International Journal of Veterinary Sciences and Animal Husbandry 2023; 8(6): 135-138

ISSN: 2456-2912
VET 2023; 8(6): 135-138

www.veterinarypaper.com
Received: 02-08-2023
Accepted: 07-09-2023

Sreeranjani Raju
PG Scholar, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Sathyabama Thangavelu
Associate Professor and Head, Department of Veterinary Physiology and Biochemistry, Veterinary College and Research Institute, Udumalpet, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Selvaraju Mani
Dean, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Selvaraj Palanisamy
Professor and Head, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Corresponding Author:
Sreeranjani Raju
PG Scholar, Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Seminogram of Salem black bucks: A pilot study

Sreeranjani Raju, Sathyabama Thangavelu, Selvaraju Mani and Selvaraj Palanisamy

Abstract
The Salem Black goat breed is a well-known for meat and its reproductive potential in north-western part of Tamilnadu. The present study was conducted to document the semen characteristics of Salem Black bucks. A total of 96 ejaculates from two Salem Black bucks (SB 882 and SB 917) maintained in the Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, VCRI, Namakkal were utilized for this study. The animals were trained for semen collection using artificial vagina. The semen samples were collected, processed and evaluated for microscopic and macroscopic quality parameters. The semen was further extended and cryopreserved for artificial insemination. The results of semen volume per ejaculate, sperm concentration, initial sperm motility, percentage of live sperm, percentage of normal sperm and percentage of abnormal sperm were ranged from 0.921±0.028 to 0.928±0.026 ml, 3232.9±56.55 to 3293±63.19 million/mL, 86.77±0.30 to 90.437±0.326%, 85.61±0.27 to 89.437±0.25%, 85.33±0.002 to 86.24±0.293%, and 13.76±0.29 to 14.70±0.22% respectively. The seminal characteristics of Salem Black bucks were found to be good enough to be adopted for advanced reproductive technologies.

Keywords: Ejaculate, motility, semen, seminal characteristics

Introduction
The Salem Black is an important meat goat breed in the north-western part of Tamil Nadu, India. The native tract of this breed is Salem, Dharmapuri, Krishnagiri, Erode, Karur and Namakkal districts of Tamil Nadu. Salem Black goats are tall animals, completely black in color and reared mainly for meat (Thiruvenkadan, 2006) [24]. Selection of buck represents the starting point in goat development program. Selection of male is much more important because a buck can produce thousands of kids a year through artificial insemination (AI), therefore the main avenue for genetic improvement is the selection of breeding male (Ahmed and Islam, 1987)[1]. The conception rate is chiefly depending upon the quality of the semen. In most situations, bucks are being kept by only a few people and animals are often genetically very poor with unknown pedigree. Moreover, same buck has been used generation after generation which has created greater chance of inbreeding and hence lowering reproductive performance along with disseminating of various venereal and infectious diseases (Husain, 2007) [9]. In order to propagate superior germplasm of Salem Black bucks, their seminal attributes were analyzed and further cryopreserved and propagated. This study is the first attempt in analyzing and documenting the seminal attributes of Salem Black bucks.

Materials and Methods
Source of experimental animal
Two Salem Black bucks (SB 882 and SB 917) maintained in the Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal were utilized for this study.

Preparation of artificial vagina
The AV used in this experiment was IMV model. The sterilized inner liner was attached inside the rubber cylinder and the edges of the inner liner were folded over the rim of rubber cylinder on either side. The space in between the rubber cylinder and inner liner was filled with hot water of 60 to 70 °C to maintain the final AV temperature of 40-45 °C. The sterile collection

cup was placed on one edge of assembled AV to collect the semen during mounting (Elamurugan *et al.*, 2020) [6].

Semen collection

The semen was collected from the bucks, one buck was used as dummy for the other during semen collection. The semen was collected twice a week (Tuesday and Friday) and at each time two ejaculates were collected. Two false mounts were given before semen collection. On the third mount, the penis was directed into AV and the semen was collected in the collection tube. The collected ejaculate was immediately transferred to the semen processing laboratory and kept at 34 °C in the water bath.

