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# Reproduction in the Female Mithun

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## Abstract

Mithun is a domesticated beef bovine species in Himalayan foothills of Southeast Asia. It inhabits at an altitude from 300 to 3000 m MSL, often under extensive grazing system. Mithun is a very fertile animal, able to produce one calf/year. Mithun can interbreed with other bovine species, but male offspring are sterile. This chapter intent is to gather and discuss available information on several aspects of reproduction in female Mithun. The morphology of the reproductive organs is different from cattle and has a longer reproductive tract and pregnancies compared to cattle. Although its estrus period is longer than in cow, Mithun usually displays silent heats, requiring a breeding bull for heat detection. Seasonal fluctuations on reproductive pattern have been reported, which are related to forage availability and quality. Calving in summer takes longer to resumption of cyclic ovarian activity than calving in winter. As Mithun is an important socio-cultural-religious-economic asset in India, to counter some short comings, new breeding strategies have been introduced like multiple ovulation and embryo transfer technology. A good understanding of different aspects of reproduction is crucial to support effective reproductive managements to enhance socio-economic status and cultural importance of tribals to preserve Mithun germ-plasm to be used in future.

**Keywords:** reproductive traits, reproductive problems, biometry of the female genitalia, reproductive physiology, assisted reproduction

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## 1. Introduction

Mithun is a unique domesticated bovine species raised in the Himalayan foothills of South/Southeast Asia. In India, they can be found in the North Eastern hilly regions, such as Arunachal Pradesh, Manipur, Mizoram, and Nagaland. It is commonly believed that Mithun originated more than 8000 years ago, from the wild Indian Gaur, *Bos gaurus* [1, 2]. The spelling

word such as Mithun (exact in pronunciation) is commonly practiced for the hilly bovine species (*Bos frontalis*); however, “Mithan” is also a common pronunciation and spelling while in some other cases, “Mythun” is another term used. Mithun are also known as “Gayal” in the Indian subcontinent. In Myanmar and Bhutan, it is called Mithan, whereas in China it is called Dulong cattle. This name probably originated from Assamese dialects.

The massive unique beautiful animal is anatomically and physiologically well adapted to altitudes ranging from 300 to 3000 mMSL. Mithun is a social animal; they establish small groups, usually containing one adult male and several females and juveniles. The multiple purpose use of Mithun is well acknowledged. It is a potential source of delicious meat [3], and it can also be used for the improvement of local, native cattle breeds. Mithun is a recent addition to the field of scientific animal husbandry. In the future, foreign exchange could be earned by exporting Mithun’s products, namely meat, milk, hide, or skin. The Gayal or Mithun is larger and heavier compared with native domestic cattle breeds. Mithun hide has almost three times the size and thickness that of native cattle [4].

Human involvement and the manipulation of biodiversity severely affected Mithun’s habitats and increase the pressure that drove them deep into the jungle, in areas farther into the border [5]. Even though Mithun is not yet considered an endangered species, it has been subjected to severe noncyclic population fluctuations on a local or national or regional basis. Mithun population is decreasing gradually, mainly due to the local unavailability of certified breeding bulls, the increase of inbreeding practices, the decline in the grazing land, and also because a suitable breeding and feeding management is still to be established in Mithun rearing areas.

A good understanding of the reproductive processes in Mithun cows and its interaction with the environmental factors is crucial to implement effective reproductive management measures, to preserve its population, and to enhance the socio-economic status and the cultural importance of Mithun in the local tribal populations. Hence, a holistic approach from all areas of animal husbandry and veterinary programs will allow to exploit the production potentiality of Mithun. The present review aims to bring the different aspects reproductive physiology of Mithun in concise manner for the benefit of Mithun farmers, breeders and technicians.

