SUCCESS STORY

Establishment of Semen Bank and Implementation of Artificial Insemination (AI) in Mithun

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PREFACE

Mithun (*Bos frontalis*), a unique bovine species, is primarily reared for meat purposes in the hilly states of Arunachal Pradesh, Nagaland, Manipur, and Mizoram but also has a great potential for milk, hide and draft power. Since time immemorial, mithun is reared under a free-range forest ecosystem without scientific supervision of health, nutrition, and reproduction.

During the last decades, due to a decrease in forest cover, commercialization of agriculture and indiscriminate slaughter of mithun, a significant decline in the mithun population has been recorded in certain pockets. Under the present free-range mithun rearing system, grazing of a limited number of mithun in a particular hill pocket is allowed without migration to other locations and vice versa. Moreover, since mithun is considered a sacred animal under tribal custom, the best mithun bulls are generally slaughtered during auspicious occasions which resulted in a significant decline in superior breeding bulls in the population leading to inbreeding in this species.

ICAR-NRC on Mithun has introduced and popularized the semi-intensive mithun rearing model and implemented it under field conditions with scientific intervention. In the present scenarios, genetic improvement of the mithun population is of utmost importance. Artificial insemination (AI) is one such technology used worldwide for livestock improvement. ICAR-National Research Centre on Mithun, Nagaland has been continuously working on the various aspects of semen collection, preservation & AI. In our continuous endeavour to augment the reproductive efficiency of mithun, we have successfully eastblished semen bank and implemented AI at Institute Mithun Farm as well as under field condition and presently, semen collection & cryopreservation are the routine farm procedures.

The success of AI largely depends on the semen quality of the bulls. High fertility bulls with sound body conformation, libido, and mating ability are usually selected for semen collection. This success story attempts to document the breeding soundness evaluation of the bull, semen collection and evaluation procedures, semen cryopreservation, and artificial Insemination in mithun. The authors would like to express their sincere support to all the contributors who directly or indirectly have helped to prepare this success story.

Authors

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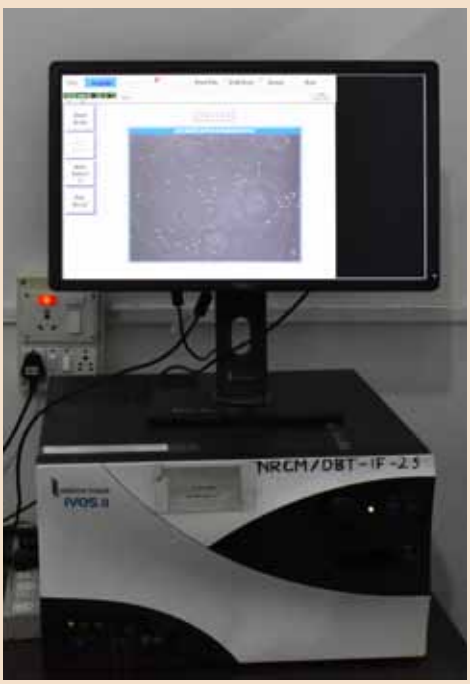
INTRODUCTION

Artificial insemination (AI) with cryopreserved semen is one such technology used worldwide for livestock improvement. It is by far the most common method of breeding intensively kept domestic livestock such as dairy cattle and buffalo. AI is increasing in horses, sheep and has been reported in other domestic animals such as dogs, goats and in conservation breeding of rare or endangered species. ICAR-National Research Centre on Mithun, Nagaland has continuously worked on the various aspects of semen collection and standardized the cryopreservation process and AI in mithun. For successful implementation of AI, it is necessary to standardize the semen collection and cryopreservation protocols for particular species.

The recent initiatives to popularize mithun as an economic beef animal emphasize the introduction of control breeding and breed improvement programme in this species. In this context, AI technique in Mithun has been standardized and implemented successfully both in farm and field condition at NRC Mithun, Nagaland. First Mithun calf under field condition was born on 6th May 2010 using a standardized AI technique at Mithun rearing area of Khonoma village in Kohima district of Nagaland. The institute has been successfully standardized the collection procedure of semen with an innovative way of spraying urine of estrus females on the dummy cow for attracting the bulls. Moreover, the method of preservation of semen has also been standardized.

Equipments under semen lab

Semen lab was established at the ICAR-NRC on Mithun with the objective of improving the genetic quality of Mithun by disseminating superior germplasm. Its main activity is mithun frozen semen production, offering high quality genetics in the form of frozen semen from superior breeding bulls. The semen is collected from the selected mithun bulls and evaluated before processing for use in artificial insemination programme. The semen lab is fully equipped with the equipments necessary for processing and cryopreservation of semen. The following equipments are present in the semen lab.

