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Biochemical evaluation of fresh semen in salem black buck

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Abstract
In this study, a sexually mature Salem Black Buck (SB6) was utilized. The semen was collected twice a week after two false mountings. The collected semen was transferred to the semen processing laboratory. The semen was evaluated macroscopically and microscopically. Then biochemical evaluation was done before further processing. The biochemical analysis showed that the average semen pH was 6.16 ± 0.30. The mean Methylene Blue Reduction Time was 4.83 ± 0.30 minutes. The average fructolytic index was 1.79±0.35. The biochemical characteristics indicate good metabolic activity, overall semen quality, and its suitability of fresh Salem Black buck semen for further processing.

Keywords: Salem Black buck, pH, MBRT, Fructolytic index

1. Introduction
Among domesticated animals, goats are highly adaptable to many environmental conditions, which makes them globally distributed. The first step of the goat improvement program is the selection of superior quality bucks. Bucks contribute to half of the genetic material to the flock (Perumal *et al.,* 2023) [12]. Fertility in male animals is evaluated by semen analysis, which assesses the number of sperm produced and their ability to fertilize the oocyte, both *in vivo* and *in vitro* conditions (Wang *et al.,* 2014) [16]. Semen analysis also indicates the functional efficiency of testicles in sperm production (Tanga *et al*., 2021) [15]. A combination of in vitro tests is necessary to assess the fertility of a semen sample, as no single test is sufficient (Sundararaman *et al.,* 2016) [14]. In this study, biochemical evaluation of semen samples was carried out. The objective of the study is to evaluate the biochemical characteristics of fresh Salem Black buck semen before further processing.

2. Materials and methods
A healthy, sexually matured Salem Black buck (SB6) maintained at the Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal, was utilized for this study. Semen collection was done between 6 am to 7 am on Tuesday and Friday during the trial period. The semen was collected from the SB6 buck after the animal's preputial area was prepared aseptically. It was collected twice a week using an artificial vagina (AV), which was a Russian model. Two false mountings were allowed before the collection. During the third mounting penis was guided into the AV, and the semen was collected. The collected ejaculates were immediately transferred to a water bath (34 °C) in the laboratory.

2.1 Biochemical evaluation of fresh semen
2.1.1 pH
The pH of a fresh semen sample was evaluated using a pH paper. A drop of fresh semen sample was placed on pH paper, and the resulting color change was compared with standard colors to determine the pH value.

2.1.2 MBRT
The Methylene Blue Reduction test was done to assess the metabolic activity of spermatozoa. In a sterile test tube fresh semen sample (0.2 ml) was taken, and Tris buffer (0.8ml) and methylene blue (0.1 ml) were mixed. Liquid paraffin (1cm layer) was placed above the mixture. The test tube was kept in a water bath at 46.5 °C to observe the color-changing time. The duration taken to change the blue color to colorless was recorded

2.1.3 Fructolytic index
Fructose concentration was measured by the colorimetric method as described by Ruthrakumar *et al.* (2023) [13]. First, the centrifugation of a fresh semen sample was done at 3000 g for 15 minutes. Following 0, 2, or 4 hours of storage at 25 °C, fructose concentration in seminal plasma was measured. 0.1 ml of fresh seminal plasma was taken and mixed with 2.9 ml of distilled water. Then 0.15 M barium hydroxide (0.5 ml) (Himedia) and 0.175 M zinc sulphate (0.5 ml) were added, completely mixed and kept at room temperature (28 °C) for 5 minutes. After that, centrifugation of the mixture was done at 3000g for 15 minutes, and the supernatant was separated. 3ml of 10 M hydrochloric acid and 1ml of 8.47mM resorcinol were added in 1ml of the supernatant and incubated at 90 °C for 10 minutes. Immediately after the incubation, the tubes were kept on ice. The fructose concentration (mmol/L) in the seminal plasma was evaluated by measuring the absorbance at 490 nm using a UV-VIS double-beam spectrophotometer().
The fructose concentration was calculated as mmol/L: absorbance value for test tube/ absorbance value of standard tube×11.12.
From the fructose concentration fructolytic index was calculated as explained by Mann (1964) [9].

3. Results and discussion
3.1 pH
Semen pH affects sperm motility and metabolic activity of the sperm, thereby influencing fertilization potential (Kumar *et al.,* 2024) [8]. In the present study, the mean semen pH was 6.16 ± 0.30, with values ranging from 5.0 to 7.0 (Table 1). Previous studies in different breeds have reported comparable findings: Ferdinand *et al*. (2012) [4] observed a pH of 6.73 ± 0.25 in West African Dwarf bucks. Patil *et al.* (2019) recorded 6.78±0.01 in Berari buck, with a range of 6.70 to 7.00, Yotov (2015) [17] and Dagli (2011) [2] reported similar values (6.0-7.0) in Bulgarian White milk and in Osmanabadi and Sirohi bucks, respectively. An optimal semen pH important for proper enzymatic activity and sperm survival as they travel through the female reproductive tract (Kachru *et al*., 2023) [5].

3.2 Methylene Blue Reduction Test (MBRT)
The enzymatic activity of the sperm, the dehydrogenase function, is evaluated by MBRT. During metabolism, hydrogen ions are released, which reduce methylene blue to leucomethylene blue (colorless form) (Chaurasia, 2023) [1]. The mean Methylene Blue Reduction Time in fresh semen of Salem Black buck was 4.83 ± 0.30 minutes, ranging from 4 to 6 minutes (Table 1). The finding was comparable with Kannan (2024) [6], who observed the reduction time of 4.66 ± 0.33 minutes in Salem Black bucks. This test reflects sperm activity and density, making it a useful indicator for estimating fertility (Chaurasia, 2023) [1].

3.3 Fructolytic index
Fructose is a major energy source of the spermatozoa (Matos-Brito *et al.,* 2013) [10]. The metabolic activity of the spermatozoa is reflected by their ability to utilize glucose, fructose, and mannose. These sugars, through a hexokinase-dependent reaction with ATP, enter the glycolytic pathway, resulting in lactic acid production via the hexose monophosphate pathway (King *et al*., 2006) [7]. Therefore, measuring the fructose concentration is important to evaluate the metabolic activity of sperm. The average fructolytic index in Salem Black buck fresh semen was 1.79±0.35 (Table 1). Reported fructolytic index in other species includes 1.02±0.37 in Kangayam bulls (Ruthrakumar *et al.,* 2023) [13] and 1.09±0.06 in HF bulls (Patel *et al.,* 2014) [11]. El-Azzazi and Yaseen (2016) [3] mentioned fructose utilization and fructolytic index as indicators of sperm metabolic activity in ram semen, while Matos-Brito *et al.* (2013) [10] reported the importance of seminal fructose concentration in supporting sperm metabolism in goats.

4. Conclusion
This present study concluded that biochemical characteristics of fresh Salem Black buck semen, including pH, MBRT, fructolytic index values indicating good metabolic activity, overall semen quality, and its suitability for further processing.

Table 1: Biochemical evaluation of fresh semen from Salem Black buck

Semen characteristics	No of samples	Grading	Values
p^H	6	p^H 5	1 (16.66%)
		p^H 6	3 (50.00%)
		p^H 7	2 (33.33%)
		Mean ± SE	6.16±0.30
MBRT	6	4 min	2 (33.33%)
		5 min	3 (50.00%)
		6 min	1 (16.66%)
		Mean ± SE	4.83±0.3
Fructolytic index	6	Mean ± SE	1.79±0.35

Conflict of Interest
Not available

Financial Support
Not available

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