Characteristics and freezability of Assam Hill goat semen

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ABSTRACT

Assam Hill goat (AHG) is an important goat germplasm found in Assam and its adjoining areas of India. The study was designed with an objective to study the semen characteristics and freezability of AHG buck semen using Tris-Egg yolk-Citrate-Fructose diluent. The mean values of fresh semen characteristics in AHG bucks viz., ejaculate volume (ml), initial sperm motility (%), sperm concentration (x10^6/ml), live sperm (%), sperm abnormality (%), HOST-reacted sperm (%) and intact acrosome (%) recorded were 0.39 ± 0.01, 77.97 ± 0.73, 3201.00 ± 143.78, 83.02 ± 0.65, 7.66 ± 0.73, 66.95 ± 0.74 and 93.34 ± 0.51, respectively. Mean values for post-thaw semen characteristics i.e., sperm motility (%), live sperm (%), HOST-reacted sperm (%) and intact acrosome (%) were 55.39 ± 0.97, 71.01 ± 0.78, 54.77 ± 0.55 and 82.16 ± 0.43, respectively. It can be concluded that AHG bucks donate acceptable quality of semen which can be frozen successfully in Tris-Egg yolk-Citrate-Fructose diluents for using in Artificial Insemination.

Key words: Assam Hill goat, Freezability, Post-thaw, Semen characteristics.

INTRODUCTION

In the developing countries, goats contribute immensely to the livelihood security of rural population. The importance of this valuable genetic resource is underexplored and the extent of their contribution to the livelihood of the rural population is not properly addressed. Research and development investments to improve the relatively low level of goat’s productivity do not match their potential importance, resulting in non-exploration of many goat breeds genetically especially in the developing countries. Assam Hill goat (AHG) is an important goat germplasm found in Assam and its adjoining areas. It is essentially meat type animal with high prolificacy but a poor milker. Most common colour of this goat is white, however, brown, black and mixed colours are not uncommon.

The lack of good quality breeding stock is a major constraint in commercialization of goat production. Selection of high fertile bucks with superior inheritance and their widespread use could improve the overall production potential of goats. Artificial insemination using semen of high fertile buck can be a good alternative for rapid genetic improvement of a flock. The quality of semen used for insemination directly influences the pregnancy rate. Therefore, present study was designed to study the semen characteristics and freezability of AHG bucks.

MATERIALS AND METHODS

Experimental animals and semen collection: The study was conducted with eight Assam Hill goat bucks aged 2 to 2.5 years maintained in All India Coordinated Research Project on Goat Improvement (Assam Hill Goat Unit) at Goat Research Station, Assam Agricultural University, Burnihat, India. Bucks were maintained under uniform dietary and managerial regime under intensive system of management in well-ventilated sheds. They were thoroughly examined for sexual and general health before selection. Semen was collected from each buck twice a week during the period from September 2013 to February, 2014 with the help of a standard artificial vagina using a restrained doe as a mount. Two false mounts were allowed before the collection of semen. A total of 64 ejaculates comprising of 8 from each buck were used for the study.

Semen evaluation: The ejaculate volume was recorded directly from the glass graduated semen collection tube and expressed in millilitre. For estimating the initial sperm motility, a fine drop of semen was diluted with 4-5 drops of prewarmed (37°C) Tris buffer that consisted of 2.422 g Tris, 1.36 g citric acid, 1 g fructose and 100 ml triple glass distilled water. A drop of diluted semen was placed on a prewarmed glass slide (37°C) with a cover slip on it and examined under a phase contrast microscope at a magnification of 400X. Initial sperm motility was recorded

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as percentage of progressively motile sperm. Sperm concentration was determined with the help of a Neubauer counting chamber after a dilution of 1:200 with a diluting fluid and expressed in million per millilitre of semen. The percentage of live spermatozoa was determined using Eosin-Nigrosin staining technique described by Blom (1977). Sperm tail and mid piece abnormality were studied by differential interference phase contrast microscopy of wet-mount semen fixed in isotonic formal saline under high power objectives. The sperm head morphology was studied by staining the spermatozoa with Williams stain and examined under microscope of 1000X under oil immersion objectives. A total of 200 sperm were examined and various sperm defects were recorded and calculated in percentage. The functional integrity of the sperm membrane was studied as per the method described by Jeyendran et al. (1984) by using Hypo-Osmotic solution (150 mOsm). The morphological changes of acrosome were studied in stained semen smear using Giemsa staining technique of Watson (1975). Two hundred spermatozoa were examined in each smear at a magnification of 1000 X of a compound microscope fitted with artificial illumination and the percentage of intact acrosome was determined.