1.	Spectrophotometer For determination of concentration	
2.	Computer Assisted Semen Analyzer (CASA) For sperm motility and velocity parameters	

3.	Straw filling and sealing machine For filling of semen straws and sealing	
4.	Straw holding cold cabinet For equilibration of semen straws at 4 °C before freezing	
5.	Biological freezer For freezing semen straws by using Liquid nitrogen	

Selection of mithun bulls for semen collection

The Mithun (*Bos frontalis*) inhabits the northeastern hilly region of India and adjoining areas of neighboring countries like Bangladesh, Myanmar, Bhutan and China. Scientific intervention into the health and production aspects of mithun is a relatively recent proposition. This is a new or alternate field of animal production aiming towards the future large-scale and extensive use of this species as a meat animal. Genetic improvement of the mithun population depends upon the selection of breeding bulls. Therefore, body conformation traits and breeding soundness evaluation (BSE) are of immense importance. BSE is a method developed to assess the breeding potential of bulls for natural mating or collection of semen for use in artificial insemination programme. Further, it is important for bull replacement in a herd to avoid inbreeding. The selected bull must be sound in its body conformation, libido, mating ability, and fertility so that he can serve many mithun cows in a short period without suffering an injury. Structural soundness is, therefore, an integral part of fertility.

Phenotypic Characteristics

The Mithun (*Bosfrontalis*) is sturdy with a heavy and well proportioned compact muscular body with a quite aggressive temperament under restraint. The body is well developed and symmetrical with distinct rippling muscles. The head looks 'V' shaped with a broad and distinct frontal bone. The colour of the coat around the head is mostly black with a grayish or white forehead and white face. The horn is massive at the base pointed outwards and slightly curved upward and tapering end. Tips of the horn are blunt and eyes are prominent, bright, and alert with black eyebrows. Males have more developed body muscles and horns than females. Unlike the hump of indigenous cattle, mithun is having a dorsal broad-based muscular crest (ridge) and more prominent in males than females. The neck is strong and well developed with pendulous dewlap which is thick and muscular with folds. The chest is broad and thick. The skin of these animals is thick, smooth, and tightly attached to the body like that of other beef cattle. The oval-shaped testicles are located outside the body cavity in the blackish-brown scrotum and have a compact prepuce. The tail of mithun is long and reaching up to the hock joint with a black or white tail switch. Legs are compact and proportionate in size, set well apart like beef cattle.

Semen collection and evaluation in mithun

Mithun breeds naturally under the free-range system, very shy, and usually do not exhibit reproductive and mounting behavior under the semi-intensive system. Similarly, mithun cows also do not exhibit a prominent sign of standing estrus. Initially, mithun semen was collected by rectal massage technique. In this method, seminal vesicles are massaged centrally and backwardly for 5 min followed by the gentle milking of ampullae one by one for 3-5 min, which results in erection and ejaculation. During collection, the initial transparent secretions are discarded and neat semen drops are collected in a graduated test tube with the help of a funnel. The massage method does not yield the optimum quality of semen in terms of volume, sperm concentration, motility, and freezability of semen. It consists of a large number of dead and damaged spermatozoa.

Mithun bulls can also be trained for mounting over the dummy (estrus mithun cows or non-estrus cows with a sprinkling of estrus female urine). ICAR-National Research Centre on Mithun standardized the technique of bull training for the collection of semen and freezing. However, it is very difficult to train the bull for mounting over dummy and semen collection by the AV method. It is even more difficult for older bulls and bulls with poor libido. Therefore, for semen collection electro-ejaculation

is the better alternative for germplasm conservation. Through electro-ejaculation, semen can be collected from older bulls, bulls with poor libido, and bulls reared under a free-range forest ecosystem. Electro-ejaculation is particularly suited for captive species and an estrous female is not required to stimulate ejaculation. It is regularly used in bulls and rams without sedation or anesthesia.

Electroejaculation is of great value by providing a means of extending the use of valuable sires and also for routine estimation of the fertility of bulls. This technique also gives access to mithun bulls reared under a free-range system. It saves the time and labour needed to train the bull to an artificial vagina and eliminates the risk to the operator and his assistants. These factors, coupled with advances in electronics, have enabled the development and wider usage of the electro ejaculator, which is giving satisfactory and reliable results with no undesirable side effects.

The choice of collection method depends on the circumstances, bull response, bull behavior, and technician confidence. Electroejaculation and Artificial Vagina are the methods most often used for semen collection in free-range or semi-intensive animals.

