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Semen quality parameters of mithun semen preserved at liquid state (5°C)

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There is an urgent need to concentrate on certain alternative way of mithun breeding to overcome breeding failure and inbreeding depression. This can be achieved easily through the intensive selection and artificial insemination (AI). Liquid storage of semen is generally used as a method to reduce metabolism to maintain or extend the viability and motility of spermatozoa over a period of time. Moreover, it has been reported that fresh bovine semen can be preserved at 5°C for 6 days successfully with tris based egg yolk extender with or without glycerol (Verberckmoes *et al*. 2004). But, there are very scanty reports available for mithun species. Therefore, the present research was focused on standardization of mithun semen preservation at liquid state (5°C) and selects the suitable time period of incubation for AI.

Apparently healthy adult mithun bulls (5) of 4 - 6 yr – old and weighing approximately 500 kg (good body condition: score 5–6) were maintained under uniform feeding, housing and management conditions. Good quality semen ejaculates (50) were collected through transrectal massage method. The samples were kept in a water-bath at 37°C immediately after collection and evaluated for routine seminal attributes. The samples were subjected to the initial dilution with pre-warmed (37°C) 1 ml of tris–egg yolk based semen extender.

Spermatozoa concentration was determined in partially diluted semen samples by haemocytometer method (Tomar 1997). Final dilution was done with pre-warmed (37°C) tris egg yolk based extender (@30×10^6/ml). The diluted semen samples were stored at 5°C for 72 h in the cold cabinet. The preserved semen samples were evaluated for SQPs at 6 h intervals for 72 h with standard assessment procedure. The count of live sperm, acrosomal integrity and morphological abnormality were determined by the trypan blue - Giemsa staining technique (Kovacs and Foote 1992). The total morphological abnormality was determined by adding the portion of head, mid piece and tail abnormalities. Sperm were classified into four categories namely, (i) live with intact acrosome, (ii) live with damaged acrosome, (iii) dead with intact acrosome and (iv) dead with damaged acrosome.

In each experiment, variations in SQPs among the different hours of incubation and among the different bulls were analyzed by one way analysis of variance (ANOVA) and between the 0 hr of incubation and time at 30% progressive motility was by student "t" test using the SPSS version 18.00. A probability value of less than 0.05 ($P<0.05$), 0.01 ($P<0.01$) was considered statistically significant.

Routine seminal attributes such as colour, consistency, volume, mass activity and spermatozoa concentration of fresh mithun semen samples were creamy white, medium, 1.8±0.08 ml, 3.4±0.09 (5 point scale) and 478±15 (×10^6/ml), respectively. The percentage of progressive motile spermatozoa decreased significantly ($P<0.01$) over the period of storage and were below 20% after 48 h of storage. Nearly 30% progressive motility of the preserved semen samples was maintained until 42 h of storage (Table 1). The percentage of live sperm with intact acrosome was decreased significantly ($P<0.01$) over the time of storage (Table 2). Approximately 32% of spermatozoa were live and with intact acrosome at the time of 30% progressive motility. The proportion of live spermatozoa with damaged acrosome did not differ significantly following preservation, but proportion of both categories of dead spermatozoa (with intact or damaged acrosome) increased significantly ($P<0.01$) after preservation at the time of 30% progressive motility (Table 2) whereas, the total and individual morphological abnormalities were increased significantly ($P<0.01$) at the time of 30% progressive motility (Table 3). But the SQPs did not vary significantly among the experimental bulls.

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Table 1. Overall variations (mean±SE) in progressive motility of spermatozoa of mithun semen samples preserved at 4°C

Stage of freezing	Progressive motility (%)
After initial dilution	74.0±1.0^{A}
After final dilution (0 h)	64.8±1.2^{B}
6 h	59.2±1.5^{BC}
12 h	54.8±1.6^{CD}
18 h	50.8±1.6^{DE}
24 h	46.4±2.6^{E}
30 h	43.2±2.4^{EF}
36 h	37.2±2.7^{F}
42 h	29.2±3.6^{G}
48 h	18.8±3.8^{H}
54 h	9.2±3.4^{I}
60 h	2.8±1.9^{I}
66 h	1.2±1.2^{I}
72 h	1.2±1.2^{I}

Superscripts (A–I) indicate values with different superscript within column differ significantly (P<0.01).

Table 2. Variations (mean±SE) in the liveability and acrosomal integrity of mithun spermatozoa after initial dilution and at the time of 30% progressive motility following storage at 4°C

Particulars	After final dilution (0 h)	At 30% of progressive motility
Live spermatozoa with intact acrosome (%)	63.8±1.6**	31.3±0.9**
Live spermatozoa with damaged acrosome (%)	4.2±0.8	3.4±0.8
Dead spermatozoa with intact acrosome (%)	3.9±0.4*	5.7±0.7*
Dead spermatozoa with damaged acrosome (%)	29.1±1.3**	59.6±1.0**

** Indicates values within row differ significantly (P<0.01) and * indicates values within row differ significantly (P<0.05).

