



Original Research Article

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Macro Seminogramm Neat Semen of a Kangayam Bull – An Ingenious Study

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The objective of the present study is to analyze the macroscopic seminal attributes in a red color Kangayam bull (No. 14 Nagulan) aged between 4 1/2 and 5 years which is maintained at Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal. The semen was collected twice weekly from a Kangayam bull for the period of three months. The collected ejaculates were immediately tested for their macroscopic parameters such as gross motility, volume, colour, density, presence of foreign material and reported.

Introduction

The Kangayam breed of cows is a dual purpose breed with maximum milk yield of 1,455 litres and it is distributed in Erode, Dindigul, Karur, Coimbatore, Salem and Namakkal districts of Tamil Nadu. It is best known for its superior draught qualities, adaptation to poor nutrition and longevity, a pair of bullocks has the capacity to haul a total load of 3787 ± 51.4 kg of sugarcane load over a distance of 10 to 20 km without taking

rest (Kandasamy, 2001). As per the estimate of 1996, the size of Kangayam population in the breeding tract was 0.479 million and it got reduced to 80,620 in 2013. Artificial insemination (AI) has become an inevitable tool in the breeding management of most of the domestic animals.

This technology helps to propagate the male germplasm in a faster way. It has been successfully implemented in breeding of bovines but is still lacking in indigenous cattle

breeds. Semen cryopreservation prolongs the life of spermatozoa by decreasing metabolism and toxin production (Curry, 2000 and Bailey *et al.*, 2003).

Kangayam cattle are in the verge of declining populations. However, recent awareness on rearing native cattle has prompted the importance of this breed. There is still long gap between the breeds and scientists to come out with good quality Frozen Semen Straws (FSS) from pedigreed Kangayam bulls.

Limited availability of Kangayam bulls in the present scenario demands the adoption of techniques like semen collection, semen preservation and artificial insemination for the conservation and perpetuation of this unique germplasm.

Seminal attributes in neat semen were analyzed in Haryana bulls (Ajendar Singh Pal *et al.*, 2020); Hallikar bulls (Prem Kumar *et al.*, 2020); Sahiwal bulls (Ahmad *et al.*, 2003); Tharparkar bulls (Bhosrekar *et al.*, 1972); Punganur bulls (Bramhaiah *et al.*, 2013); Gir bulls (Chikhaliya *et al.*, 2018); Kankrej bulls (Patel *et al.*, 2013); Red Sindhi bulls (Ritesh Tiwari *et al.*, 2012); Ongole bulls (Rao *et al.*, 2000) and Boer crossbred bucks (Manivannan *et al.*, 2017). This study is the first attempt in analyzing the macroscopic seminal attributes in a Kangayam bull.

Materials and Methods

Source of experimental animal

Kangayam Bull No. NK14 named Nagulan, aged 5 years which is maintained at Frozen Semen Bank, Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal was utilized for this study.

Preparation and sterilization of semen collection equipment

Glass wares

Glasswares such as conical flask, beaker and graduated semen collection vials were soaked in sodium dodecylbenzene sulfonate (Labolene[®], Fisher Scientific, Mumbai) detergent solution for 20 minutes initially. Then they were washed in tap water followed by rinsing with single distilled water. The glassware were allowed to dry in incubator and covered with aluminium foil. Finally, they were sterilized in hot air oven at 160°C for 30 minutes and kept ready for use.

Rubber wares

The rubber wares such as artificial vagina (AV), inner liner and director cone were soaked in sodium dodecyl benzene sulfonate solution for 20 minutes initially. Then they were washed in tap water followed by rinsing with single distilled water and sterilized in autoclave at 5 psi for 20 minutes and kept ready for use.

Preparation of AV

The AV used in this experiment was Russian 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.

Preparation of bull

In the previous day of semen collection, the long preputial hairs around the preputial

orifice of bull no.14 (Nagulan) were clipped to maintain the length of 2 cm. The semen collection was carried out at 6.00 AM before feeding. On the day of semen collection, the prepuce was washed with running tap water followed by normal saline. Then the preputial orifice was wiped with sterile napkin.

Semen collection

The semen was collected from the bull no. NK 14. Other Kangayam bulls maintained in the semen station were used as dummy during semen collection. The semen was collected twice in a week (Tuesday and Friday) and each time two ejaculates were collected. The bull NK14 was allowed to mount on the dummy. Two false mounts were given before semen collection during every time. On the third mount, the penis was directed in to AV and the semen was collected in the collection Tube. The collected ejaculate was immediately transferred to semen processing laboratory and kept at 34°C in the water bath. Totally eight ejaculates were collected for the evaluation and production of frozen semen straws.

Macroscopic evaluation of fresh semen

Gross motility: 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.

Volume: The volume of the ejaculate was measured in milliliter directly from the graduations of the collection tube.

Color: 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.

Density: 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 D to DDDD. Samples which had DDDD and DDD density were used for processing.

Sperm concentration: Concentration of spermatozoa in the semen sample was measured by using Accucell photometer (IMV Technologies, France) (Plate. 4) at 530 nm wave length after diluting 10 µl of semen with 3.990 ml of 0.152 M sodium chloride. Semen samples which had above 500 million spermatozoa per ml and above were taken for further processing.

