

Original Research Article

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Characterization of Mongrel Dog Semen of Mizoram

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ABSTRACT

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The objective of this study was to characterize mongrel dog semen of Mizoram so as to have a definite specification to enable further use in different assisted reproductive technology and help in differentiating normal from abnormal samples. Colour, ejaculate volume, pH, progressive motility, sperm concentration, live sperm percent, intact acrosome and membrane integrity (hypo osmotic swelling test) were assessed. Characterization of these parameters was comparable with those reported from other mongrels and medium sized breeds. Milky white coloured semen was observed. Specific semen characteristics obtained (mean \pm S.E.) were ejaculate volume 2.95 ± 0.41 ml; pH 6.59 ± 0.76 ; Progressive motility $93.30 \pm 1.65\%$; sperm concentration $298.73 \pm 35.46 \times 10^6$ /ml; live sperm $90.84 \pm 1.8\%$; intact acrosome $97.37 \pm 0.77\%$ and HOOST reacted sperm $93.27 \pm 0.53\%$.

Introduction

It is said that dogs are man's best friend which is attributed to an increase in the trend of keeping dogs as companion animals as they have a reputation of being loyal to their owners. Moreover, dogs are mostly preferred as pet animals as compared to any other as they are faithful and easily-trained. Even in Mizoram scenario, the population of dog is gradually increasing. According to the quinquennial livestock census, 2007 which is mentioned in the statistical handbook of Mizoram, 2013, the total population of dogs in Mizoram was 35302. Many true dog lovers

of Mizoram had expressed their desire to have a reliable alternative breeding technique in case of breeding failure through natural service.

Artificial insemination have been proved to be one of the most practiced and cost – effective assisted reproductive techniques in livestock industry and keeping in view its relevance for field use, it can be a reliable breeding technique for dogs as well. However, the knowledge of morphological, physical and biochemical characteristics of

the semen is a pre-requisite to effectively utilize the technique. Even though, characteristics of dog semen in general are more or less similar, there are slight differences in specifications. Therefore, the present research is done to study the characteristics of mongrel dog semen.

Materials and Methods

Animals and housing

Three mongrel dogs between the ages of 1-2 years were used in the present study. The dogs were reared under hygienic condition and with proper supply of nutritious feed and drinking water in the concrete flooring animal house with access to outdoor for run and exercise. They were thoroughly examined for both general and reproductive health and found to be clinically fit before inclusion in the study.

Semen collection and evaluation

Semen from dogs was collected by digital manipulation following the method described by Christiansen (1984) with slight modifications without using an estrous bitch as a teaser in a glass graduated tube. Initial friction movements are made with a gloved hand and the penile sheath is gently pulled back behind the bulbus glandis. When penile erection starts, a constant pressure is maintained caudal to the bulbus with the fingers encircling a penis like a ring at this level with slight manipulation at short regular intervals. This results in erection and eventually ejaculation is achieved. The first and second fractions of the ejaculate were collected in the same tube and are used for evaluations of different parameters.

A total of 42 ejaculates from 3 mongrel dogs were collected by digital manipulation and examined for colour, volume, pH, progressive

sperm motility, live sperm, sperm concentration, intact acrosome and HOSST-reacted sperm. The colour of semen was recorded based on visual observation immediately after collection in glass graduated semen collection tube. The combined volume of first and second fractions of ejaculate was recorded in millilitre directly from the glass graduated semen collection tube. The pH of ejaculated semen was recorded by using a pH paper. A fine drop of diluted semen was placed on a clean pre-warmed glass slide (37 °C) by placing it over a Biotherm on which a cover glass was placed and examined under microscope at a magnification of 400X and sperm motility was recorded from 0 to 100 on visual appraisal based on the percentage of progressively motile spermatozoa.

The percentage of live spermatozoa was determined using Eosin-Nigrosin staining technique described by Blom (1950). The staining solution was prepared by mixing 1 part 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 pre-warmed (37 °C) staining solution and allowed to stand for 30 seconds. A thin smear was then prepared out of the mixture on a clean grease-free glass microslide with the help of another slide with smooth edge and 200 spermatozoa were examined in different fields of the smear under oil immersion objective of the microscope at a magnification of 1000X for determining the percentage of live spermatozoa. Spermatozoa which were stained or partially stained were considered as dead and those that were not stained were considered as live.

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

Compostion of diluting fluid

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

The functional integrity of the sperm membrane was studied by using a Hypo-Osmotic solution as per the method described by Jeyendran *et al.*, (1984).

Composition of hypo-osmotic solution (100 mOsm/L osmolality)

Trisodium Citrate - 0.49 g
Fructose - 0.99 g
Double glass distilled water ad - 100 ml

A total of 200 spermatozoa were examined in different fields at a magnification of 400X using a phase contrast microscope for determining the status of sperm swelling.

The incidence of intact acrosome was studied following the technique of staining with Giemsa stain adopting some modifications.

Preparation of Giemsa stain

Giemsa stain powder (3.80 g) was grounded with 375 ml of absolute methanol (AR 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 was 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 (AR Grade) was added in it and the stain mixture was then allowed to be ripen for about one

week by keeping it in an incubator at 37 °C. During the period of ripening it was shaken daily for a few minutes. The stocked Giemsa stain ready to use was then transferred to the refrigerator at 5 °C.

