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A comparative study on seminal attributes of Sirohi and Beetal bucks

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Abstract
A total of 126 ejaculates, 21 from each of six bucks comprising three Beetal and three Sirohi bucks, collected by artificial vagina were used to study various semen characteristics. The semen characteristics *viz.*, ejaculate volume, mass activity, initial sperm motility, live sperm, sperm concentration, sperm number per ejaculate, cold shock resistance index, live intact acrosome and sperm abnormalities were studied by conventional methods. The overall mean values of different characteristics of fresh semen in Beetal and Sirohi bucks for ejaculate volume, mass activity, initial sperm motility, live sperm, sperm concentration, sperm number per ejaculate, cold shock resistance index, live intact acrosome, head abnormalities, mid piece abnormalities, tail abnormalities and sperm with proximal and distal cytoplasmic droplet were 1.08 ± 0.05 and 1.18 ± 0.04 ml, 3.71+ and 3.92+, 82.94 ± 0.73 and 84.60 ± 0.61 per cent, 85.57 ± 0.52 and 86.03 ± 0.48 per cent, 3356.73 ± 83.06 and 3176.94 ± 53.92 million per ml, 3595.17 ± 122.08 and 3669.00 ± 126.42 million, 6.72 ± 0.22 and 6.77 ± 0.20 per cent, 85.18 ± 0.39 and 84.05 ± 0.41 per cent, 1.65 ± 0.13 and 1.50 ± 0.12 per cent, 0.25 ± 0.02 and 0.30 ± 0.03 per cent, 2.73 ± 0.18 and 2.53 ± 0.13 per cent, 0.43 ± 0.03 and 0.48 ± 0.04 per cent and 0.86 ± 0.12 and 0.61 ± 0.04 per cent respectively. The different semen characteristics did not differ significantly between breeds; however, the differences between bucks within the breed were significant for ejaculate volume, live intact acrosome, mid piece abnormalities, tail abnormalities and sperm with distal cytoplasmic droplet.

Keywords: Beetal, Sirohi, mass activity, cold shock resistance.

Introduction
The goat (*Capra hircus*) is one of the main sources of earnings for landless and marginal farmers for whom it is a saviour during distress. Goat is known as "poor man's cow" because of its smaller size and different managemental advantages. It can survive on almost all types of grass and tree leaves without any expenses. India is the second largest goat producing country in the world with a population of 140.54 million which constitutes 16.31 per cent of the world's goat population. Goat occupies an important place in the rural economy of Assam with a total population of 43, 20,000 (18th Livestock census 2007) [2] that constitutes 3.07 per cent of the country's goat population and 25.08 per cent of the state's livestock population. The people of Assam are mostly non-vegetarian and prefer chevon (goat meat) which is free from religious and social taboo. Thousands of goats are being slaughtered every year for meat purpose and people in general prefer the meat of male goats. This has resulted in acute scarcity of male goats for breeding. Goat farming has now shown an increasing trend and now a days one of the main livelihood of poor families especially female Self Help Groups (SHG).
Being smaller in body size, the native goats of Assam are of poor production potentialities in terms of meat and milk in comparison with that of other recognized goat breeds of India *viz.* Beetal, Jamunapari, Sirohi, Barbari, etc. This can be improved by crossbreeding with bucks of superior breeds of goat through Artificial Insemination. For last about three decades Beetal bucks of Punjab maintained at Goat Research Station, Assam Agricultural University, Burnihat are being used to breed Assam goats and for this purpose Beetal buck semen is processed and preserved in frozen state. Of late Animal Husbandry and Veterinary Department, Government of Assam has shown keen interest in introducing Sirohi bucks particularly because of its popularity and adaptability in the hill districts of Karbi Anglong and North Cachar Hills. The success of A.I. depends on quality of frozen semen which in turn depends on quality of fresh semen and extender used in freezing of semen. Although semen characteristics of Beetal bucks under the agro-climatic condition of Assam was studied by several workers (Dewry, 2014 and Hazarika, 2014) [6, 8], no systematic study was conducted yet

on semen characteristics of Sirohi bucks.
Since the hot and humid climate of Assam is very much different from that of Rajasthan, the native home of Sirohi bucks, it is indeed necessary to study the semen quality of Sirohi bucks maintained in Assam. Keeping in view the above facts, the present study had been planned to study the semen characteristics of Sirohi and Beetal bucks.