Macroscopic evaluation of fresh semen

Immediately after semen collection, the gross motility of the semen sample was assessed by naked eye based on the swirling movement observed in the collection cup. The collection tube and semen samples were grossly examined by naked eye for the presence of foreign materials if any. The volume of the ejaculate was measured in milliliter directly from the graduations of the collection tube. The color of the semen sample was assessed visually by keeping creamy color as standard and further graded based on the intensity of samples. Thick, milky and creamy color ejaculates were taken for further analysis. A drop of pooled semen sample was placed on clean and grease free glass slide and visually examined for opacity and the semen samples were graded from 1 to 5. Samples which had 3-5 density were used for processing. Concentration of spermatozoa in the semen sample was measured by using Accucell photometer (IMV Technologies, France) at 530 nm wave length after diluting 10 µl of semen with 3.990 ml of 0.152 M sodium chloride (Elamurugan *et al.*, 2020) [6].

Microscopic evaluation of fresh semen

A drop of neat semen kept in a clean grease free glass slide and examined under microscope at 10x and 40x reveals individual sperm motility. Live and dead sperms and abnormality in semen samples were assessed by making smear on clean grease free glass slide and stained using eosin and nigrosin stains. Then the smears were examined at 100x in oil immersion of bright field microscope.

Statistical analysis

The results were analysed using IBM SPSS Statistics 20. For comparison of volume, sperm concentration, sperm motility, live sperm, normal sperm and abnormal sperm between two buck semen samples, the unpaired t-test was applied (Petrie and Watson, 1999) [16]. Data were reported as mean (±SE). Values were considered to be statistically significant at $p<0.05$.

Results and Discussion

Macroscopic and microscopic seminal parameters in Salem Black bucks have been tabulated in Table 1. The average semen volume in Salem black bucks (Table 1) in present study 0.925±0.019 ml which was higher when compared with Chegu goats 0.85±0.07 ml (Sharma and Sood, 2019) [18], Gaddi bucks 0.66±0.04 ml (Sharma, 2018) [19], Pashmina bucks 0.62 ml (Mohan *et al*., 1980) [12], Chegu bucks 0.47 ml (Thakur *et al.*, 2005) [23], West African Dwarf bucks 0.38–0.44 ml (Waidi *et al.*, 2007) [25]. However, it was lower than Blanca Andaluza bucks 0.87-0.97 ml (Gallego-Calvo *et al.*, 2015) [8], Blanca-Celtiberica bucks 1.16 ml (Jimenez Rabadan *et al.*, 2013) [10], Boer bucks 1.15 ml (Yodmingkwan *et al.*, 2016) [26], Majorera bucks 1.21±0.03 ml (Batista *et al.*, 2009) [2] and Blanca de Rasquera bucks 1 ml in 1 year and 1.8ml in 2 year old (Tabarez *et al.*, 2017) [22]. Sharma and Sood, 2019 [18] reported that the seminal plasma contributes to about 70 per cent of the volume of the ejaculate and an increase or decrease, in the semen volume mainly depends on the quantities of fluids secreted by the epididymis and the accessory glands.

The colour of Salem Black buck semen was to be found creamy in the present study. Creamy colour of buck semen was reported by Olurode *et al*., 2018 [14] and Dagli, 2011 [5] in West African Dwarf and Sirohi bucks respectively. However, some researchers reported yellowish (Bezjian *et al.*, 2013) [3] and yellowish white colour semen (Bras, 2012) [4] in goat. The colour pattern of the neat semen is the species specific and is also dependent on the sperm concentrations and presence of pigmented proteins and carotenoids in the seminal plasma (Patil *et al.*, 2019) [15].

The Sperm concentration of Salem Black bucks semen was recorded as 3263.1±42.35 million per ml (Table 1). The value recorded in the present study is in agreement with the different authors who reported total sperm concentration in various goat breeds [Sundararaman and Edwin, 2003 [21] in Boer Grade half-bred bucks, Gacitua and Arav, 2005 [7] in Saanen bucks and Yotov, 2015 [27] in Bulgarian White milk breed]. Higher value of sperm concentration was reported in Gaddi bucks 3401.0± 247.2 million per ml (Sharma 2018) [19] and, Blanca Andaluza bucks 3336-7776 million per ml (Gallego Calvo *et al.*, 2015) [8]. Lower value of sperm concentration was reported in Chegu bucks 2238.5±231.0 million per ml (Sharma and Sood, 2019) [18], Chegu bucks 1759.3± 79.79 million per ml (Thakur *et al.*, 2005) [23], West African Dwarf bucks 1700 million per ml (Waidi *et al.*, 2007) [25] and Blanca-Celtiberica bucks 2845±142.64 million per ml (Jimenez-Rabadan *et al.*, 2013) [10]. The sperm concentration in goats is influenced by factors such as the frequency of semen collection, the age of the buck, and the prevailing season (Sundararaman and Edwin, 2003) [21]. Gacitua and Arav, 2005 [7] and Batista *et al*., 2009 [2] reported the significant variation among bucks for sperm concentration.