Semen collection methods

1. Artificial vagina method

The artificial vagina method is the desirable method of collection as it allows the evaluation of a natural ejaculate and also has the additional advantage of assessment of the natural mating ability of the bull. However, it is also recognized that for a majority of mithun bulls as well as beef and dairy bulls, semen collection is only possible using either massage and/or electroejaculation methods. The massage

Training of Mithun bulls

Collection of semen through Artificial Vagina (AV) method in Mithun

method has a limitation when large numbers of bulls are to be collected therefore become impractical.

2. Massage Method

In this technique, semen was collected by rectal massage of ampullae along with seminal vesicle and pelvic urethra. The focus should be given more to the ampullae. This technique has been successfully used in mithun bulls.

Collection of semen through Rectal massage (RM) method in Mithun

3. Electro-ejaculation method

The Electro-ejaculation technique is highly successful for mithun semen collection. More than 90 percent of mithun bulls respond to electro-ejaculation. However, the semen quality may vary from bull to bull or at different time intervals in the same bull. As poor semen samples can be the result of poor collection technique, extended sexual inactivity, or contamination (especially urine). Successive samples of semen may be collected at 5-15 minute intervals by electro-ejaculation. It is observed that the quality of ejaculate improved upon subsequent collection.

Collection of semen through Electro-Ejaculation (EE) method in Mithun

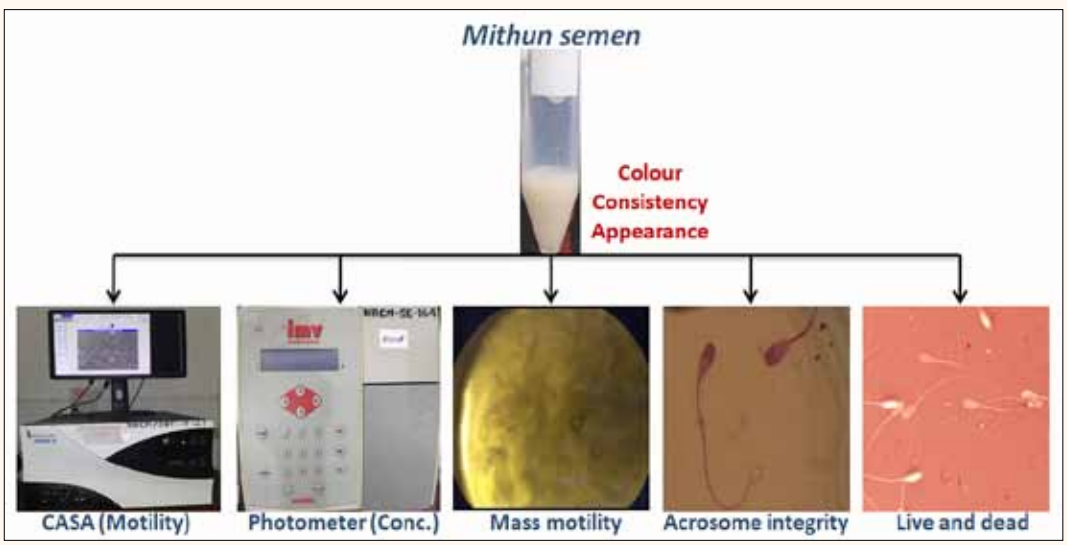
Semen evaluation

- Mithun semen is collected from the young, fertile bull by AV (Artificial Vagina) or electro-ejaculation method.
- Just after collection, semen is brought to the laboratory at 35 ^{o}C for evaluation.
- Semen volume, pH, sperm concentration, mass motility, individual motility, live sperm count, abnormal sperm percentage are checked.
- Semen samples that meet the standard criteria are frozen and can be kept for several years inside liquid nitrogen without any change in fertility and utilized for AI.

Seminal characteristics (mean ± SEM) of fresh ejaculates collected through Rectal massage (RM), Artificial vagina (AV), and Electroejaculation (EE) methods