Materials and methods
Three Sirohi and three Beetal bucks aged three to eight years maintained at Goat Research Station, Assam Agricultural University, Burnihat were used as experimental animals in the study. The bucks were thoroughly examined for sexual and general health before being selected for the present study. The bucks were stallfed and maintained under uniform feeding and managemental practices throughout the period of study.

Collection of semen
Semen was collected from each buck once or twice a week with the help of a standard artificial vagina using a restrained doe as a mount (Figure 1).

Fig 1: Collection of semen from Sirohi buck by artificial vagina method

Evaluation of fresh semen
Immediately after collection the semen was evaluated for ejaculate volume, mass activity and initial sperm motility. A total of 126 ejaculates comprising 21 ejaculates from each of six bucks were further evaluated for live sperm, sperm concentration, sperm number per ejaculate, cold shock resistance index, live intact acrosome and sperm abnormalities.

Ejaculate Volume
The ejaculate volume was recorded directly from the glass graduated semen collection tube and was recorded in millilitre.

Mass activity
It was estimated by placing a drop of fresh semen on a prewarmed (37ºC) glass slide and examined without cover slip under low power objective at a magnification of 100X using a compound microscope. The scoring of mass activity was done on the basis of wave pattern described by Zemjanis (1970) [18] as follows:

Observation	Numerical scale
Waves not present, sperm cells immotile	0
Waves not present, sperm cells motile	1+
Barely distinguishable waves in motion	2+
Waves apparent, moderate motion	3+
Dark, distinct waves in rapid motion	4+

Initial sperm motility
A fine drop of semen was diluted with 4-5 drops of prewarmed (37°C) tris buffer for estimation of initial sperm motility. A drop of diluted semen was then placed on a prewarmed glass slide (37°C) and examined under a cover slip at a magnification of 400X using a compound microscope. Initial sperm motility was recorded from 0 to 100 based on the percentage of progressively motile sperm.

Live sperm
The percentage of live spermatozoa (Figure 2) was determined using Eosin-Nigrosin staining technique described by Blom (1977) [3]. The staining solution was prepared by mixing 1part of 5 per cent Eosin and 4 parts of 10 per cent Nigrosin stain and kept at 5°C in a refrigerator. Immediately after collection, one drop of fresh semen was mixed with 4 drops of prewarmed (37°C) staining solution and allowed to stand for 30 seconds. A thin smear was then prepared on a clean grease-free glass slide with the help of smooth edge of another slide and 200 spermatozoa were examined in different areas of the smear under oil immersion objective at a magnification of 1000X using a compound microscope for determining the percentage of live spermatozoa. Spermatozoa which were not stained were considered live and stained or partially stained were considered as dead.

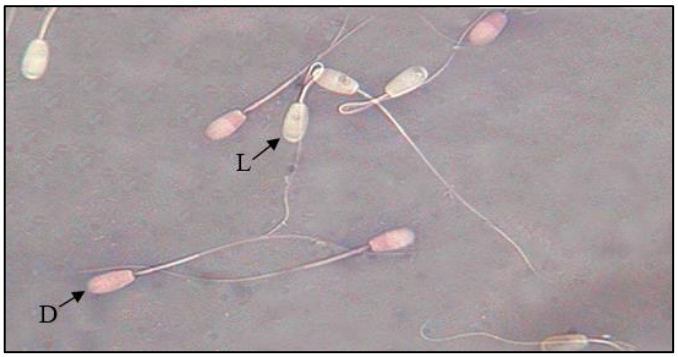

Fig 2: Live (L) and Dead (D) sperm

Sperm concentration
The sperm concentration was determined with the help of Neubauer counting chamber after a dilution of 1:200 with a diluting fluid and expressed in million per millilitre of semen.

Composition of diluting fluid

Eosin-Y	0.05g
Sodium chloride (NaCl)	1.00g
Formalin	1 ml
Distilled water ad	100 ml

Sperm number per ejaculate
The sperm number per ejaculate was calculated by multiplying the sperm concentration per millilitre of semen with ejaculate volume.

Cold shock resistance index
Fresh semen, 0.2ml was transferred into a small glass test tube. The glass test tube was then placed into a beaker containing finely crushed ice (0ºC) for 5 minutes. At the end of 5 minutes, the semen sample was warmed up by placing the test tube in a beaker containing water at 37°C. For determination of cold shock resistant spermatozoa, a drop of cold shocked semen was mixed with 4 drops of Eosin-

Nigrosin stain and a thin smear was prepared. After drying, the smear was examined under oil immersion objective at a magnification of 1000X using a compound microscope. Two hundred spermatozoa were counted in each smear at different areas for determination of percentage of live sperm after cold shock.