Presence of foreign material: Immediately after semen collection, the collection tube and semen samples were grossly examined by naked eye for the presence of foreign materials if any.

Results and Discussion

A total of 24 semen ejaculates, obtained from Kangayam bull No. 14 (Nagulan) by artificial vagina method were evaluated for macroscopic semen attributes such as the ejaculate volume (ml), gross motility (0-5 scale), sperm color, semen density (D to DDDD), sperm concentration (millions/ml) and presence of foreign materials. The mean values of these parameters of neat semen have been depicted in Table 1.

Macroscopic characteristics of fresh semen

Gross motility: The gross motility of all the ejaculates of Kangayam bull semen ranged from 4 to 5.

Volume: The volume of Kangayam semen of present study ranged from 3.0 to 4.1 ml with a mean (\pm S.E) of 2.02 ± 0.16 ml.

Color: The color of present study Kangayam bull semen varied from thick milky yellow to creamy yellow color. Out of 24 samples examined, 20 samples (50 per cent) were in creamy yellow and 4 samples (50 per cent) were in thick milky yellow in color.

Density: The density of Kangayam bull semen varied from DDD to DDDD. Out of 24 samples 21 samples exhibited higher density of DDDD (50 per cent) and 3 samples had medium density of DDD (50 per cent).

Sperm concentration: The concentration of Kangayam bull semen was assessed by using Accucell photometer and the results were presented in Table 1. The concentration of buck semen ranged from 998 to 1210 million per ml with a mean (\pm SE) of $1113.75 \pm$ million per ml.

Presence of foreign materials: Out of 24 semen samples none of the samples had foreign materials.

Table.1 Macroscopic evaluation kangayam neat semen

| Semen characteristics | No of samples | Grading | Values | Per cent |
|-----------------------------------|---------------|----------------------------|---------------------|----------|
| <i>Macroscopical Evaluation</i> | | | | |
| Volume of semen (ml) | 24 | Maximum | 4.10 | - |
| | | Minimum | 3.00 | - |
| | | Mean | 3.65 ± 0.14 | - |
| Color | 24 | Creamy yellow | 20/24 | 50 |
| | | Thick Milky yellow | 4/24 | 50 |
| Density | 24 | DDD | 3/24 | 50 |
| | | DDDD | 21/24 | 50 |
| Concentration(millions/ml) | 24 | Maximum | 1280.00 | - |
| | | Minimum | 998.00 | - |
| | | Mean | 1113.75 ± 38.35 | - |
| Foreign materials | 24 | No of contaminated samples | Nil | - |

The volume of semen varies from breed to breed (Ahmed *et al.*, 1993) and influenced by a number of factors such as age, breed, weight and season. Laing (1988) reported that a bull of high fertility produced greater semen volume than that in an in a lower fertility bull. Variation in semen volume has been reported among different species and within a species and even the same male at different time of collection. The volume of semen was reported to vary with breed, age (Rao and Shreemanarayana, 1996), body size, size of

accessory sex gland, frequency of collection, exercise, teasing, level of nutrition, effect of season (Saxena and Tripathi, 1981 and Dhami *et al.*, 1998), and method of collection.

In the present studies the other factors responsible for variation of semen volume are same for all bulls except that level of teasing could have been a factor for variation in semen volume. The mean volume of Kangayam bull semen was 3.65 ± 0.14 ml observed for the period of three months in 24

ejaculates. The number of viable bovine spermatozoa deposited in the female reproductive tract influences the fertilizing ability of the cow up to an upper threshold level (Gerard and Humblot, 1991). Sperm concentration in ejaculate is one of the important criteria of semen characteristics to qualify fertile males for breeding purposes (Grafferet *et al.*, 1988). Accurate determination of the number of spermatozoa per milliliter of semen is extremely important.

The sperm concentration is highly variable semen characteristics and when combined with ejaculated semen volume it determines number of females that can be inseminated with optimal number of sperm cells (Hafez, 1987). Present study documents the sperm concentration of the Kangayam bull semen in 24 ejaculates is the mean of 1113.75 ± 38.35 million/ml. In a study (Kumar, 2011 and Singh, 2012) in Haryana bull semen the sperm concentration (millions/ml) ranges between 938.60 ± 41.39 to 2890.0 ± 118.51 which was differ compare to the present investigation. The results differ due to effect of age as well as of the season (Das *et al.*, 2007). A slight difference in semen concentration may be due to degree of sexual stimulation. Gross motility of sperms has been an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI and it has been positively correlated with keeping quality, freezability and fertility of that sample (Bhoiteet *et al.*, 2008).

The gross motility of neat semen of Kangayam bull varied from 4 to 5 with an overall mean of 4.88 ± 0.07 (0-5 scale). The density of the Kangayam bull semen was reported to be DDDD in 21 out of 24 ejaculates in our study. Majority of the ejaculates was light cream and cream colored as reported by Kanchan and J.S. Matharoo, 2015. The present study documented the colour of the Kangayam bull semen especially Sevalai variety was creamy yellow. There should not be any foreign materials in the neat semen which indicates the hygienic semen

collection. Thus, the present study reports the macroseminogram of the Kangayam bull semen for the first time.

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