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Physico morphological attributes of mithun semen

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Seminal attributes varied widely, not only between the bulls, but also between the ejaculates within the same bull and varied between seasons (Raval and Dhami 2010). Semen volume, sperm concentration, percentage of dead, abnormal spermatozoa and motility of spermatozoa are recognized as the important indices of semen quality and are significantly correlated with freezability and/or fertility (Fiaz *et al*. 2010). Correlation relationship among the seminal attributes was highly significant and positively correlated among the motile spermatozoa, live sperm and intact acrosome and these attributes could be applied for practical utility in routine semen evaluation to predict preservability, freezability and fertilizing ability of spermatozoa (Bhoite *et al.* 2005). Perusal of literatures revealed, paucity of information on physico morphological attributes of good and poor quality semen in mithun bulls. Hence, the objective of this study was to assess the physico morphological attributes of good and poor quality semen in mithun bull to pursuit future sperm preservation protocols in this precious species.

Apparently healthy mithun bulls (10), approximately 3- to 5- yr old, with average body weight of 500 kg and with good body condition (score 5–6), were selected from the herd in mithun farm, NRC on Mithun, Jharnapani, Nagaland. Ejaculates (50), collected through rectal massage method, were split into good and poor quality semen based on the mass activity and individual motility of fresh semen. Mass activity 3+ and above and individual motility 70% and above were considered as good quality ejaculates whereas less than 3+ and 70%, respectively, was considered as poor quality ejaculates. The percentage of sperm motility, viability (eosin nigrosin staining technique), total sperm abnormality, acrosomal integrity (giemsa staining technique), plasma membrane integrity (hypo-osmotic swelling test), nuclear integrity (Fuelgen's staining technique), vanguard distance travelled by sperm in bovine cervical mucus (bovine cervical mucus penetration test) were determined as per standard procedure.

The results were analysed statistically with the student 't' test using the SPSS (version 15.0; SPSS, Chicago, IL) and expressed as the mean ± SEM. Differences with values of ($P < 0.05$) were considered to be statistically significant after arcsine transformation of percentage data. Correlation among the physico morphological attributes was established with Pearson's correlation coefficient. Differences at $P < 0.05$ were considered to be statistically significant.

The colour of the semen lies in normal colour range of semen for the mithun species and the consistency varies from thin milky to creamy. The colour was creamy white in good quality and watery white in poor quality ejaculates (Table 1). Similar result was observed by Karunakaran *et al.* (2007). The volume of ejaculate was significantly ($P< 0.05$) higher in good quality ejaculates than in poor quality ejaculates (Table 1). Volume of ejaculate in the present study was higher than that of Karunakaran *et al.* (2007) and Bhattacharyya *et al.* (2005). The results of present study were lower than to those reported by Mohanty (1999) and Loyi (2008) in cattle. In massage method of semen

Table 1. Mean (±SE) physico morphological attributes of good and poor quality mithun semen

Physico morphological attributes	Good quality semen (n=25)	Poor quality semen (n=25)
Volume (ml)	1.91 ± 0.78^a	1.35 ± 0.71^b
Colour	Creamy White	Watery to Medium
Concentration ($\times 10^6$/ml)	623.04 ± 7.59^a	509.93 ± 6.51^b
Mass activity (0–5 scale)	3.18 ± 0.70^a	2.15 ± 0.45^b
Individual motility (%)	81.44 ± 2.07^a	57.28 ± 1.31^b
Livability (%)	84.65 ± 2.17^a	63.37 ± 1.89^b
Acrosomal integrity (%)	88.68 ± 2.25^a	68.86 ± 1.96^b
Total sperm abnormality (%)	7.15 ± 1.13^a	16.16 ± 1.74^b
HOST (%)	86.66 ± 2.25^a	65.31 ± 1.91^b
CMPT (mm/h)	23.93 ± 1.62^a	18.16 ± 0.94^b
Nuclear integrity of spermatozoa (%)	86.72 ± 2.22^a	66.13 ± 1.75^b
pH	6.92 ± 0.36^a	7.10 ± 0.42^b

Means with different superscript within rows differ significantly ($P < 0.05$); N, number of ejaculates.

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collection, the bulls responded more, were getting more volume of good quality semen ejaculates, but those bulls had longer protrusion and ejaculation time leads to less quality and less volume of semen. In the present study, some bulls consistently produced poor quality ejaculates. Volume of the semen mainly depends upon the secretory function of the accessory sex glands and its function as well as individual response.

The mass activity and individual motility were significantly (P< 0.05) higher in good quality than poor quality ejaculates (Table 1). Mass activity and individual motility were positively correlated (P< 0.05) with individual motility, livability, acrosomal integrity, BCMPT and nuclear integrity and negatively correlated (P< 0.05) with total sperm abnormality in good (Table 2) and in poor quality ejaculates (Table 3). Similar report was observed by Karunakaran *et al.* (2007), Bhattacharyya *et al*. (2009) and lower value was reported by Bhattacharyya *et al*. (2005). Similar observation was reported in cattle by Gebreselassie *et al.* (2012). Sethi *et al*. (1989) concluded that bulls donating larger volume of neat semen with higher mass activity are supposed to produce freezable semen. Less mass activity in present study may be due to collection through massage method, individual responses and concentration of live spermatozoa as well as effect of climatic variation. Ejaculates having individual motility 70% or more were considered as good quality. Similar condition was reported in other domestic species. The poor semen quality in crossbred and Murrah buffaloes and the poor semen freezability in exotic and crossbred bulls are the major reasons for disposal of breeding bulls (Suryaprakasam and Rao 1993).

