | | |
| Sirohi | 48 | 34 | 70.83 | | |
| Jamnapari | 48 | 31 | 64.58 | 0.44 | 0.80 |
| Barbari | 48 | 32 | 66.66 | | |

Table 2. Average mean values of microbial load (CFU/mL) of bucks of different breed and age in various seasons

| Effect/Factor | Microbial Load (Mean±SEM) | 95% CI | F value | p value | $n^2$ |
|---|---|---|---|---|---|
| Breeds | | | | | |
| Sirohi | $458.360^{B} \pm 9.354$ | 439.563–477.157 | | | |
| Jamnapari | $413.824^{A} \pm 9.774$ | 394.193–433.455 | 6.034 | 0.003** | 0.074 |
| Barbari | $419.283^{A} \pm 10.228$ | 398.758–439.808 | | | |
| Seasons | | | | | |
| Rainy | $475.690^{A} \pm 10.933$ | 453.796–497.583 | | | |
| Summer | $393.673^{B} \pm 4.676$ | 384.298–403.048 | 30.576 | 0.001*** | 0.295 |
| Winter | $404.028^{B} \pm 6.838$ | 390.146–417.909 | | | |
| Age | | | | | |
| 2.5-3.5 year | $406.900^{A} \pm 4.330$ | 398.199–415.601 | | | |
| 3.5-5 year | $423.396^{A} \pm 9.534$ | 404.215–442.577 | 7.046 | 0.001** | 0.085 |
| ≥5 Year | $456.732^{B} \pm 12.563$ | 431.555–481.909 | | | |

CI=Confidence Interval, means for microbial load with different superscripts (A, B, C) in column within each effect differ significantly ($p<0.05$) from each other

difference were recorded in microbial load among the three breeds of buck with Sirohi bucks (458.36 ± 9.35 CFU/mL) exhibiting higher bacterial counts than Barbari (419.28 ± 10.23) and Jamnapari (413.82±9.77 CFU/mL). An age-wise analysis indicated that older bucks (≥5 years: 456.73±12.56 CFU/mL) had significantly greater microbial loads compared to younger groups (2.5–3.5 years: 406.90±4.33 CFU/mL; 3.5–5 years: 423.40 ± 9.53 CFU/mL). Seasonal variation was also highly significant ($p<0.001$), with Tukey's HSD confirming that microbial loads during the rainy season (475.69 ± 10.93 CFU/mL) were substantially higher than those observed in winter (404.03 ± 6.84 CFU/mL) and summer (393.67 ± 4.68 CFU/mL). However, no significant difference was found between the bacterial loads in winter and summer. Collectively, these findings highlighted that genetic background (breed), physiological status (age), and environmental seasonality significantly influenced microbial load, with the rainy season exerting the most pronounced effect. The analysis revealed that sperm motility, sperm viability, and sperm plasma membrane integrity were adversely affected by the increase in bacterial load in semen (Table 3). However, the seminal parameters remained within permissible limits. Analysis of variance indicated that as bacterial load increased in the semen of all breeds, the quality of semen was compromised. In this study, Jamnapari bucks exhibited significantly higher percentages of sperm motility (81.740±0.855), sperm viability (83.580±0.837), and sperm plasma membrane integrity (70.820±0.605) compared to Barbari and Sirohi bucks (Table 3). The increase in sperm viability in Jamnapari bucks was in significantly different from that in Barbari bucks.

Semen contaminated with pathogens pose a significant risk to fertility and reproductive effectiveness. The present regarding the prevalence of bacteriospermia in various goat breeds aligned with the findings of Gangwar *et al.* (2021). However, the prevalence of bacteriospermia was notably higher in other species, with reports of 97% (Yaniz *et al.* 2010) and 99% (Gączarzewicz *et al.*2016) in ram semen, and 63% (Bresciani *et al.*2014) in boar semen. Conversely, a lower prevalence of 33.2% (Moretti *et al.* 2009) and 35.3% (Vilvanathan *et al.* 2016) has been earlier reported in