Semen attributes	RM Method	AV Method	EE Method
Volume (ml)	1.85±0.72[a]	6.17±0.13[b]	5.04±0.16[c]
Colour	Watery to Milky white	Milky to creamy white	Milky to creamy white
Consistency	Thin	Thick and creamy	Thick
Concentration (x10^6/ml)	349.83±3.44[a]	687.63±5.60[b]	643.10±14.33[c]
Mass activity (0-5 Scale)	2.46±0.09[a]	4.53±0.09[b]	3.13±0.12[c]
pH	6.63±0.30	6.60±0.03	6.61±0.02
Individual motility (%)	75.60±0.64[a]	80.06±1.04[b]	76.23±0.80[a]
Liveability (%)	79.66±0.64[a]	82.53±0.96[b]	80.26±0.72[a]
Acrosomal integrity (%)	73.66±0.87[a]	82.50±0.85[b]	74.93±0.45[a]
Plasma membrane integrity (%)	55.50±0.54[a]	80.90±2.54[b]	76.50±0.55[b]
Total sperm abnormality (%)	11.40±0.46[a]	7.83±0.27[b]	9.90±0.39[c]
Nuclear Integrity of spermatozoa (%)	83.06±0.35	82.40±0.40	80.70±2.53

a, b, c means significant within rows, P<0.05



Semen evaluation

Cryopreservation of mithun semen

Mithun semen cryopreservation is of utmost necessity for the conservation and wider distribution of superior quality genetic material keeping in view the hilly terrain and inaccessible areas of mithun habitat. In addition, the recent initiatives to popularize mithun as an economic beef animal or component of an alternative animal production system by rearing under a semi-intensive system demand the adoption of the controlled breeding programme. In this context, effective semen cryopreservation protocol has been standardized for mithun to adopt artificial insemination (AI).

Cryopreservation by conventional or controlled freezing

After semen collection and evaluation, it is diluted with tris egg yolk citrate glycerol (TEYG) or a commercial extender. Before freezing, the fresh semen samples are analyzed for initial progressive motility, live sperm count, acrosomal integrity and hypoosmotic sperm swelling test (HOST).The ejaculates with ≥ 70 % progressive motility, ≥ 2.5 mass activity and ≥ 500 millions/mL concentration are considered for freezing. Tris-egg yolk extender is prepared by dissolving 3.025 g tris buffer, 1.67 g citric acid and 1.25 g fructose in 50 mL of distilled water by stirring and the volume is adjusted to 73 mL with distilled water. To this, the sterilized mixture of egg-yolk and glycerol is added @ 20 % and 7 %, respectively. If a commercial extender is employed, it is diluted according to the manufacturer's instructions. The freshly collected semen is diluted with an extender to make the final sperm concentration of 80 million/mL.

Conventional freezing

The extended semen is packed and sealed into a 0.5 mL straw and cooled up to 5 °C by keeping them in a cold handling cabinet for 60 min. Once the temperature reached 5 °C, the semen straws are equilibrated for 4 h. Conventional freezing is performed using an isothermal box filled with liquid nitrogen (LN_2). Semen straws are placed horizontally 4 cm above the LN_2 levels to expose LN_2 vapours for 10-12 min. After initial freezing on LN_2 vapours, the straws are immersed into liquid nitrogen for storage.

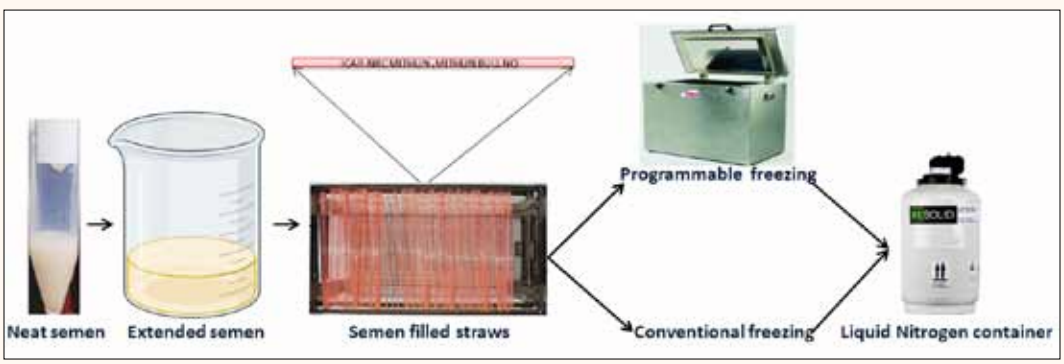
Controlled freezing

In the control freezing system, the straws are placed evenly on racks inside the bio-freezer. The bio-freezer equipment is programmed with the following rate: 5 °C/min from 5 to -10 °C, 20 °C/min from -10 to -100 °C and 40 °C/min from -100 to

-140 °C. After initial freezing in the bio-freezer, the straws are immersed into liquid nitrogen for storage.

