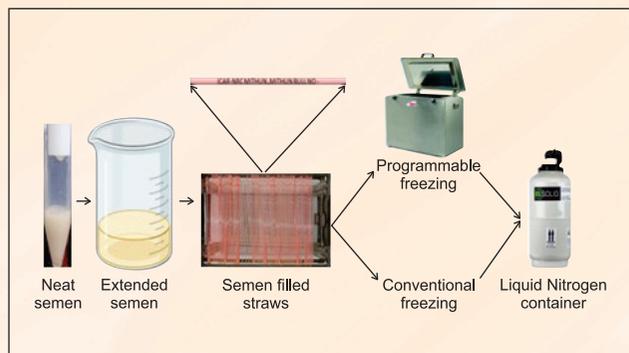


Mithun semen evaluation



Mithun semen processing and cryopreservation

# MITHUN SEMEN COLLECTION & CRYOPRESERVATION

## Cryopreservation of semen

1. The collection tube remains capped until processed.
2. The 1:1 diluted semen is kept in a thermo-controlled water bath at 35°C under laminar air flow unit.
3. After examination of sperm concentration and initial motility, the semen is extended further after 7 minutes of cooling at 20°C with dilutor maintained at the lab temperature.
4. 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.
5. The freezing is carried out as per the recommended protocols for freezing cattle and buffalo semen using a biological freezer or conventional freezer.
6. 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 (37°C) before transferring to the laboratory in a thermos

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Artificial insemination (AI) in mithun is a high potential assisted reproductive technology, which facilitates the propagation of elite germplasm. The advantage of AI over natural service is that it facilitates rapid genetic improvement by allowing the use of elite bulls. 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 (e.g. free-range mithun bulls).

## Semen collection

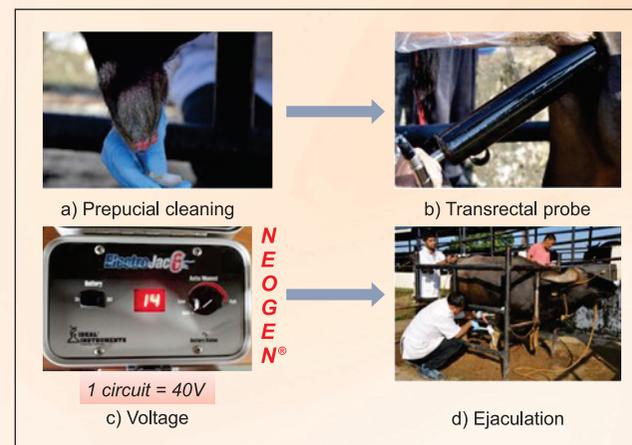
### Artificial vagina method

Semen collection from mithun bulls using AV requires three people: to handle the estrus female or teaser bull, to control bull, and to collect semen. An estrus female or teaser bull is used as a mount animal, it should be a calm and of the appropriate size for the bull being collected. The collection area should have a non-slippery floor to avoid injuries and for efficient ejaculation. The rear quarters of the estrus female or teaser are washed with a disinfectant before collection. The bull's preputial hairs are clipped in preparation for using the AV. The sanitary precautions are intended to minimize microbial contamination of the semen being collected. Mithun are typically collected 2 or 3 times per week, with 2 or 3 ejaculates per collection day. The artificial vagina (AV) uses thermal and mechanical stimulation to stimulate ejaculation. The AV consists of a rubber cylinder, rubber liner, rubber cone, and semen collection tube. AV is assembled and the liner of the AV is filled with water at 42-46°C, and the inner surface is lubricated with K-Y jelly. An insulating cone is placed over the end from which the collection tube protrudes to avoid subjecting the semen to temperature shock. False mounting is an effective way to sexually stimulate the bull, providing two false mounts with two minutes of active restraint and one additional false mount maximizes the volume and concentration of

sperms. During the third or fourth mount, the shaft of the penis is diverted into AV, and semen is collected successfully after thrust by the bull.

### Electroejaculation method

Electroejaculation is an adequate alternative method used in mithun bulls that fail to mount and from free-range bulls. The bull's preputial hairs are clipped in preparation for electroejaculation, cleaned with a disinfectant, and dried using paper towels. A gloved and lubricated hand is inserted transrectal to evacuate faeces and to sexually stimulate the bull. An electroejaculator (EE) is used to collect semen. The probe is inserted and controlled by a trained technical person; another person controls an EE remote. The third person handles the collection cone to collect semen from the bull. The EE will be set on automatic mode; the electric current of a low volt is passed into the probe. After insertion of the rectal probe, EE is switched on and continued until ejaculation. The initial clear watery discharges are avoided and thicker milky ejaculates are collected. The animals which failed to ejaculate in single stimulation are subjected to second stimulation. The semen collection by this method is easy and of lower quality compared to AV.



### Rectal massage method

The rectal massage method is the simplest technique of semen collection by massaging the seminal vesicles and ampullae of vas deferens by trained personnel. It is useful in lame animals and bulls that fail to mount. After the preparation of bulls, a gloved and lubricated hand is inserted transrectal to evacuate faeces and to sexually stimulate the bull. The seminal vesicles are massaged centrally and backwardly for 5 min followed by the gentle milking of ampullae one by one for 3 to 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. If collected regularly, the bulls may become accustomed to this technique in 3-4 weeks.

### Semen evaluation

Semen is evaluated grossly for volume, colour, consistency, and abnormal appearance. The presence of small "clots" or blood can indicate inflammatory conditions of accessory glands or genital tract. Mithun semen is evaluated for following parameters before cryopreservation.

Parameter	Normal values
Colour and consistency	Milky white and thick
Ejaculate volume	3-5 ml
Sperm concentration	600 - 800 million/ml
Total sperm per ejaculate	3-4 billion
Mass motility	> 3 (Scale of 1 - 5)
Progressive motility	>70%
Live and dead sperm count	>80% live
Morphology	>80% normal

The microbial examination, biochemical, metabolic tests, and physical test (resistance to cold and heat shocks) are additional evaluation test performed.