Live intact acrosome

The incidence of live intact acrosome (Figure 3) was studied in stained smears using Nigrosin-Eosin-Giemsa staining technique (Tamuli and Watson, 1994) [15]. Two hundred spermatozoa were examined in each smear at a magnification of 1000X of a compound microscope to determine the percentage of live intact acrosome

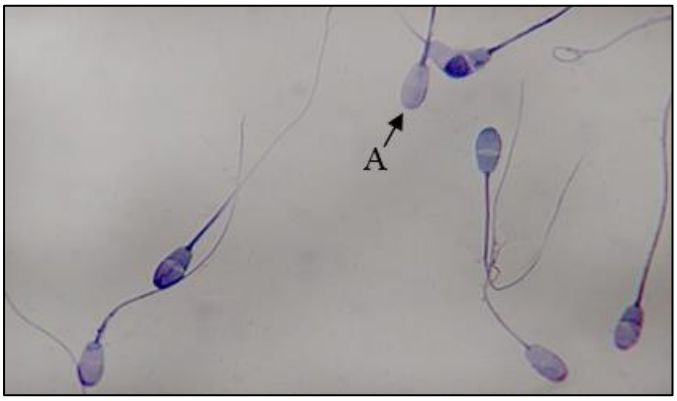

Fig 3: Live intact acrosome (A)

Nigrosin- Eosin-Giemsa staining procedure

One drop of semen was placed on a warmed (35ºC) glass slide, and mixed with two drops of pre-warmed (35ºC) Eosin-Nigrosin stain (Blom, 1977) [3] and left for 30 seconds. A thin uniform smear out of the mixture was made on a clean grease-free glass slide and dried. The dried smear was fixed in tartrate phosphate buffer with 10 per cent Formaldehyde solution for 10 minutes. The fixed smear was washed under slow running tap water for 10 minutes and then rinsed with distilled water. The smear was then stained with Giemsa working solution for 60 minutes, rinsed with distilled water and dried in air. The stained slide was examined under oil immersion lens of a compound microscope at 1000X magnification. Two hundred spermatozoa were examined and four categories of spermatozoa *viz.*, Live intact acrosome, Live reacted acrosome, Dead intact acrosome and Dead reacted acrosome were identified and percentage of spermatozoa with Live-intact acrosome was determined

Tartrate phosphate buffer (pH 7.0)

Potassium sodium tartrate (77mM)	2.179g
Sodium dihydrogen orthophosphate (50mM)	0.700g
Potassium dihydrogen orthophosphate (25mM)	0.340g
Distilled water ad	100ml

Preparation of stock Giemsa stain (Watson, 1975) [17]

Giemsa stain powder (3.8g) was grounded with 375ml of absolute methanol (A.R. grade) in a pestle and mortar in seven fractions. Before addition of each fraction of methanol the mixed portion was pipetted out and transferred into a bottle. The amount transferred into the bottle was recorded. After entire quantity of stain was transferred into the bottle, it was found that some quantity of methanol evaporated out during the process of mixing. The amount of methanol that was evaporated was then added into the bottle. A total of 125 ml of glycerol (A.R. grade) was added and the stain mixture was then allowed to be ripen for about one week by keeping it in an incubator at 37ºC. During this period of ripening it was shaken daily for a few minutes. The stock Giemsa stain ready for use was then stored in a refrigerator at 5ºC.

Sorensen's phosphate buffer (Lillie, 1965) [11]

Sorensen's phosphate buffer was prepared by mixing 17ml of 0.1M potassium phosphate monobasic (KH_2PO_4) solution and 33ml of 0.1M sodium phosphate anhydrous (Na_2HPO_4) solution. The pH of the buffer was adjusted at 7.0 using a pH meter.

0.1 M Potassium Phosphate Solution

Potassium phosphate monobasic (KH_2PO_4)	13.60g
Triple glass distilled water ad	100 ml

0.1 M Sodium Phosphate Solution

Sodium phosphate anhydrous (Na_2HPO_4)	14.19g
Triple glass distilled water ad	100 ml

Giemsa Working Solution

Giemsa stock solution	3 ml
Sorensen's phosphate buffer (pH 7.0)	2 ml
Triple glass distilled water	35 ml

Sperm abnormalities

Sperm head abnormalities

The head abnormalities of spermatozoa were studied using Carbol fuchsin eosin stain as per the method of Lagerlof (1934) [10]. Immediately after collection, one drop of semen was diluted with 4 drops of sodium citrate buffer and a thin smear was prepared on clean, grease-free glass slide. The semen smear was air dried and then stained with Carbol fuchsin eosin stain. Two hundred spermatozoa were examined in each smear at a magnification of 1000X of a compound microscope to determine various abnormalities of sperm head.

