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# Semen Preservation and Artificial Insemination in Bengal Goat at Field Level

***M. Karunakaran*, Ajoy Mandal, Mohan Mondal, C. Bhakat, S. Garai***
ICAR- National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal, India

***Abstract***

*Semen ejaculates (n=72) were collected from five Black Bengal Bucks by artificial vagina method. Semen samples were evaluated for volume, sperm cell concentration, motility and morphology. Semen samples were preserved using Tris –Egg yolk-Citrate buffer in liquid state at refrigeration temperature. Rural youths (n=21) were trained on artificial insemination (AI) in goats and preserved semen was used for AI in rural villages of West Bengal and kidding rate was recorded. Black Bengal buck semen was characterized by 0.75 ±0.25 ml volume, 1522 ± 35.30 X $10^6$/ml sperm cell concentration, 85.40 ± 8.20% progressive forward motility and 92.0 ± 1.90% normal morphology. Significant (P<0.01) reduction in sperm motility was observed during storage at refrigeration temperature and sperm motility reduced to less than 40% after 96 h of storage. Out of 146 does inseminated with preserved semen, 69 does became pregnant and delivered kids with 47.26% kidding rate. It was concluded that Bengal buck semen can be preserved using Tris –Egg yolk- Citrate buffer at liquid state in refrigeration temperature and can be used for artificial insemination in remote villages over a four days of storage with acceptable kidding rate.*

***Keywords:*** *Goat, semen, preservation, insemination*

****Author for Correspondence*** E-mail: drmkarunakaran@gmail.com

## INTRODUCTION

Goats have been considered as poor man's cow because of its immense contribution in rural economy. Goat has been reared since the time immemorial. Goat keeping provides source of regular income to the poor, landless and marginal farmers. It is reared for the purpose of meat, milk, leather and in some places for fibre. Goat meat is a great source of consumable meat which is very tasty and nutritious. Skin of goat plays a vital role in leather industry. Goat milk is easily digestible and has medicinal values. Goats are very part of rural family as there is no religious taboo to rear and to eat goat meat, require less space, clean animal, easy to maintain, needs very low investment and has high market demand round the year. Bengal goat is precious germplasm of West Bengal and the breed is distributed in West Bengal, Bihar, Jharkhand, Orissa, parts of NEH states and Bangladesh. The breed of goat is characterized by early sexual maturity, high fertility and fecundity, good mothering ability, high adaptability. Thrive well with available feed and fodder. Most of the goat rearers are small, marginal farmers and landless labourers (86.10%) and 91.3% of the goats were looked after by women [1]. Most of house hold has small units of 2 to 4 goats. Black Bengal breed of goat is well known for its excellent meat quality all over Eastern India and there is very heavy demand for the meat from this breed. Male goats (bucks) are castrated at an earlier age to get better growth rate and to avoid the development goaty odour in the meat. The males are slaughtered for meat at less than 12 months of age to get tender meat. This resulted in less number of males available for breeding in the villages. The buck: doe ratio is 1.13: 88.7 against the recommended 1:20 [1]. The farmers are not able to breed the females in right time of estrus which ultimately leads to poor conception rate or less number of kids produced. In addition due to continuous use of same breeding males over a longer period in the same locality may leads to inbreeding depression [2]. It is therefore, important to make the availability of quality preserved goat semen from elite bucks at village level for breeding the goats. Selection of good quality bucks and their wide spread use could improve the overall potential

  

production of goats. The selected best quality bucks could only be exploited rapidly through using artificial insemination (AI). AI has gained widespread acceptance in dairy cattle sector all over the world. However, interest in AI in goat is gaining importance due to non-availability of quality breeding male from the desired breed. Conception rate is one of the key factors deciding the success of AI. It is important to evaluate the semen characters and fertilizing capacity of preserved semen as there is limited information on the various semen attributes of Bengal bucks. The present study was carried out to evaluate the neat and diluted semen quality and determine fertility following AI in Bengal goats.