Steps involved in cryopreservation of mithun semen

- The tube containing the freshly collected semen is recorded for volume, initially diluted in a 1:1 ratio with an extender placed in the thermos (35 °C) before transferring to the laboratory in a thermos. The collection tube remains capped until processed.
- The 1:1 diluted semen is kept in a thermo-controlled water bath at 35 °C under Laminar Air Flow Unit.
- After examination of sperm concentration and initial motility, the semen is diluted further (final sperm concentration of 80 million/mL) after 7 minutes of cooling at 20 °C with extender maintained at the lab temperature.
- Filling and sealing of semen into sterile straws is done under Laminar Air Flow Unit. The filling nozzles and rubber tubing used are always fresh.
- The freezing is carried out as per the above-described protocols using a biological freezer or conventional freezer. Figure represents the processing and cryopreservation of mithun semen



Processing and cryopreservation of mithun semen

Number of semen straws cryopreserved from 2015 to 2021

Particulars	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22	Total
Mithun semen straws	800	1200	1500	1000	1000	900	1000	**7400**

Number of straws distributed

Particulars	Number of frozen straws
Nagaland Livestock Development Board (NLDB)	50

Mithun Bulls, phenotypic and seminal characteristics

Bull no. 001

Date of birth - 10/3/2010, **Bodyweight** – 645 kg

Phenotypic characteristics

The Mithun bull is heavy with a well proportioned compact muscular body. The head is 'V' shaped with broad frontal bone and horns that emerge from the sides of the frontal bone are massive at the base and pointed outwards. The bull has a prominent broad-based muscular crest (dorsal ridge) along with well developed muscular neck. The dewlap is less pendulous which is thick and muscular with folds. The colour of the body coat is piebald (a mixture of black and white), with a white forehead, black muzzle, and white leg stockings. This bull is sturdy with legs set well apart.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	5±0.62	Total motility (%)	78.5±4.2
Colour	Watery to Milky white	FPM (%)	52.5±3.6
Concentration (x10^6/ml)	800±45.44	VAP (µm/s)	125.6±8.1
Mass activity (0-5 Scale)	3.46±0.1	VSL (µm/s)	90.25±5.7
pH	6.5±0.40	VCL (µm/s)	242±10.1
Individual motility (%)	72.60±5.6	ALH (µm)	10.75±1.2
Liveability (%)	78.66±4.4	BCF (Hz)	24.5±2.2
Acrosomal integrity (%)	72.86±2.3	Straightness (%)	72±1.5
Plasma membrane integrity (%)	67.50±4.8	Linearity (%)	38.6±2.2
Total sperm abnormality (%)	7.40±0.26	Rapid velocity (%)	78.2±3.6
		Static velocity (%)	14±1.5

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 028

Date of birth – 30/8/2013, **Bodyweight –** 605 kg

Phenotypic characteristics

The Mithun bull is sturdy with a well-proportioned compact body. The head is broad with well-developed frontal bone, wide nostrils, horns that emerge from the sides of the frontal bone moves straight backward which are massive at the base and pointed outwards. The colour of the horn is white at the base and black at the end. The bull has a prominent lean dorsal ridge along with a muscular neck. The dewlap is pendulous which is thick and muscular with folds. The colour of the body coat is jet black with an ash forehead, black muzzle, and characteristic white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	4.2±1.3	Total motility (%)	80.2±6.3
Colour	Watery to Milky white	FPM (%)	65±10.2
Concentration (x10^6/ml)	787±12.04	VAP (µm/s)	132.2±5.7
Mass activity (0-5 Scale)	2.1±0.02	VSL (µm/s)	100.1±8.1
pH	7.0±0.25	VCL (µm/s)	260±5.3
Individual motility (%)	65±6.8	ALH (µm)	9.2±2.1
Liveability (%)	76.32±6.7	BCF (Hz)	24.35±4.5
Acrosomal integrity (%)	76.03±5.9	Straightness (%)	70±2.3
Plasma membrane integrity (%)	59.6±6.1	Linearity (%)	40.2±3.7
Total sperm abnormality (%)	8.89±1.62	Rapid velocity (%)	65±4.2
		Static velocity (%)	21±2.7

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 247

Date of birth - 17/7/2012, **Bodyweight** – 580 kg

Phenotypic characteristics

The compact muscular body with rippling muscles is the main feature of this Mithun bull. The massive horns emerge at the right angle to the frontal bone which remains straight and pointed at the tip. The colour of the horn is white at the base and black at the end. The muscular crest (dorsal ridge) is prominently visible along with a thick muscular neck. The dewlap is largely pendulous which is thick and muscular with folds. The colour of the body coat is jet black with a shaded ash colour forehead and characteristic white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	3.6±2.5	Total motility (%)	85.66±4.2
Colour	Watery to Milky white	FPM (%)	72.5±6.8
Concentration (x10^6/ml)	752.1±35.65	VAP (µm/s)	120.9±10.1
Mass activity (0-5 Scale)	2.7±0.2	VSL (µm/s)	95.7±2.2