Composition of stain

(a) Stock fuchsin solution:

Basic fuchsin		5g
Ethanol (96%)	:	50ml

The solution was filtered

(b) Phenol solution

Liquid phenol (90%)	:	20ml
Distilled water		340ml

(c) Eosin solution

Eosin-Y	:	1.5g
Ethanol (96%)		150ml

Preparation of stain

Stock fuchsin solution 30ml was added to 300 ml of phenol solution. From this mixture, 300 ml was taken and mixed with 150 ml of Eosin solution and stored at room temperature or at 37°C for 14 days. The stain solution was then stored after filtration.

Sperm mid-piece and tail abnormalities

The mid-piece and tail abnormalities of spermatozoa and proximal and distal cytoplasmic droplets were studied in wet preparation by using Buffered Formal Saline Solution under Phase Contrast Microscope. Immediately after collection, one fine drop of semen (37°C) was mixed with 1.5ml buffered formal saline (37°C) in a 2ml plastic vial. Formalin kills the spermatozoa immediately and preserves them in their original morphological states. One drop of semen mixture was taken on a clean grease-free glass slide with the help of a Pasteur pipette, covered with a cover slip and allowed to settle down and the drop was focused under low power and then under high power objective of the microscope. Two hundred spermatozoa were examined to determine various mid piece and tail abnormalities and proximal and distal cytoplasmic droplets.

Composition of buffered formal saline (Hencock, 1957) [9]

Ingredient	Quantity
Disodium hydrogen phosphate	6.19 g
Potassium dihydrogen phosphate	2.54 g
Formalin 40%	125 ml
Sodium chloride	5.41 g

Results and Discussions

The mean values of different semen characteristics *viz.*, ejaculate volume, mass activity, initial sperm motility, live sperm, sperm concentration, sperm number per ejaculate, cold shock resistance index, live intact acrosome and sperm abnormalities in Beetal and Sirohi bucks are presented in Table 1 and 2.

Table 1: Semen characteristics (MEAN* □SE) in different Beetal and Sirohi bucks

Semen characteristics	Beetal				Sirohi			
	Buck I	Buck II	Buck III	Overall	Buck I	Buck II	Buck III	Overall
Ejaculate volume (ml)	1.19 ±0.10	1.05 ±0.08	1.00 ±0.04	1.08 ±0.05	1.34 ±0.07	1.09 ±0.06	1.11 ±0.07	1.18 ±0.04
Mass activity (0 to 4+ Scale)	3.71+	3.71+	3.71+	3.71+	3.95+	3.86+	3.95+	3.92+
Initial sperm motility (%)	82.14 ±1.40	83.81 ±1.03	82.86 ±1.36	82.94 ±0.73	85.24 ±1.27	84.29 ±0.99	84.29 ±0.93	84.60 ±0.61
Live sperm (%)	86.04 ±0.73	86.22 ±0.89	84.44 ±1.02	85.57 ±0.52	87.67 ±0.59	84.68 ±0.99	85.73 ±0.77	86.03 ±0.48
Sperm concentration (million/ml)	3162.95 ±128.74	3429.00 ±139.96	3478.24 ±158.49	3356.73 ±83.06	3059.81 ±85.71	3269.52 ±106.59	3201.48 ±84.76	3176.94 ±53.92
Sperm number per ejaculate (million)	3673.43 ±215.46	3612.29 ±298.63	3438.43 ±155.07	3574.71 ±131.63	4062.86 ±267.97	3508.00 ±192.28	3436.14 ±167.57	3669.00 ±126.42
Cold shock resistance index (%)	6.75 ±0.27	6.53 ±0.36	7.04 ±0.39	6.72 ±0.22	6.07 ±0.21	7.00 ±0.41	7.09 ±0.45	6.77 ±0.20
Live intact acrosome (%)	86.89 ±0.68	84.17 ±0.58	84.46 ±0.64	85.18 ±0.39	83.07 ±0.27	84.59 ±0.82	84.48 ±0.85	84.05 ±0.41

*21 observations

Table 2: Per cent sperm abnormalities (MEAN* □SE) in different Beetal and Sirohi bucks

