Indian Journal of Animal Sciences **88** (8): 918–920, August 2018/Short communication

Effect of nocturnal collection of semen on its quality parameters in mithun

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Received: 11 December 2017; Accepted: 16 May 2018

Key words: Computer assisted sperm analysis, Mithun, Nocturnal semen collection, Semen quality parameters,

Artificial semen collection and frozen semen bank play important roles in production of large quantity of good quality semen doses in the prescribed time to inseminate many females with single ejaculate. It is believed that the mithun is cold loving animal and during hot hours or seasons, they enter and retire into their deep cold forest. Mithun is reared under the extensive free-range rearing system in the forest although it was domesticated in longer period ago. In general, natural breeding practice is followed in mithun with various limitations like reproductive disorders and infertility, etc that affect the mithun population. Mithun is not a endangered bovine species but is suffering severe non-cyclical population fluctuations due to various reasons including lack of proper breeding management and breeding bulls. Therefore, immediate breeding policy is required to protect mithun by various quarters of the society. The one is to formulate the effective breeding protocol and artificial insemination breeding programme to preserve the mithun germplasm. There are various factors such as collection method (Bhattacharyya *et al*. 2009), season (Perumal *et al*. 2015), vaccination (Perumal *et al*. 2013), breed (Rajoriya *et al*. 2013), pathological conditions (Perumal *et al*. 2016) and scrotal and testicular parameters (Perumal *et al*. 2017) etc. that significantly affect the semen production and its quality, freezability and fertility of sperm as well as the breeding bulls. In general, it is believed that the majority of natural mating occurs during night time in the extensive free range rearing system of mithun in the forest (naturally night breeder). Therefore, a study was designed to study the semen quality profiles of the ejaculates collected during night time (nocturnal hours) versus day time to understand the possibility of semen collection in night time in mithun.

Three mithun bulls with good health, 4 to 6 yr of age, weighing 495 to 510 kg with good body condition score (5–6) managed in mithun research farm, ICAR-National Research Centre on Mithun, Medziphema, Nagaland, India were selected for the present investigation. These bulls were maintained under uniform housing, feeding, lighting and other managemental conditions. Each and every animal in the experiment was fed as per the farm schedule and offered *ad lib.* clean drinking water. During the present study, all the experimental protocols followed were as per the rules and regulation given by the Institutional Animal Care and Use Committee regulations.

A total of 30 ejaculates (15 each from day and night) were collected from the mithun (attempted twice a week) and semen ejaculates of similar properties were pooled to eliminate individual differences for night and day separately. In day time, the semen was collected at 8.00 AM and night time collection at 11.00 PM. Semen ejaculates were collected from the bulls through trans-rectal massage method. Briefly, seminal vesicles were massaged centrally and backwardly for 5 min followed by the gentle milking of ampullae one by one for 3–5 min, which resulted into erection and ejaculation. During collection, the initial transparent secretions were discarded and neat semen drops were collected in a graduated test tube with the help of a funnel. The andrological parameters such as success rate, protrusion time, ejaculation time and length of penis at the time of ejaculation were evaluated. Immediately after collection, the samples were preserved at 37°C in a water bath and evaluated for the routine semen quality parameters (SQPs) such as volume by graduated test tube, colour, sperm concentration by spectrophotometer, mass activity and pH. Samples were allowed to the preliminary dilution with pre-warmed (37°C) Tris citrate glycerol extender (TCG). The partially diluted samples were then brought to the laboratory in an insulated flask containing warm water (37°C) for further processing. The mass activity (Nikon, Eclipse 80i; magnification 100× with thermo stage maintained at 37°C), percentage of sperm motility was assessed by phase contrast microscope (Nikon, Eclipse 80i; magnification 400× with thermo stage maintained at 37°C), viability by eosin nigrosin staining (Tomar 1997), total sperm abnormality (Tomar 1997), acrosomal integrity by Giemsa staining (Watson 1975), the plasma membrane integrity by hypo-osmotic swelling test (HOST) (Jeyendran *et al*. 1984) and nuclear integrity by Feulgen's staining technique (Barth and Oko 1989) were determined as per standard procedure. Sperm motility and velocity parameters were assessed by computer assisted sperm analyser (CASA) by Hamilton Thorne Sperm Analyser (HTM-IVOS, Version 10.8,

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Hamilton Thorne Research, Beverly, MA, USA). CASA parameters such as total motility (TM), forward progressive motility (FPM), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), curvilinear velocity (VCL), beat cross frequency (BCF), linearity (LIN) and straightness (STR) were estimated. A minimum of 200 spermatozoa were analysed with minimum of two different drops from each semen sample for each ejaculate. The number of objects incorrectly identified as spermatozoa were deleted manually and analysed accordingly. All the samples were examined in duplicate.

The results were analysed statistically and expressed as the mean±SEM. Means were analyzed by student t test between the day and night time collection using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL) for the physico morphological attributes and CASA parameters using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL). Differences with values of $P<0.05$ were considered to be statistically significant after arcsine transformation of percentage data by using SPSS 15.