Semen attributes	Values	CASA Parameters	Values
pH	6.9±1.1	VCL (µm/s)	211.2±15.5
Individual motility (%)	63±5.1	ALH (µm)	8.3±1.8
Liveability (%)	80.64±3.7	BCF (Hz)	33.5±2.6
Acrosomal integrity (%)	84.6±2.3	Straightness (%)	79.2±1.1
Plasma membrane integrity (%)	65.35±4.9	Linearity (%)	47±3.3
Total sperm abnormality (%)	7.9±1.35	Rapid velocity (%)	90.2±3.7
		Static velocity (%)	6±3.5

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 107

Date of birth – 22/5/2012, **Bodyweight** – 528 kg

Phenotypic characteristics

The Mithun bull is having a small compact muscular body. The head is 'V' shaped and horns that emerge from the sides of the frontal bone are massive at the base and curve by pointing outwards. The bull has a prominent dorsal ridge along with a muscular neck. The dewlap is less pendulous which is thick and muscular with folds. The colour of the body coat is jet back, with an ash forehead, black muzzle, and white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	5.2±3.6	Total motility (%)	82.7±6.5
Colour	Watery to Milky white	FPM (%)	65.5±4.6
Concentration ($x10^6$/ml)	600±25.3	VAP (µm/s)	121.8±5.2
Mass activity (0-5 Scale)	2.5±0.1	VSL (µm/s)	83.66±3.5
pH	7±0.2	VCL (µm/s)	213.9±6.5
Individual motility (%)	70±3.4	ALH (µm)	8.6±1.2
Liveability (%)	75.26±2.8	BCF (Hz)	34.2±1.7
Acrosomal integrity (%)	82.3±3.1	Straightness (%)	89±2.5
Plasma membrane integrity (%)	55.6±6.7	Linearity (%)	48±1.1
Total sperm abnormality (%)	6.8±2.75	Rapid velocity (%)	85±2.5
		Static velocity (%)	6.3±2.0

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 297

Date of birth – 10/11/2015, **Bodyweight** – 555 kg

Phenotypic characteristics

The Mithun bull is active with a well-built body. The head is 'V' shaped and horns that emerge from the sides of the frontal bone and curve by pointing outwards. The dorsal ridge is lean along with the muscular neck and the dewlap is less pendulous. The colour of the body coat is jet back with grey shade on the back, ash forehead, black muzzle, and white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	4±4.2	Total motility (%)	86.5±5.4
Colour	Watery to Milky white	FPM (%)	67.5±5.6
Concentration ($x10^6$/ml)	590±12.5	VAP (µm/s)	138.15±8.2
Mass activity (0-5 Scale)	2±0.3	VSL (µm/s)	95.1±3.5
pH	6.9±0.24	VCL (µm/s)	252.15±10.2
Individual motility (%)	59.5±5.4	ALH (µm)	9.75±2.2

Semen attributes	Values	CASA Parameters	Values
Liveability (%)	72.3±4.5	BCF (Hz)	29.7±1.5
Acrosomal integrity (%)	80.2±4.1	Straightness (%)	70.5±2.8
Plasma membrane integrity (%)	60.2±3.7	Linearity (%)	40±1.6
Total sperm abnormality (%)	12.5±3.2	Rapid velocity (%)	84.5±1.8
		Static velocity (%)	8.2±1.1

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 285

Date of birth – 5/12/2015, **Bodyweight** – 510 kg

Phenotypic characteristics

The Mithun is well built with lean muscle mass. It has the characteristic white face and chin. The horns are massive that emerge from the sides of the frontal bone and curve by pointing outwards. The dorsal ridge is lean along with the muscular neck and the dewlap is less pendulous. The colour of the body coat is jet back with a slight grey shade on the back, white forehead, white muzzle, and white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	4.5±1.1	Total motility (%)	95±6.1
Colour	Watery to Milky white	FPM (%)	74.66±2.5
Concentration (x10^6/ml)	530±25.3	VAP (µm/s)	114.46±2.6
Mass activity (0-5 Scale)	2±0.1	VSL (µm/s)	87.2±4.1
pH	6.85±0.42	VCL (µm/s)	237.6±8.2
Individual motility (%)	65±6.7	ALH (µm)	10.3±1.1
Liveability (%)	80.3±2.3	BCF (Hz)	25.8±1.8
Acrosomal integrity (%)	86.3±4.7	Straightness (%)	77±3.4
Plasma membrane integrity (%)	50.5±8.6	Linearity (%)	37.33±2.2
Total sperm abnormality (%)	7.9±2.2	Rapid velocity (%)	92.33±3.7
		Static velocity (%)	4.5±3.3