The concentration is important for the production of maximum number of semen straw for getting maximum number covered. In the present study, the sperm concentration was significantly (P< 0.05) higher in good than in poor quality ejaculates (Table 2). However, the difference in concentration between these 2 groups has so far not been studied in this mithun species. But average values of concentrations in both the groups were well within the range of overall sperm concentration. Similar report was observed in mithun bulls (Bhattacharyya *et al*. 2005, Karunakaran *et al*. 2007) and cattle (Loyi 2008, Gebreselassie *et al.* 2012). Sperm concentration is greatly influenced by individual bull factor, breed and environmental factors and age (Swain and Singh 2004). The difference in sperm concentration may be due to managemental practices such as restraint before collections, frequency of semen collection and seasonal variations (Singh and Prabhu 1983).

The percentage of live spermatozoa is important parameter while selecting ejaculates for freezing to get more number of post-thaw live spermatozoa. In the present study, the percentage of live spermatozoa of ejaculate was significantly (P< 0.05) higher in good than in poor quality ejaculates (Table 2). The livability of spermatozoa was positively correlated (P< 0.05) with mass activity, individual motility, acrosomal integrity, plasma membrane, nuclear integrity and BCMPT and negatively correlated (P< 0.05) with total abnormality in good (Table 2) and in poor quality semen (Table 3) as reported earlier in mithun (Bhattacharyya *et al.* 2005, Karunakaran *et al.* 2007). Similar results were reported in cattle (Gebreselassie *et al.* 2012, Loyi 2008). The variation in the results could be due to different breeds involved and age. This may be due to breed effect and technical skill of the observer (Tomar *et al.* 1985). The abnormal sperm percentage of spermatozoa was significantly (P< 0.05) lower in good than in poor quality ejaculates (Table 2). It was negatively correlated (P< 0.05) with mass activity, individual motility, acrosomal integrity, livability, plasma membrane integrity in good quality semen (Table 2). In the present study, percentage of total sperm abnormality was higher than earlier reports (Karunakaran *et al.* 2007). Roberts (1982) opined that in fertile semen sample, the total sperm abnormality should not exceed more than 20 %. In the present study, the total sperm abnormality was calculated from caput, mid-piece and tail abnormalities. Moreover, the sperm abnormality may vary due to method of collection, temperature shock and technique employed. Hence, the difference between the good and poor quality ejaculates may be attributed to these factors (Bishop *et al.* 1954).

Table 2. Correlation coefficient among the physico-morphological attributes of good quality ejaculates in mithun bulls

No	Attributes	1	2	3	4	5	6	7	8	9	10	11
1	Volume	1.00	–0.10	0.28	0.18	0.31	0.29	–0.12	0.21	0.36	0.30	0.02
2	Concentration		1.00	0.23	0.14	0.32	0.36	–0.47	0.25	0.12	0.32	–0.09
3	Mass activity			1.00	0.87*	0.85*	0.83*	–0.65*	0.59	0.84*	0.86*	–0.18
4	Individual motility				1.00	0.88*	0.76*	–0.66*	0.70*	0.84*	0.77*	–0.41
5	Livability					1.00	0.90*	–0.72*	0.86*	0.84*	0.88*	–0.47
6	Acrosomal integrity						1.00	–0.81*	0.76*	0.78*	0.94*	–0.41
7	Abnormality							1.00	–0.69*	–0.60	–0.75*	0.36
8	Plasma membrane integrity								1.00	0.75*	0.68*	–0.56
9	Cervical mucus penetration test									1.00	0.80*	–0.46
10	Nuclear integrity										1.00	–0.35
11	pH											1.00

* Correlation coefficients were significant, P< 0.05.

Table 3.Correlation coefficient among the physico-morphological attributes of poor quality ejaculates in mithun (*Bos frontalis*) bulls

No	Attributes	1	2	3	4	5	6	7	8	9	10	11
1	Volume	1.00	–0.59	–0.65	–0.14	–0.47	–0.28	0.29	–0.32	–0.30	–0.28	0.32
2	Concentration		1.00	0.42	0.15	0.42	0.32	–0.04	0.40	0.31	0.27	–0.39
3	Mass activity			1.00	0.43	0.81*	0.64	–0.46	0.71*	0.69*	0.65*	–0.45
4	Individual motility				1.00	0.59	0.67*	–0.14	0.52	0.43	0.54	–0.24
5	Livability					1.00	0.77*	–0.50	0.90*	0.81*	0.86*	–0.51
6	Acrosomal integrity						1.00	–0.36	0.74*	0.54	0.77*	–0.30
7	Abnormality							1.00	–0.41	–0.66	–0.43	0.09
8	Plasma membrane integrity								1.00	0.77*	0.80*	–0.54
9	Cervical mucus penetration test									1.00	0.62*	–0.37
10	Nuclear integrity										1.00	–0.40
11	pH											1.00

* Correlation coefficient were significant, P< 0.05.