In the present study, the results revealed that SQPs differed among the different times of incubation period from time of immediately after dilution to 72 h. Cold storage (liquid preservation) of semen is used to reduce metabolism and to maintain sperm viability over an extended period of time. But the quality of semen is deteriorated during this extended storage period. The results of the study indicated that mithun spermatozoa can be preserved at liquid storage in tris based extender (Karunakaran *et al*. 2007). Earlier results in semen cryopreservation study in gaur and mithun indicated that semen can be preserved in liquid as well as in frozen state in tris based extender and maintain its fertilization potential (Karunakaran *et al*. 2007, Mondal *et al*. 2010, Baruah *et al*. 2013). Moreover, it is also reported that tris extender is the common extender to preserve the sperm of other domestic animal species semen efficiently in liquid state and that semen can be able to preserve its

Table 3. Variations (mean±SE) in the morphological abnormalities of mithun spermatozoa after initial dilution and at the time of 30% progressive motility following storage at 4°C

Morphological abnormalities (%)	After final dilution (0 h)	At 30% of progressive motility
Head	0.5±0.2**	3.7±0.3**
Mid piece	0.1±0.1**	0.6±0.1**
Tail	4.8±0.3**	10.2±0.6**
Total	5.4±0.3**	14.5±0.8**

** indicates values within row differ significantly (P<0.01).

progressive motility more than 30% in 42 h of incubation (Verberckmoes *et al*. 2004, Pervage *et al*. 2009, Frydrychová *et al*. 2010).

The results of SQPs of mithun semen in the present study were consistent with earlier research reports in mithun and other similar bovine species (Karunakaran *et al*. 2007, Bhattacharya *et al*. 2009, Baruah *et al*. 2013) and also there was gradual decrease of progressive motility, livability, acrosomal integrity and increase of total morphological abnormalities over the period of incubation from initial dilution up to 72 h of incubation at 5°C (Karunakaran *et al*. 2007, Baruah *et al*. 2013). Livability and acrosomal integrity were observed more than 32% at the time of 30% progressive motility. The result also revealed that there was significant difference observed between the immediately after dilution (0 h) and time at 30% of progressive motility for the SQPs such as live spermatozoa with intact acrosome (P<0.05), dead spermatozoa with intact acrosome (P<0.01) and dead spermatozoa with damaged acrosome (P<0.05) (Table 2). Similarly, total morphological abnormality was observed less than 10% at the time of 30% progressive motility (Karunakaran *et al*. 2007, Baruah *et al*. 2013) and head, mid-piece, tail and overall total morphological abnormality differed significantly between dilution at 0 h and time at 30% of progressive motility (Table 3). Further, it was reported that cattle and mithun semen can be preserved at liquid state in tris based extender up to 3 days (Verberckmoes *et al*. 2004, Karunakaran *et al*. 2007). Earlier reports in bovine species such as in cattle and mithun suggested that semen can maintain progressive motility, livability and acrosomal integrity more than 30% in 3 days of semen preservation, stored in tris extender at liquid state (Karunakaran *et al*. 2007, Baruah *et al*. 2013). But in cattle, it was reported that progressive motile spermatozoa and live sperm concentration considerably improved more than 30% in liquid storage (5°C) in tris semen extender for a week without presence of glycerol (Verberckmoes *et al*. 2004).

In the present study, total morphological abnormality increased over an extended period of time of storage (Karunakaran *et al*. 2007, Baruah *et al*. 2013). Abnormalities such as head, midpiece and tail observed to be highest after 72 h of storage and were significantly differed between the

immediately after dilution and 30% progressive motility in respective mithun bulls. Similar reports were observed in earlier studies in mithun and cattle (Karunakaran *et al*. 2007, Perumal *et al.* 2011, Baruah *et al*. 2013). Earlier study in cattle and buffalo also showed that total and specific morphological abnormalities increased significantly in liquid as well as in cryopreserved semen as compared to fresh semen (Perumal *et al.* 2011, Baruah *et al*. 2013).