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 294

Date of birth – 16/5/2015, **Bodyweight** – 525 kg

Phenotypic characteristics

The Mithun is well built with lean muscle mass. It has the characteristic white face and chin. The horns are massive that emerge from the sides of the frontal bone and curve by pointing outwards. The dorsal ridge is lean along with the muscular neck and the dewlap is less pendulous. The colour of the body coat is jet back with a slightly grey shade on the back, white forehead, white muzzle, and white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	4.0±2.3	Total motility (%)	81.14±4.3
Colour	Watery to Milky white	FPM (%)	57.5±6.7
Concentration (x10^6/ml)	700±10.6	VAP (µm/s)	127.63±4.8
Mass activity (0-5 Scale)	2.2±0.2	VSL (µm/s)	86.2±2.5

Semen attributes	Values	CASA Parameters	Values
pH	6.9±0.2	VCL (µm/s)	281.66±5.1
Individual motility (%)	55±5.5	ALH (µm)	11.86±2.1
Liveability (%)	78.5±2.5	BCF (Hz)	22.6±1.7
Acrosomal integrity (%)	83.6±1.5	Straightness (%)	68.66±3.2
Plasma membrane integrity (%)	48±4.1	Linearity (%)	31.66±4.3
Total sperm abnormality (%)	9.3±1.2	Rapid velocity (%)	72.66±2.5
		Static velocity (%)	20.66±1.9

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Bull no. 347

Date of birth – Purchased (Approx. 6 yrs), **Bodyweight** – 510 kg

Phenotypic characteristics

The young mithun is having a small compact body. The horns are small in length with a broad base that emerges from the sides of the frontal bone with the tapering end. The dorsal ridge is lean and non-prominent and the dewlap is pendulous. The colour of the body coat is jet back with slight grey shades on the ridge and back region, ash forehead, black muzzle, and white leg stockings.

Seminal characteristics (mean ± SEM) of fresh ejaculates

Semen attributes	Values	CASA Parameters	Values
Volume (ml)	4.2±1.6	Total motility (%)	75.5±6.1
Colour	Watery to Milky white	FPM (%)	60.2±5.2
Concentration ($x10^6$/ml)	620±30.5	VAP (µm/s)	117.3±2.3
Mass activity (0-5 Scale)	2.5±0.1	VSL (µm/s)	91.85±1.2
pH	6.95±0.4	VCL (µm/s)	262.11±3.5
Individual motility (%)	65±5.6	ALH (µm)	11.86±2.1
Liveability (%)	72.6±4.2	BCF (Hz)	24.35±1.6
Acrosomal integrity (%)	76.2±5.7	Straightness (%)	79.42±4.9
Plasma membrane integrity (%)	55±3.5	Linearity (%)	36.85±3.6
Total sperm abnormality (%)	10.3±1.2	Rapid velocity (%)	80.42±1.8
		Static velocity (%)	16.14±2.5

Forward progressive motility (FPM), average path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), amplitude of lateral displacement of the sperm head (ALH) and beat cross frequency (BCF).

Artificial Insemination (AI) in Mithun

Artificial insemination (AI) is the technique in which semen is collected from the superior bulls and introduced into the female reproductive tract at the proper time with the help of instruments. Artificial insemination (AI) in mithun is a high-potential assisted reproductive technology, which facilitates the propagation of elite germplasm. The major advantage of AI over natural service is that it facilitates rapid genetic improvement by allowing the use of elite bulls and control of venereal diseases. For AI, semen is most commonly collected using an artificial vagina from mithun bulls. Electroejaculation is an alternative method used in bulls that fail to mount or are too fractious for easy handling.

Advantage of AI

1. Maximum utilization of superior males. Through natural mating, a bull can mate to 100 – 150 cows whereas, in AI, a bull can inseminate about 1500 – 2000 cows.
2. Genetic improvement of a herd by insemination using superior bulls.
3. Reduces the incidence of sexually transmitted diseases.
4. Overall growth rate and productivity of the herd will improve.

An ideal time for AI in Mithun

The estrus period in Mithun has a wide range of 36 h to 72 h, as a thumb rule, animals coming in heat in the morning should be inseminated in the next morning and those coming in heat in the evening should be inseminated in the next evening. Ideally, animals should be inseminated 24 h after the onset of heat symptoms at least two times at an interval of 12 h apart.