The percentage of acrosomal integrity of spermatozoa was significantly higher in good than in poor quality ejaculates (Table 2). Acrosomal integrity was positively correlated with mass activity, livability, nuclear, plasma membrane integrity and BCMPT and negatively correlated (P< 0.05) with total sperm abnormality in ejaculates (Table 2). Similar observation was reported in cattle (Gebreselassie *et al.* 2012). The acrosomal integrity depends on the factors like age of the bulls, temperature, frequency of semen collections, sexual excitement before collection (Javed *et al.* 2000). The study of sperm membrane functional status is of particular importance since an intact and functionally active membrane is required for maintenance of sperm motility, metabolism, capacitation, acrosome reaction and successful fertilization. During the HOST, the biochemically active spermatozoa when exposed to hypo-osmotic stress due to influx of water, undergoes swelling and subsequently increase in volume to establish equilibrium between the fluid compartment within the spermatozoa and extracellular environment. The osmotic changes induce typical morphological alteration characterized by presence of swollen area in the tail region. These changes have been considered as an indicator of the membrane integrity and normal functional activity of spermatozoa (Jeyendran *et al.* 1984). The HOST positive sperm per cent of ejaculate was significantly (P< 0.05) higher in good than in poor quality ejaculates (Table 2). Plasma membrane integrity was positively correlated (P< 0.05) with mass activity, livability, acrosomal integrity, nuclear integrity and BCMPT and negatively correlated (P< 0.05) with total sperm abnormality in good (Table 2) and poor quality ejaculates (Table 3). In cattle, it was reported by Gebreselassie *et al*. (2012) and Perumal *et al.* (2011) that good quality has higher plasma integrity value than poor quality semen. Routine semen evaluation has certain limitations for comprehensive prediction of fertility of bull semen. The HOST highlights the permeability of sperm membrane to hypo osmotic solution and the projection of higher value is a valid indication of intact membrane and sample with higher value is regarded as potent for establishing pregnancy.

Per cent nuclear integrity of spermatozoa was significantly (P< 0.05) higher in good than in poor quality ejaculates (Table 2) in the present study. Nuclear integrity was positively correlated (P< 0.05) with mass activity, livability, acrosomal, plasma membrane integrity and BCMPT and negatively correlated with total sperm abnormality in good (Table 2) and poor quality ejaculates (Table 3). Perusal of literature did not reveal any study on nuclear integrity/DNA fragmentation in good and poor quality ejaculates in mithun species. So the appropriate comparison could not be substituted by this study. The *in-vitro* sperm penetration test is considered as a sperm function test that measures the ability of sperm in the semen to swim up into a column of cervical mucus. The vanguard distance travelled by sperm was significantly (P< 0.05) higher in good than in poor quality ejaculates (Table 1). BCMPT was positively correlated (P< 0.05) with mass activity, individual motility, livability, acrosomal, nuclear and plasma membrane integrity and negatively correlated (P< 0.05) with total sperm abnormality in good (Table 2) and poor quality ejaculates (Table 3). Perusal of literature did not reveal any study on BCMPT in good and poor quality ejaculates in mithun species, similar study was conducted in cattle that good ejaculates have higher vanguard distance travelled by sperm than by poor quality sperm (Perumal *et al.* 2011). The difference of distance travelled between good and poor ejaculates would be possible due to the difference in the forward progressive motility and loss of mitochondrial membrane potential.

It was concluded from the study that most of the seminal attributes were significantly higher in good quality ejaculates in comparison to the poor quality ejaculates in mithun. This indicated that good quality sperm has higher structural stability than the poor quality sperm that leads to good quality sperm has higher functional sperm structures to move faster and forward direction to fertilize.

SUMMARY

The present study was undertaken to assess the volume, colour, concentration, mass activity, individual motility, viability, total sperm abnormality, acrosomal integrity,

plasma membrane, nuclear integrity, vanguard distance travelled by sperm in bovine cervical mucus (BCMPT) of ejaculates and hydrogen ion concentration (pH) in good and poor quality ejaculates in mithun. Ejaculates (50) were collected from matured mithun bulls and based on the mass activity and individual motility; the semen samples were split into good and poor quality. The result revealed that these parameters varied significantly between good and poor quality semen. The mass activity was positively correlated with individual motility, livability, acrosomal integrity, plasma membrane, nuclear integrity and BCMPT and negatively correlated with total sperm abnormality. It was concluded that most of the seminal parameters were significantly higher in good quality than in poor quality ejaculates. Good quality sperm has higher functional sperm structures to move faster and forward direction to reach the site of fertilization and successful fertilization.

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