## 2. Taxonomical classification of Mithun

Based on the available literature, the taxonomy of Mithun is presented below [6]:

- Kingdom: Animalia
- Phylum: Chordate
- Sub-phylum: Vertebrata
- Class: Mammalia (mammals)

- Sub-class: Eutheria (placental mammals)
- Order: Ungulate (hoofed mammals)
- Sub-order: Artiodactyla (even-toed animals)
- Pecora/Ruminantia (true ruminants)
- Family: Bovidae (hollow-horned animals)
- Sub-family: Bovinae
- Genus: *Bos*
- Species: *frontalis*
- Scientific Name: *Bos frontalis*

### 3. General description

There is no clear-cut historical record on the origin and domestication of Mithun. European studies documented, for the first time, the existence of this animal during the nineteenth century. The history of domestication of this animal has been reconstructed based on the native people's traditions and present daily practices. Native traditions suggest that Mithun is an early domesticated form, descending of related wild animals that were attracted to human beings by its craving for salt. Simultaneously, the human motives focused on obtaining an animal to sacrifice for meat. Three different hypotheses have been proposed on the origin of this mountain animal: (1) Mithun is the domesticated form of the wild Gaur [1, 2, 6, 7]; (2) Mithun is a hybrid of bull Gaur with a zebu cow [8] or either a *B. indicus* or *B. taurus* cow [9]; or (3) Mithun is a descendant of a wild Indian bovine now extinct.

As the male originating from a cross between Mithun and cattle is usually sterile, the second hypothesis can be discarded, since there is little chance for Mithun to be a stable hybrid of Gaur and cattle. Hybridization practices with domestic cattle, however, make it more complicated to understand the origins of domestication and Mithun identification. The third hypothesis is questionable as there is little similarity in the appearance of pure Mithun and common cattle available in Mithun-inhabited areas. Conversely, the physical features of Mithun and Gaur are similar and it is difficult, even for an expert, to differentiate whenever Mithun and Gaur are kept together. Moreover, no sterility barrier exists between Mithun and Gaur, and both animals possess 29 pair of chromosomes. Due to their resemblances, some animal taxonomists had once mistaken the Mithun as a type of domesticated Indian Gaur [10]. The fact is that many morphological, physiological, and reproductive parameters are different in Mithun compared with cattle, strongly support the hypothesis that the Mithun is a domesticated form of wild Gaur; whose domestication occurred some 8000 years ago. Even according to the latest taxonomical classification, both Mithun and Gaur are classified under the same species *Bos* [1, 2, 7]. Two different "breeds" of *B. frontalis* have been reported in the northeastern region of India [11].

The divergence time estimated for *B. gaurus* and *B. frontalis* provide further support in favor of the hypothesis that assumes its origin from an extinct wild progenitor.

Result from genetic studies display contradictory information on the origins of Mithun, which could be related to the existence of different genetic subpopulations that are originated by the habitat fragmentation, which would allow the breed to present different genetics adrift. Baig et al. [12] reported the occurrence of two haplotypes of *B. indicus* and one of *B. taurus* in *B. frontalis*, reinforcing the gene flow occurring between the domestic cattle and *B. frontalis* population. This genetic introgression of domestic cattle poses a severe threat to the diversity of *B. frontalis* in their respective regions [10, 13]. Nevertheless, Shan et al. [14] speculated that Mithun was neither a domesticated type of Gaur nor a descendant of Gaur and cattle crosses; whereas, Lan et al. [15] reported that the restriction type of mtDNA of Mithun was the same as that of zebu, so the maternal origin of Mithun had a close relationship with zebu while Y chromosome of Mithun was the same with Gaur. They speculated that Mithun might be the descendant of male gaur and female zebu. Based on the partial sequences of cytochrome *b* (*Cyt b*) gene, Ma et al. [10] reported that the Mithun was not a crossbreed descendant, but an independent species of *Bos* from *Bos indicus*, *Bos taurus*, and *Bos gaurus*. Li et al. [13] reported based on the complete *Cyt b* gene sequences that Mithun could be grouped into three embranchments: one embranchment clustering with *Bos gaurus*, another clustering with *Bos taurus*, and the third clustering with *Bos indicus*. Phylogenetic analysis indicated that Mithun might be the Gaur in the domesticated form and also that a significant proportion of the Mithun bloodlines in China were mixed with other related species of bovine [16]. Based on the mtDNA and *SRY* gene sequences, Gou et al. [17] reported that the Mithun from the Yunnan province originated from the hybridization between a male *Bos frontalis* and female *Bos taurus* or *Bos indicus*. Tanaka et al. [18] reported based on the mitochondrial *Cytb* gene that the ultimate maternal origin of Mithun was Gaur and indicated that it was directly domesticated from Gaur. Baig et al. [12] argued that the *B. frontalis* should be treated as a separate species/sub-species based on the mitochondrial DNA (mtDNA) analysis, and not merely as a domestic form of *B. gaurus*.