Sorensen's phosphate buffer

Sorensen's phosphate buffer was prepared by mixing 17ml of 0.1 M potassium phosphate monobasis (KH₂PO₄) solution and 33 ml of 0.1 M sodium phosphate anhydrous (Na₂HPO₄) solution. The p^H of buffer was adjusted at 7.0 using a pH meter.

0.1 M potassium phosphate solution

Potassium phosphate monobasic (KH₂PO₄) - 13.609 g

Triple glass distilled water ad - 1000 ml

0.1 M sodium phosphate solution

Sodium Phosphate anhydrous (Na₂HPO₄) - 14.198 g

Triple glass distilled water ad - 1000 ml

Giemsa working solution

Giemsa stock solution - 3 ml
Sorensen's phosphate buffer (p^H7.00) - 2 ml
Triple glass distilled water - 35 ml

Modified Hancock's fixative

Sodium chloride (NaCl) - 1.5 g
Sodium bicarbonate (NaHCO₃) - 0.1 g
Formalin 41 % (HCHO) - 12.5 ml
Triple glass distilled water ad - 100 ml

Modified Giemsa staining procedure

A drop of semen was placed on a clean grease free slide.

A smear (thin smear in case of undiluted semen and thick smear in case of extended semen) was prepared and allowed to dry in air.

The slide was then fixed in modified Hancock's fixative for 30 minutes.

The slide was rinsed in slow running tap water for 20 minutes.

It was then allowed to dry in air.

The slides were stained in Giemsa working solution overnight.

It was then rinsed in distilled water, air dried and mounted using DPX mountant.

The slide was then examined under a compound microscope at a magnification of 1000x using oil immersion objective.

A total of 200 spermatozoa were counted to determine the percentage of intact acrosome.

Results and Discussion

Colour of the semen

The colour of the semen was clear and watery in the first fraction and milky white and opaque in the second or sperm-rich fraction. The colour of the semen is influenced by the concentration of spermatozoa in the semen. The findings is in agreement with the observations on colour of ejaculates in normal dogs reported by Kadirvel (1998) in mongrel and Michael *et al.*, (2007) in pooled semen of Golden Retriever, Alaskan Malamute and crossbred dogs.

Ejaculate volume

The over-all mean volume of first and second fractions of the ejaculate in the study was 2.95

± 0.41 ml. This findings was in agreement with that recorded by early workers in mongrel (Daiwadnya *et al.*, 1995; Alamo *et al.*, 2005) and crossbred (Yildiz *et al.*, 2000) dogs. The present findings is slightly higher than those reported by Taha *et al.*, (1981) in adult Beagle dogs and Raul *et al.*, (2009) in toy breeds. However, it was more lower than those reported from pooled semen of Golden Retriever, Alaskan Malamute and crossbred dogs by Michael *et al.*, (2007).

pH

The over-all mean value of pH from three mongrel dogs was 6.59 ± 0.76 which are in close proximity with that recorded by Daiwadnya *et al.*, (1995) in mongrel dogs, Ponglowhapan *et al.*, (2004) in pooled semen of different breeds.

Progressive motility

The over-all mean progressive motility of mongrel dog semen obtained in the study was 95.30 ± 1.65 %. This finding was in close proximity with the observations made by Verstegen *et al.*, (2005) in Beagle dogs and Neagu *et al.*, (2010) in mongrel dogs. The present findings were higher than those observations made by Kadirvel (1998) and Nair *et al.*, (1999) in mongrel dogs.

Sperm concentration

The over-all mean concentration of spermatozoa recorded in mongrel dog semen in the study was recorded as 298.73 ± 35.46 millions per ml which were also in the range as those reported by Daiwadnya *et al.*, (1995) in mongrel dogs and Prinosilova *et al.*, (2006) in pooled semen of different breeds of dogs. The present findings were higher than observations reported by Pinto and Kozink, (2008) in fresh matured dogs' semen. However, it was lower than those reported by

Yildiz (2000) in crossbred dogs and Raul *et al.*, (2009) in toy breeds.

Live sperm

The over-all mean percentage of live spermatozoa in mongrel dog semen in the study was 87.84 ± 1.8 which was in close proximity with the reports of Kadirvel (1998) in mongrel dogs, Rijsselaere *et al.*, (2002) in Anglo - Normand and crossbred German Shepherd.

However, there is variation from reports made by Daiwadnya *et al.*, (1995) in mongrel dogs and Kim *et al.*, (2010) in Beagle dogs.

Intact acrosome

The overall mean incidence of intact acrosome in the present study was 97.37 ± 0.77 per cent in mongrel dogs which is in close agreement with that reported by Rijsselaere *et al.*, (2002) in pooled semen of Anglo – Normand and crossbred German Shepherd, Ceylan and Serin (2006) in pooled semen of different breeds.

But variation is observed from reports made by Michael *et al.*, (2007) in pooled semen of Golden Retriever and crossbred dogs and Raul *et al.*, (2009b) in both toy breed and large breed of dogs.

HOSST-reacted sperm

The over-all mean HOSST – reacted sperm in semen of mongrel dogs was 93.27 ± 0.53 % which was in close accordance with the values reported by Rota *et al.*, (1995) in fresh pooled semen of different breeds, Nair *et al.*, (1999) in mongrel dogs and Veznik *et al.*, (2003) in different breeds of dogs. The findings varied from those reported by Nizanski (2006) in pooled semen and Das (2012) in Labrador semen. In regards to the results obtained the physical characteristics of

mongrel dog semen of Mizoram were found to be more or less comparable with that of other mongrel dogs and breeds of medium sized dogs.

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