The procedure of AI in Mithun

In Mithun, the best method of insemination is the "Recto vaginal method of insemination"

- The Mithun in heat will be restrained in the Travis.
- The inseminator will get ready by wearing a plastic apron and gloves.
- The frozen semen straw after thawing (keeping the semen straw in 37 ^{0}C water for a minute to convert frozen semen into liquid) is loaded in a sterilized AI gun covered with a plastic sheath.

- The vulval region of the animal should be sterilized using the potassium permanganate disinfectant (1:1000) and wipe dry with a paper towel.
- The inseminator will insert the lubricated gloved hand into the rectum and back rack the animal to remove the dung, the hand will be further extended to catch hold the cervix through the rectal wall.
- The AI gun loaded with semen straw is passed through the vulva to 'vagina and cer-vix and observed with the hand in the rectum that the AI gun reaches the cervix, then the semen is deposited by injecting the gun, and after depositing the semen the gun is removed, the empty straw and sheath are discarded.

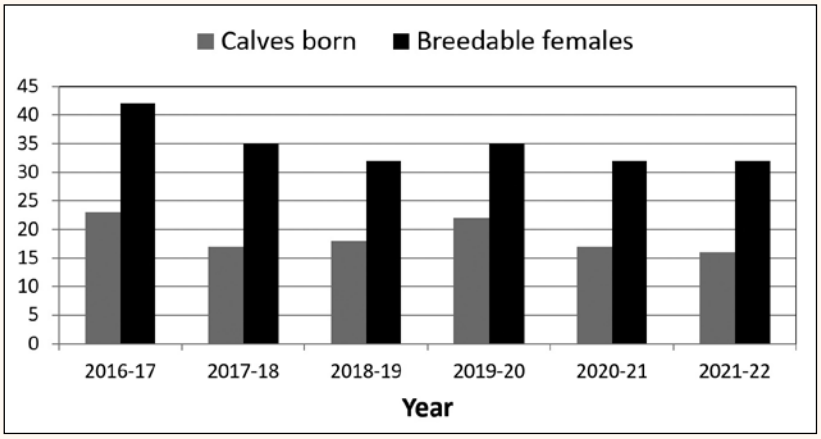
Estrous Synchronization and Fixed Time AI (FTAI) in Mithun

Synchronization of estrus has the potential to shorten the calving season, increase calf uniformity, and enhance the possibilities for using AI. The primary obstacle in synchronizing estrus and achieving optimum pregnancy rates in suckled mithun cows is overcoming postpartum anestrus. Numerous estrous synchronization protocols using PGF2α, GnRH, and/or progestin have been developed that induce cyclicity and successfully synchronize estrus in cattle. To further enhance the use of estrous synchronization, the protocols need to limit time and labor, which can be achieved by using protocols that minimize or eliminate the detection of estrus by employing fixed-time AI (FTAI). The development of FTAI protocols that eliminates estrus detection is an attractive reproductive management tool. One of the limiting factors in the application of artificial insemination (AI) in mithun is the difficulty in estrus detection. A small number of females exhibit estrus behavior, the signs of estrus are discrete, and it occurs mainly at night. Therefore, the use of hormonal protocols associated with FTAI makes a reproduction in these animals more advantageous and practical, especially during seasonal anestrus. FTAI is also convenient from the farm management point as we can plan the calving according to the availability of green fodder and favorable season. Co-synch protocol is used for estrus synchronization of mithun cows and heifers after at least 65 days of parturition in pluriparous animals and after attaining maturity (>24 to 30 months) in case of heifers. In the Co-synch protocol (PGF2α is administered 7 d after GnRH followed by a second GnRH injection and FTAI at 48 h).

Artificial insemination in institute mithun farm

The shy nature of mithun bull and a less pronounced estrous sign of mithun female make semen collection and artificial insemination (AI) in mithun a tiresome task. Nevertheless, AI is an important step for the implementation of any genetic improvement programme. ICAR-NRC on standardized the semen collection using artificial vagina and electroejaculation and developed a protocol for the freezing of mithun semen using a controlled bio-freezer. To address the problem of detection of estrus in mithun, successfully validated the estrus synchronization protocol ('Ovsynch') for timed AI. From the year 2016 onwards, implemented estrus synchronization and 100% AI in the Mithun farm.

Artificial Insemination (AI) in Mithun



Graphical representation of the number of calves born through timed AI

AI calves born at Institute Mithun farm, Medziphema

Artificial insemination in field

Particulars	Number of mithun synchronized	Appeared in estrus	AI
1. Molvom village, Dimapur, Nagaland	10	06	06
2. Gidemi village, Phek, Nagaland	05	05	05 (3 calves born)




Azadi Ka Amrit Mahotsav

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किसानों का हमसफर
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