Sperm abnormalities	Beetal				Sirohi			
	Buck I	Buck II	Buck III	Overall	Buck I	Buck II	Buck III	Overall
Head abnormalities	1.39 ±0.14	1.53 ±0.15	2.02 ±0.33	1.65 ±0.13	1.83 ±0.27	1.40 ±0.16	1.27 ±0.14	1.50±0.12
Mid piece abnormalities	0.33 ±0.03	0.14 ±0.01	0.28 ±0.02	0.25 ±0.02	0.38±0.07	0.19 ±0.01	0.32 ±0.03	0.30 ±0.03
Tail abnormalities	2.42±0.22	2.28±0.30	3.48±0.36	2.73±0.18	2.53±0.24	2.47±0.16	2.59±0.26	2.53±0.13
Sperm with proximal cytoplasmic droplet	0.46±0.04	0.39±0.05	0.43±0.05	0.43±0.03	0.52±0.10	0.38±0.05	0.54±0.05	0.48±0.04
Sperm with distal cytoplasmic droplet	0.66±0.08	0.58±0.04	1.33±0.32	0.86±0.12	0.83±0.09	0.51±0.04	0.49±0.05	0.61±0.04

*21 observations

Ejaculate volume

The ejaculate volume recorded in Beetal bucks was comparable with the findings of Akela (2006) [1] in Beetal bucks. The ejaculate volume obtained in Sirohi bucks was similar with the findings of Singh *et al*. (1985) [13] in Barbari bucks.

Mass activity

The mean mass activity was reported (scale 0-4) to be 3.82+ (Akela, 2006) in Beetal bucks and 4.02 ± 0.0143 (Sinha *et al*., 1981) [14] in Jamunapari bucks. The variations recorded in mass activity of semen could be due to difference in breed, season and scale and method adopted for evaluation.

Initial sperm motility

The initial sperm motility noted in the present study was close to the value recorded by Dutta Borah (2005) [7] in Beetal and Saraswat *et al*. (2012) [12] at pre-freezing in Sirohi bucks.

Live sperm

The values observed in the present study were comparable with the findings of Akela (2006) [1] in Beetal bucks (89.43 ± 1.17%) and Saraswat *et al*. (2012) [12] at pre-freezing in Sirohi bucks (89.10 ± 0.53%).

Sperm concentration

The overall mean concentration of spermatozoa (3356.73 ± 83.06 million/ml in Beetal and 3176.94 ± 53.92 million/ml in Sirohi bucks) recorded in the present study was comparable with the findings reported by Dutta Borah (2005) [7] in Beetal bucks (3342.65 ± 52.37 million/ml).

Sperm number per ejaculate

The overall mean sperm number per ejaculate in Beetal and Sirohi bucks was found to be 3574.71 ± 131.63 and 3669.00 ± 126.42 million respectively. These values were comparable with that reported by Dutta Borah (2005) [7] in Beetal bucks.

Cold shock resistance index

The values found in the present study were lower than that reported in Beetal and Assam local bucks (Borgohain, 1981) [4] but higher than that reported by Akela (2006) [1] in Beetal and Assam local bucks. This might be due to inherent differences in the resistance of spermatozoa to cold in different individuals in different breeds and/or method designed for rendering cold shock to spermatozoa.

Live intact acrosome

The values of live intact acrosome obtained in the study were higher than the values of live intact acrosome reported by Dewry (2014) [6] and Hazarika (2014) [8] at pre-freezing in Beetal buck semen extended with tris extender. The present value of live intact acrosome was lower than the values of intact acrosome reported in fresh semen of local bucks of Andhra Pradesh (Deka, 1984) [5]. Perusal of available literature revealed no information on incidence of live intact acrosome in fresh semen, hence the obtained values in Beetal and Sirohi bucks could not be suitably compared.

Sperm abnormalities

Traldi (1983) [16] observed that the most common sperm abnormalities in Moxoto goats at the onset of sexual maturity were those of sperm head and tail. The incidences of the three sperm parameters found in the present study were comparable with that reported by Dutta Borah (2005) [7] in Beetal breed. The incidences of sperm mid piece and tail abnormalities recorded in the present study were also comparable with the values obtained by Borgohain (1981) [4] and Akela (2006) [1] in Beetal and Assam local. The variation in the incidences of spermatozoan abnormalities might be due to differences in breed, age, season and plane of nutrition and/or methods employed for determination of sperm abnormalities.

Conclusions

Semen characteristics of Beetal and Sirohi bucks were within normal range and did not differ significantly.

Disclosure statement

No potential conflict of interest was reported by authors.

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