Table 3. One-way Anova analysis for effect of breed on semen characteristics in goat bucks (n=30)

| Seminal Attributes | Breeds | Mean±SEM | *F-value* | *P value* |
|---|---|---|---|---|
| Progressive motility (%) | Barbari | $78.320^{A}$±0.825 | 6.888 | 0.0014** |
| | Jamnapari | $81.740^{B}$±0.855 | | |
| | Sirohi | $76.960^{A}$±1.109 | | |
| Sperm viability (%) | Barbari | $80.780^{AB}$±0.901 | 7.156 | 0.0011** |
| | Jamnapari | $83.580^{B}$±0.837 | | |
| | Sirohi | $78.620^{A}$±1.040 | | |
| Plasma membrane integrity (%) | Barbari | $66.820^{A}$±0.706 | 13.73 | 0.0001*** |
| | Jamnapari | $70.820^{B}$±0.605 | | |
| | Sirohi | $65.780^{A}$±0.826 | | |

CI=Confidence Interval, means for microbial load with different superscripts (A, B, C) in column within each effect differ significantly ($p<0.05$) from each other

human semen samples. The differences in level of bacterial contamination may be attributed to factors such as species, age, breed, season (Sannat *et al.*2015), anatomy, physiology, and management practices (Yaniz *et al.* 2010). Additionally, unsanitary conditions during the collection, transportation, and processing of semen may also contribute to these variations (Mitra *et al.*2016). Nevertheless, the bacterial load can be significantly reduced by implementing hygienic practices related to packaging, handling, sterilization, and fumigation protocols (Althouse, 2008; Sannat *et al.*2015), in addition to performing preputial washing (Meena *et al.* 2015).

Statistical analysis indicated a significant disparity in bacterial counts among the three breeds of bucks, showing that Jamnapari buck semen had a notably lower bacterial load compared to Barbari and Sirohi (Table 2). In contrast to these findings, Gangwar *et al.* (2021) reported that Jamnapari buck semen had a substantially higher bacterial load than that of Barbari and Jhakrana bucks. It may be due to the comparatively higher hair growth around the prepuce, which could have led to the contamination. The observations regarding breed-specific variation in microbial load are supported by Brown *et al*. (1974), who noted variable concentration of natural inhibitors in seminal plasma among individual bulls and breeds. They found that Holstein-Friesian cattle have higher concentrations of these inhibitors in their seminal plasma compared to Hereford cattle. Furthermore, Bhatt *et al.* (2012) indicated that significant differences in bacterial load within the semen of different breeds may be linked to the levels of cytokine production in the cells of those breeds, which contributes to their inherent resistance to infection. However, the bacterial load in the semen of all breeds was determined to be within acceptable limits.

The present study examined the levels of bacteria in semen samples collected from bucks across different age groups. We found that bacteriospermia was significantly higher in older bucks, specifically those over 5 years old, compared to their younger counterparts (2.5-3.5 year) (Table 2). Young and elderly bulls may experience a higher bacterial burden due to their compromised immune systems, which makes them more vulnerable to infections. The findings were aligned with those of Gangwar *et al.* (2021), who reported that microbial load was significantly higher in older bucks compared to younger ones among various Indian goat breeds. Conversely, Ghoneim *et al.* (2014) found that middle-aged camels (9-13 years) exhibited a greater bacterial load than both younger (4-8 years) and older (14-18 years) animals.

Seasonal effects arise from various factors, including cleanliness, ambient temperature, relative humidity, and photoperiod. Rainy season had a significantly higher microbial load compared to the summer and winter seasons. In agreement with our observations, Gangwar *et al.* (2021) noted a non-significantly higher microbial load compared to the summer and winter seasons. In addition, Azawi and Ismaeel (2012) reported the highest microbial load (138.3±21.6) in ram semen during July. During the rainy season, there is an increase in bacterial contamination in semen, plausibly attributed to difficulties in maintaining the cleanliness of the bull's bedding and lanes. Which might have resulted in a greater accumulation of dirt on the legs, flanks, and preputial orifice under such conditions. Research indicates that summer sunlight significantly inhibits bacterial growth, while prolonged exposure to winter light notably reduces bacterial proliferation (El-Tayeb *et al.*2007). Sunlight, particularly the red light, is associated with the activation of reactive oxygen species, which can be toxic to catalase-negative bacteria.