**Semen processing and freezing:** The ejaculates were diluted (1:5) with Tris buffer having pH 6.8 consisting of 2.42 g Tris, 1.36 g citric acid, and 1.0 g fructose and up to 100 ml of triple glass distilled water. The seminal plasma was separated by centrifugation (washing) for seven minutes at 700 × g at room temperature. The Tris extender was prepared in two fractions, fraction A (50 ml) and fraction B (50 ml). Fraction A composed of Tris buffer 33.6 ml, distilled water 6.4 ml and egg yolk 10 ml. Fraction B composed of of Tris buffer 33.6 ml, glycerol 6.4 ml and egg yolk 10 ml. The centrifugate was extended (1:5) with the fraction A of the Tris extender. For extension, the initial volume of semen was cooled gradually to 5ºC @ 1ºC per 3 minutes in a cold handling cabinet. The Tris extender maintained at 5ºC was then added (amount equal to the fraction A) in two steps at an interval of 15 minutes to the initially extended semen. The extended semen was then maintained at 5ºC in the cold handling cabinet for 4 hours of equilibration. The filling of straws was done by suction at 4º-5ºC in a cold handling cabinet. Thirty minutes before the end of 4 hours equilibration at 4º-5ºC, the straws were taken out from the water and wiped dry using pre-cooled (4º-5ºC) towel. After drying, the straws were arranged in a freezing rack horizontally. On completion of equilibration period the rack containing straws was transferred into a freezing container and exposed to liquid nitrogen vapour for 10 minutes and finally the straws were collected in a goblet full of liquid nitrogen and transferred to liquid nitrogen container for storage.

After 7 days of storage in liquid nitrogen, the frozen semen was thawed in warm water (37ºC) for 30 seconds for evaluation. Each semen sample was evaluated for sperm motility, live sperm, HOST–reacted sperm and intact acrosome after freezing as per the methods discussed previously.

Data obtained were analyzed as per Statistical Software Package (SAS, Cary, NC, USA, 2010).

**RESULTS AND DISCUSSION**

The fresh semen characteristics viz., ejaculate volume, initial sperm motility, sperm concentration, live sperm, HOST-reacted sperm and intact acrosome and the mean values of post-thaw sperm motility, live sperm, HOST-reacted sperm and intact acrosome in Assam Hill goat bucks are presented in Table 1.

It was observed that the values of fresh semen characteristics in respect of ejaculate volume, initial sperm motility, sperm concentration live sperm and intact acrosome recorded in Assam Hill goat bucks in the present study were in agreement with that of Borgohain (1981) and Akela (2006) except that the present value for initial sperm motility was lower than that reported by Borgohain (1981). The initial sperm motility reported by Borgohain (1981) for Assam local bucks was 87.20 per cent. The percentage of HOST–reacted sperm in Assam Hill goat bucks in the present study was lower than that reported by Sarma et al. (2011) in Beetal bucks (83.58 ± 1.01%). The present values in respect of initial sperm motility, live spermatozoa and sperm abnormality were very close to the that reported by Mahto et al. (2012) which might be due to the fact that these two breeds, Black Bengal and Assam Hill goat are most similar phenotypically except for the differences in geographical location.

In the present study analysis of variance showed significant variations between the bucks for different characteristics in fresh semen characteristics except for sperm concentration. In accordance with the present study Sarma et al. (2011) reported significant variation in ejaculate volume between Beetal bucks. They further observed that sperm concentration differ significantly between bucks, but initial sperm motility, live sperm, HOST–reacted sperm and incidence of intact acrosome did not differed significantly between bucks. The findings in respect of ejaculate volume and sperm concentration in the present study were in agreement with that of Pradhan et al. (2013) in Black Bengal bucks but they did not find significant buck difference in sperm motility in freshly collected semen. This variation
might be due to difference in breed, age of the bucks and the season of year of studies.

The present values for post-thaw sperm motility, live sperm and intact acrosome were in accordance with that of Saikia (2006) for Assam local goats. The mean percentage of post-thaw sperm motility, live sperm and intact acrosome were also comparable with that reported by Sinha (1989) in Beetal bucks. However, Lalramdintluanga (2012) reported much lower values for post-thaw sperm motility (47.75 ± 1.08%), live sperm (52.62 ± 1.12%), intact acrosome (78.47 ± 0.52%) and HOST–reacted sperm (46.10 ± 0.90%) in Beetal bucks maintained in the environmental conditions of Assam. Gangwar et al. (2014) reported similar post-thaw semen quality in Sirohi bucks. In contrary to the present findings, Khalili et al. (2009) reported much lower values of post-thaw sperm motility (35.45 ± 0.69%), viability (43.07 ± 0.83%), acrosomal abnormality (16.11 ± 0.49%) and HOST–reacted sperm (35.65 ± 0.74%) in Markzhou goat.

Ranjani et al. (2015) also reported much lower values of post-thaw sperm motility (34.16 ± 1.53%), live sperm (38.33 ± 2.09%), intact acrosome (35.66 ± 2.53) and HOST–positive sperm (37.50 ± 1.70) than that in the present study using Tris-Egg yolk-Citrate-Fructose (TCF) diluent with 20 per cent egg yolk and 6 per cent glycerol in Jammunapari goat semen. This might be due to differences in breed, climatic condition, and processing and freezing technique.

It can be inferred from the present study that AHG bucks donates acceptable quality of fresh semen, which can be frozen satisfactorily for use in Artificial Insemination programmes. AHG buck semen has good freezability using Tris-Egg yolk-Citrate-Fructose diluents with 20 per cent egg yolk and 6 per cent glycerol added to semen in two fractions and following a 4 hours equilibration period.

REFERENCES