The individual sperm motility percentage of Salem Black buck ranged between 80 to 98 percent keeping the mean 88.60±0.26 percent (Table 1). The value recorded in the present study is in agreement with the different authors such as Dagli, 2011 [5] in Osmanabadi and Sirohi and Yotov, 2015 [27] in Bulgarian white milk breed. Whereas, higher individual motility was also reported in exotic breed by Olayemi *et al*., 2011 [13] and Bras, 2012 [4] in Stud breed. However, much lower individual motility value of 50.00 ± 5.50 was recorded by Bezjian *et al*., 2013 [3] in Makhor buck. The individual motility seems to be a specific breed character (Patil *et al.*, 2019) [15].

Average fresh semen livability observed in present study (Table 1) was 87.53±0.234. However, 90 per cent and above normal live sperm count reported by Bras, 2012 [4] in Stud and Olayemi *et al.*, 2011 [13] in West African Dwarf buck. However, much lower value of 48.9 ±6.0.was recorded by Bezjian *et al*., 2013 [3] in Makhor buck. The variation in the live sperm count may be due to seasonal fluctuation or ambient temperature existing in the goat shed (Patil *et al.*, 2019) [15].

Similarly, the average morphological abnormalities (14.23±0.19) in neat semen of present study (Table 1) was in agreement with the findings of Singh *et al.*, 2016 [20] (13.37-

16.81%). However it was lower than those of Black Bengal bucks and Chegu bucks 23.3% (Thakur *et al.*, 2005) [23] and much higher than those of Boer bucks 1.54-1.71% (Yodmingkwan *et al.*, 2016) [26], Jamunapari buck 2.84±0.49 (Ramachandran *et al.*, 2015) [17] and Jakhrana bucks 12.18-13.73% (Kumar *et al.*, 2016) [11]. The variation in the abnormal sperm count may be due to semen volume, pH of dilutor, age of the buck, season and frequency of semen collection (Patil *et al.*, 2019) [15]. The most probable reason for morphological abnormalities seems to be the physical and chemical environments to which a spermatozoon is exposed during the preservation (Sharma and Sood, 2019) [18]. Post thaw livability and abnormality of the Salem Black bucks will be assessed in mere future.

Table 1: Seminal attributes (Mean ± SE) of Salem Black buck (n=96)

Buck No.		Volume (ml)	Sperm concentration (million/ml)	Sperm motility (%)	Live sperm (%)	Normal sperm (%)	Abnormal sperm (%)
1	Min	0.5	2014	81	84	80	6
	Max	1.5	4789	98	95	94	20
	M±SE	0.928±0.026	3232.9±56.55	90.44±0.33	89.44±0.25	86.24±0.293	13.76±0.29
2	Min	0.5	2501	80	80	81	10
	Max	2	4871	94	95	90	19
	M±SE	0.921±0.028	3293.2±63.19	86.77±0.30	85.61±0.27	85.32±0.002	14.70±0.22
Sig level		NS	NS	**	**	**	*
Overall Mean		0.925±0.019	3263.1±42.35	88.60±0.26	87.53±0.234	85.78±0.187	14.23±0.19

(*, $p<0.05$; **, $p<0.01$; NS, Non-signifiant)

Conclusion

Thus, the current study is the first ever attempt in the evaluation of major fresh seminal attributes of Salem Black bucks. This study concluded that the seminal characteristics of Salem Black bucks were found to be good enough to be adopted for advanced reproductive technologies.

Acknowledgement

The authors are highly thankful to the Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal for providing necessary help for the study.

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