The result of the present study revealed that the andrological parameters, viz. success rate, protrusion time, ejaculation time, length of penis, SQPs such as volume, colour, concentration, mass activity, total motility, viability, acrosomal integrity, plasma membrane integrity, nuclear integrity and pH (P=0.05) differed non-significantly between the day and nocturnal collection of semen (Table 1). Non-significantly higher success rate, shorter protrusion time, shorter ejaculation time, higher length of penis and higher total sperm abnormalities were observed in nocturnal than day collection whereas other parameters were observed non-significantly higher in day than in nocturnal collection. Similarly, CASA parameters such as FPM, TM, VSL, STR, ALH and BCF (P=0.05) differed non-significantly between two groups of semen collection in mithun (Table 2). Similar observation was reported by Yates (2002) in Holstein and Brahman bulls. Based on the results, it was confirmed that night collection is not necessary to get higher quality semen because non-significant difference was observed between the day and nocturnal semen collection in mithun. But the higher success rate, shorter protrusion and ejaculation time were observed in nocturnal collection indicating mithun express more sex libido at night time (Amann and Almquist 1976). Moreover, during day time, there were different farm operations including the movement of tractors, running of grass cutter machine, labourers noise pollution and morning light and temperature that affected the semen ejaculation process whereas during night time, there was much less interruptions for the semen collection team than at day time (Kerruish 1955).The main advantage of the nocturnal semen collection was that the semen collection teams were the only people at the semen collection site. Disturbances from outsiders were non-existent because of the late hours.

The present study concluded that though there was more success rate, shorter protrusion time and ejaculation time in nocturnal semen collection, good quality ejaculate was obtained in day time collection. The results suggest that nocturnal semen collection for semen preservation and artificial insemination is neither necessary nor recommended in mithun species. Moreover, outside factors in the semen collection centre need to be kept to minimum during the day time semen collection to get high quality semen. However, further research is needed by using the artificial vagina method of semen collection to confirm the findings of the present study.

Table 1. Effect of semen collection time on semen quality parameters in mithun

Semen quality parameters	Day time collection	Night time collection
Success rate (%)	87.00	90.00
Protrusion time (sec)	115.63±5.63[a]	98.72±4.85[a]
Ejaculation time (sec)	169.87±6.87[a]	155.56±5.73[a]
Length of penis (inch)	10.23±1.41[a]	10.46±0.97[a]
Volume (ml)	1.91±0.78[a]	1.86±0.89[a]
Colour	Creamy White	Creamy White
Concentration ($\times 10^6$/ml)	623.04±7.59[a]	612.42±8.21[a]
Mass activity (0-5 Scale)	3.18±0.70[a]	3.15±0.73[a]
Total motility (%)	81.44±2.07[a]	79.76±3.53[a]
Livability (%)	84.65±2.17[a]	83.21±3.08[a]
Acrosomal integrity (%)	88.68±2.25[a]	87.76±3.39[a]
Total sperm abnormality (%)	7.15±1.13[a]	7.98±2.28[a]
Plasma membrane integrity (%)	86.66±2.25[a]	85.83±3.36[a]
Nuclear integrity (%)	86.72±2.22[a]	85.12±3.09[a]
pH	6.92±0.36[a]	6.85±0.21[a]

Means with different superscripts within rows differ significantly (P<0.05).

Table 2. Effect of semen collection time on motility and velocity parameters by computer assisted sperm analyser in mithun

Motility and velocity parameter	Day time collection	Night time collection
Forward progressive motility (%)	60.63±2.55[a]	58.76±3.21[a]
Non progressive motility (%)	17.98±2.11[a]	17.88±2.67[a]
Total motility (%)	78.62±2.16[a]	77.23±3.34[b]
Static sperm (%)	21.38±2.28[a]	23.55±3.21[a]
Curvilinear velocity (VCL) (µm/sec)	186.13±4.31[a]	189.13±4.13[a]
Straight line velocity (VSL) (µm/sec)	89.32±2.72[a]	89.63±3.22[a]
Average path velocity (VAP) (µm/sec)	114.64±3.85[a]	116.01±3.63[a]
Linearity (LIN) (%)	48.17±0.95[a]	47.95±1.64[a]
Straightness (STR) (%)	78.25±1.75[a]	77.13±2.32[a]
Wobble (WOB) (%)	61.48±1.29[a]	62.15±1.48[a]
Amplitude of lateral head displacement (µm)	8.61±1.18[a]	8.73±1.26[a]
Beat/Cross frequency (BCF) (Hz)	28.26±1.35[a]	28.65±1.89[a]

Means with different superscripts within rows differ significantly (P<0.05)

SUMMARY

A study was conducted to assess the effect of nocturnal versus day time semen collection on semen quality profiles in mithun. Three healthy adult mithun breeding bulls were selected from mithun breeding farm, ICAR-NRC on Mithun, Medziphema, Nagaland. A total of 30 ejaculates (15 ejaculates each from nocturnal [11 PM] and day time [8 AM]) were collected by tran-rectal massage method and evaluated for the andrological parameters, semen quality parameters, and velocity and motility parameters were measured by computer assisted sperm analyser (CASA). The result revealed that there was non-significant difference between the day and nocturnal semen collection with respect to andrological, semen quality and CASA parameters. However, the andrological parameters were non-significantly higher in nocturnal than day time collection indicating that sex libido was more in night time. The study concluded that nocturnal semen collection for semen preservation and artificial insemination is neither necessary nor recommended for mithun species. However, semen collection by artificial vagina method in day versus nocturnal is needed to confirm the findings of the present study.

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