Our results revealed that progressive motility, livability and intactness of acrosome of spermatozoa were more than 35% in 40 h of incubation in tris extender stored at liquid state (5°C) and morphologically abnormal spermatozoa were significantly increased over a period of incubation (Karunakaran *et al*. 2007). The *in vitro* andrological investigations revealed that post thaw progressive motility at least 40% with at least 75% normal sperm morphology were considered as the minimum standard for selection of mithun semen for cryopreservation as in other bovine species (Karunakaran *et al*. 2007, Perumal *et al.* 2011, Baruah *et al*. 2013). Further, it was established that cattle and mithun spermatozoa with 35 - 45% post thaw motility were able to establish pregnancy through artificial breeding in cow (Perumal *et al*. 2011, Karunakaran *et al*. 2007). Similarly, in the present study, result revealed that mithun spermatozoa could be able to maintain its fertilizing potential up to 36 h of *in vitro* storage at refrigerated temperature (Karunakaran *et al*. 2007, Dhali *et al*. 2008, Baruah *et al.* 2013).

The study has concluded that the mithun sperm can be preserved in tris based extender in liquid storage at refrigeration temperature upto 36 h of incubation. Further study is required to confirm the present findings and improve the SQPs with suitable semen extender to preserve the mithun semen in liquid as well as cryopreserved state for artificial breeding purpose.

SUMMARY

A study was conducted to assess the effect of liquid semen preservation on semen quality parameters (SQPs) such as forward progressive motility, livability, acrosomal integrity and total morphological abnormality at refrigerated temperature in mithun. Good quality ejaculates (50) were collected and preserved at refrigerated temperature (5°C) for 72 h using tris egg yolk citrate based extender (TEYCE). Seminal attributes, viz. colour, consistency, volume, mass activity and sperm concentration of fresh semen samples were creamy white, medium, 1.8±0.08 ml, 3.4±0.09 (5 point scale) and 478±15 ($\times 10^6$/ml), respectively. SQPs were assessed after initial dilution, after final dilution and up to 72 h at 6 h intervals. The progressive motility, live sperm with intact acrosome decreased significantly over the period of storage and were below 20% after 48 h of storage. Total morphological abnormalities increased significantly at the time of 30% progressive motility. The study concluded that the mithun sperm can be preserved in tris based egg yolk extender in liquid storage at refrigeration temperature (5°C) for 36 h.

REFERENCES

Baruah K K, Dhali A, Mech A, Bora B, Das J, Bora R, Mondal M, Sarmah B C, Deka B C, Rajkhowa C. 2013. Effect of concentration and addition method of glycerol on the quality of cryopreserved mithun (*Bos frontalis*) spermatozoa. *Journal of Animal Physiology and Animal Nutrition (Berl)* **97**(6):1051–58.

Bhattacharyya H K, Goswami B K, Bujarbaruah K M, Deka B C and Biswas B K. 2009. Collection and characterization of semen in mithun (*Bos frontalis*) bulls. *Theriogenology* **72**: 699–703.

Dhali A, Karunakaran M, Mech A, Nath N, Prakash B, Rajkhowa C and Mishra D P. 2008. Birth of the first mithun (*Bos frontalis*) calf through artificial insemination. *Animal* **2**(6): 879–82.

Frydrychová S, Èeøovský J, Lustyková A, Rozkot M. 2010. Effects of long-term liquid commercial semen extender and storage time on the membrane quality of boar semen. *Czech Journal of Animal Science* **55**(4): 160–66.

Karunakaran M, Dhali A, Mech A, Khate K, Rajkhowa C and Mishra D P. 2007. Preservation of mithun (*Bos frontalis*) semen at refrigeration temperature. *Animal Reproduction Science* **101**: 257–64.

Kovacs A and Foote R H. 1992. Viability and acrosome staining of bull, boar and rabbit sperms. *Biotechnology and Histochemistry* **67**: 119–24.

Mondal M, Karunakaran M, Lee K B and Rajkhowa C. 2010.Characterization of Mithun *(Bos frontalis)* ejaculates and fertility of cryopreserved sperm *Animal Reproduction Science* **118**: 210–16.

Perumal P, Selvaraju S, Selvakumar S, Barik A K, Mohanty D N, Das R K, Das S and Mishra P C. 2011. Effect of pre-freeze addition of cysteine hydrochloride and reduced glutathione in semen of crossbred Jersey bulls on sperm parameters and conception rates. *Reproduction in Domestic Animals* **46**(4):636–41.

Pervage S, Hassan M R, Ershaduzzaman M and Khandoker M A M Y. 2009. Preservation of liquid semen and Artificial Insemination in native sheep. *Journal of Bangladesh Agricultural University* **7**(2): 305–08.

Tomar N S. 1997. *Artificial Insemination and Reproduction of Cattle and Buffaloes.* Saroj prakashan, Allahabad, India.

Verberckmoes S, Van Soom A, Dewulf J, De Pauw I and De Kruif A. 2004. Storage of fresh bovine semen in a diluents based on the ionic composition of cauda epididymal plasma. *Reproduction in Domestic Animals* **39**: 410–16.