Moreover, in the present study, Pearson's correlation analysis indicated that sperm motility percentage, sperm viability percentage, and the percentage of intact sperm membranes were negatively correlated with bacterial contamination in buck semen (Table 4-6). These findings aligned with Gangwar *et al.* (2021), which demonstrated that various seminal attributes including sperm motility,

Table 4. Correlation among bacterial load and semen parameters in Barbari bucks

| Seminal Attributes | Progressive motility (%) | Sperm viability (%) | Plasma membrane integrity (%) | Microbial load (CFU/mL) |
|---|---|---|---|---|
| Progressive motility | 1 | 0.961 | 0.451 | -0.606 |
| Sperm viability | | 1 | 0.429 | -0.480 |
| Plasma membrane integrity | | | 1 | -0.460 |
| Microbial load | | | | 1 |

Table 5. Correlation among bacterial load and semen parameters in Jamnapari bucks

| Seminal Attributes | Progressive motility (%) | Sperm viability (%) | Plasma membrane integrity (%) | Microbial load (CFU/mL) |
|---|---|---|---|---|
| Progressive motility | 1 | 0.712 | 0.792 | -0.652 |
| Sperm viability | | 1 | 0.725 | -0.559 |
| Plasma membrane integrity | | | 1 | -0.526 |
| Microbial load | | | | 1 |

Table 6. Correlation among bacterial load and semen parameters in Sirohi bucks

| Seminal Attributes | Progressive motility (%) | Sperm viability (%) | Plasma membrane integrity (%) | Microbial load (CFU/mL) |
|---|---|---|---|---|
| Progressive motility | 1 | 0.961 | 0.512 | -0.750 |
| Sperm viability | | 1 | 0.509 | -0.657 |
| Plasma membrane integrity | | | 1 | -0.474 |
| Microbial load | | | | 1 |

viability, acrosomal integrity, and plasma membrane integrity were adversely affected by increased bacterial loads in the semen of Barbari, Jamnapari, and Jhakrana goats. Similarly, the findings are also consistent with Shukla (2005), regarding significant negative correlation between bacterial count and sperm motility and viability in the cryopreserved semen. Moreover, Jamnapari bucks demonstrated relatively high-quality semen, with lower bacterial contamination compared to Sirohi and Barbari bucks. This observation aligned with the findings from various researchers (Ahmed *et al.*2001; Akhter *et al.*2008), who reported a significant negative correlation between bacterial count and sperm functional markers in buffalo bull semen. Moreover, motility and viability of human sperm has been reported to decline significantly when infected *in vitro* by *Staphylococcus epidermidis* (Merino *et al.*1995). Several studies (Diemer *et al.*1996; Bielanski and Vajta, 2009; Prieto-Martínez *et al.*2014) have also demonstrated that the presence of these bacteria is crucial, as they can adhere to spermatozoa and decrease sperm motility. They can damage acrosomes by producing toxins (Morrell, 2006) and induce DNA damage (Gonzalez-Marin *et al.*2011) through the generation of reactive oxygen species (Morrell, 2006). Diemer *et al*. (2003) reported that *E. coli* presence hinders the progressive motility of spermatozoa. Observations from electron microscopy revealed numerous adhesions of *E. coli* to spermatozoa, which resulted in morphological damage and subsequent immobilization. Retrospective tests indicated that these bacteria have adhesive properties, allowing them to attach to flagella and pili via mannose receptors (Moretti *et al.*2014). This situation may ultimately contribute to reduced fertility rates and the culling of breeding animals, which can lead to significant financial losses for the dairy sector (Moustacas *et al.*2010). Therefore, it is crucial to actively prevent microbiological contamination from entering semen processing and storage facilities, as well as artificial insemination activities (Eaglesome and Garcia, 1997).

The prevalence of bacterial contamination in semen was recorded as 70.00%, 56.66%, and 63.33% in Sirohi, Jamnapari, and Barbari bucks, respectively. Seasonal variation was significant with summer and winter showing lower bacterial contamination compared to the rainy season. Therefore, winter and summer may be considered more suitable seasons for semen collection. Among the breeds studied, Sirohi bucks exhibited comparatively higher bacterial counts. Increased bacterial contamination beyond permissible limits adversely affected sperm motility, viability, and plasma membrane integrity. The observed negative association between microbial load and semen quality parameters may impair breeding efficiency, artificial insemination outcomes, and increase the risk of uterine infections. These findings highlighted the importance of maintaining strict hygienic practices during semen collection, processing, transportation, and storage.

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