## MATERIALS AND METHOD

### Experimental Bucks and their Management

The study was carried out at ICAR- National Dairy Research Institute, Eastern Regional Station, Kalyani, West Bengal. The work was carried out as per the approval of Institute Research Council. Five Black Bengal bucks (*Capra hircus*) aged 18–30 months, weighing 18–25 kg reared separately were used in the study. Bucks were allowed for natural grazing for four to six hours daily. In addition, each buck was supplied with green grass (2–3 kg fresh weight) in the evening with *ad libitum* drinking water. Bucks were vaccinated against *Peste des petits ruminant*, goat pox, foot and mouth disease, and enterotoxaemia. The animals were treated three times a year with albendazole @ 10 mg/kg body weight orally. Ivermectin @ 0.2 mg/kg bodyweight was injected subcutaneously for controlling ectoparasites when requires.

### Semen Collection and Evaluation

The bucks were trained to ejaculate in an artificial vagina (AV) at homosexual mount [3, 4]. Briefly, AV consists of an outer casing (15×5.5 cm) with good insulation properties containing an inner liner of thin rubber. The liner was extended at least 2–3 cm beyond the end of the outer casing and folded back and secured with rubber bands to form a watertight jacket. The jacket was two-thirds filled with water at 50°C (to achieve 45°C inside AV) through a tap on the side of the AV and inflated by blowing air through, which was then closed. The penis end of the AV was lubricated with non-spermicidal gel. At the outer end of the AV, a plastic cone with a calibrated plastic tube was fixed. Before collection, the prepuce of the buck was wiped clean to reduce contamination. An estrus doe was secured in a collection bail and her rear end was cleaned. The operator crouched or knelt at the right of the doe and held the AV in the right hand along its flank and with the open end facing towards the male and downwards at an angle of 45°C. The donor bucks given two false mount before collection of ejaculates. When the male mounted, the erect penis was directed into the open end of the AV to vigorous upward and forward thrust. The buck was allowed to withdraw his penis immediately after ejaculation. The graduated tube was separated from the cone and its mouth closed with a plastic cap and labelled. The tube was immediately placed in a beaker containing lukewarm water (37°C). Total of seventy two ejaculates were collected from the bucks. The routine evaluation of fresh semen was done immediately. The volume of the ejaculate was measured using a micropipette.

### Concentrations

Sperm cell concentration in the buck semen ejaculate was estimated by haemocytometer method [3]. Semen sample was diluted @ 1:200 using 2% eosin solution and a drop was loaded in the chamber of haemocytometer. Sperm cells present within five large squares (four squares in the corner, one in centre) were counted carefully. The concentration of the sperm cells was calculated by multiplying total number of cells counted with dilution factor and expressed in millions per milliliter.

### Motility

A drop (6 µl) of diluted semen was placed on a clean glass slide and covered with a cover slip. The proportion of spermatozoa moving progressive forward was estimated at 400X magnifications [3].

### Sperm Cell Morphology

Morphological evaluations of sperm cells were carried out by using rose bengal stain. Two drops of 3 per cent rose bengal stain and 1000 µl of Tris buffer were added to semen samples. The contents were mixed and kept at 37°C for 10 min. Then the sperm cells were washed twice in Tris buffer by centrifugation (560 g for 5 min) and sperm cells suspended in

 

Tris buffer were placed on a glass slide and covered with cover slip. A minimum of 200 cells were analyzed at 100X using a microscope [5].

**Preparation of Extender and Reagents**

The semen diluent was prepared by dissolving Tris (2.42 g), citric acid (1.34 g), and glucose (1.0 g) in 80 ml distilled water. The stock solutions were sterilized by autoclave and preserved at + 4 to + 7°C for a maximum of one week. On the day of semen collection, fresh egg yolk was added (20%, v/v) with penicillin (1000 iu/ml) and streptomycin sulfate (1 mg/ml). The collected semen samples were extended with Tris –Egg yolk-Citrate buffer to provide a concentration of $200X10^6$ spermatozoa per ml and preserved at refrigeration temperature. Sperm motility and membrane integrity were evaluated initially 2 h after extension of semen (day 0) and then at every 24 h interval over a period of seven days. On each day an aliquot of sample was taken and kept in a water bath at 37°C for 15 min before evaluation for sperm motility.

**Artificial Insemination**

Training of rural youths on artificial insemination in goats was carried out in Association with Sanjevani Khamar, Hooghly, West Bengal. Preserved semen was distributed in thermos flasks with ice through bus/train over 150 km distances to different districts of West Bengal state namely, Hooghly, Bardhman, Purulia, West Midnapur, Malda, Mursidabad, South Dinajpur and Nort 24 Parganas. Preserved semen was used for AI up to five days of storage. 0.5 ml of preserved semen was used for AI of a doe after 12 h of onset of estrus. 146 inseminations were included in the present study to assess the fertility. Fertility rate was calculated as the percentage of kidding by number of inseminated does.

**Statistical Analysis**

Statistical analysis to compare sperm cell motility during different days of storage was carried out by paired t test [6].

## RESULTS AND DISCUSSION

**Volume of Ejaculate**

Average volume of the semen ejaculate was 0.75 ±0.25 ml. Apu et al. [7] reported 0.58± 0.03 while Khandoker et al. [8] reported 0.43±0.03 to 0.45 ± 0.22 ml. This difference could be due to individual variation between animals and season, as seasonal variation affects libido and sexual activity in goats [9].

**Sperm Concentration**

Average sperm cell concentration of Black Bengal buck was $1522 \pm 35.30X10^6$/ml. The result in the present study was lower than the findings of Apu et al. [7] who obtained 2678.33 ± 30.59. This difference in sperm concentration might be due to more volume of the semen ejaculate harvested than the other reports.

**Sperm Motility**

Progressive forward motility of the fresh ejaculate was 85.40 ± 8.20%. This observation was higher than those reported by Apu et al. [7], Afroz [10] and Das et al. [11]. Sperm motility is influenced by the variation in age, frequency of collection, climatic conditions and semen handling methods [7].

**Sperm Morphology**

Per cent of sperm cells with normal morphology in the semen ejaculate was 92.0 ± 1.90% which was in agreement with Apu et al. [7], Afroz [10] who reported 91.39 ± 0.24% and 91.16 ± 0.36%, respectively in Black Bengal buck semen.

**Sperm Motility during Storage**

Progressive forward motility in preserved in Tris –Egg yolk- Citrate buffer at refrigeration temperature is depicted in the Table 1. There significant ($P<0.01$) reduction in the sperm motility as the duration of storage increases. The causes of declining motility might be related with reduced metabolism, exhaustion of reserved energy in spermatozoa, effect of metabolic end products such as spermicidal endotoxins etc. [12].

***Table 1:*** *Motility in Preserved Buck Semen during Storage at Refrigeration Temperature.*

| Storage period | 24 h | 48 h | 72 h | 96 h | 120 h | 144 h | 168 h |
|---|---|---|---|---|---|---|---|
| Motility (%) | $61.5^a$ | $52.1^b$ | $43.7^c$ | $36.2^d$ | $28.8^d$ | $19.7^e$ | $13.2^e$ |

*Means with different superscripts in a row differ significantly ($P<0.01$)*

**Fertility**

Out of 146 does inseminated 69 does became pregnant and delivered kids i.e., 47.26% kidding rate was observed in the study. Apu et al. [7] reported about 58.9% kidding rate. The lower kidding rate in the present study may be because the preserved semen was used for AI over four days storage and AI was carried out at farmers field level distributed over 150 km away from the semen collection point and was carried out trained workers. But in case of the other studies preserved semen was used for AI on the same day at farm level.

## CONCLUSION

Bengal buck semen can be preserved using Tris –Egg yolk- Citrate buffer at liquid state in refrigeration temperature and can be used for artificial insemination in remote villages over a four days of storage with acceptable kidding rate. This practice will help to meet breeding requirement of goats maintained by small farmers as well as to protect the purity of Bengal goat breed from indiscriminate breeding with nondescript bucks.

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