#### 4. Distribution of Mithun

Mithun has a limited geographical distribution. It is mainly found in the tropical rainforest of North-eastern hill States of India (Arunachal Pradesh, Nagaland, Manipur, and Mizoram), with also small numbers of animals in Myanmar, Bangladesh, China, and Bhutan. However, as per the nineteenth Livestock Census of India [19], the total population of Mithun in Indian sub-continent was 297,289 (**Table 1**).

In the moist forests of the northeastern Himalayan foothills, Mithuns are free-ranging animals; as they do not have an established breeding plan, they are sometimes described as semi-domestic animals [20]. The Naga people encourage interbreeding between the wild Gaur bull and their Mithun cows and regard this practice as an improvement on the breed [20, 21]. In east Bhutan and Arunachal Pradesh, Mithuns are particularly prized for sire the local cows (*Bos indicus* type) [20, 22]. In Bhutan, particularly in the eastern regions, the farmers are crossing the Mithun bulls with local Siri females, as the female crossbred offspring present higher milk yields, the milk

Indian states	1997	2003	2007	2012
Arunachal Pradesh	124,194	184,343	218,931	249,000
Nagaland	33,445	40,452	33,385	34,871
Manipur	16,660	19,737	10,024	10,131
Mizoram	2594	1783	1939	3287
Total	176,893	246,315	264,279	297,289

**Table 1.** Trends in Mithun population in North-East India.

is being used mainly for butter and cheese. The Arunachal Pradesh tribes also use Mithun for breeding of local cows. The  $F_1$  hybrids produced, named as Jatsha/Jatsa (male), are very strong and docile and are used for plowing, whereas the females, named as Jatsham/Jatsamin, yield more milk than purebred Mithun or local cows. Jatsham is a valuable dairy stock for these people [20, 22], but Jatsha have fertility problems. Because of male infertility, the Bhutanese farmers use to backcross the female hybrids to domestic cattle bulls for four generations [23]. In the  $F_2$  generation (male Mithun  $\times$   $F_1$  female), the males, called as Nupsa, are used for plowing; whereas the females, called as Nupsamin, are reared for the increased milk production [24].

Mithun is the only wild relative of zebu cattle found in the hilly regions of Chittagong in Bangladesh, especially in the Bandarban district. Some tribal families raise Mithun with native cattle and their cross natural offspring—called “Tang gaur”—can be found in the local markets. In Bangladesh, these animals are mainly used as a high-price sacrificial animal during religious festival [25]. In India, the Mithun female cross is called Phre [26]. In the eastern Himalayas, Mithuns are bred with Dzo (the product of a cross between a yak and a cow) or with yaks. Those crosses evidence the usual hybrid fertility/sterility pattern and therefore are used mainly for traction and milk production (males and females, respectively) [24].

## 5. Biometry of the genitalia of Mithun cow

The reproductive tract of the Mithun cow is longer than the cow's and although lying in the pelvic cavity, it hangs forward into the abdominal cavity. The vulva, the external opening of the urogenital tract, is smaller compared to the cow. The vulvar cleft (the ventral slip between the two labia) nests the clitoris, also less developed than in cattle [27, 28]. The segment showing the most important changes in size is the uterine horns, which is influenced not only by age, but also by parity and the time length elapsed from the last calving [27]. They are usually about 20 cm long in a virgin heifer, but may reach up to 40 cm long in older cows. The uterine horns curve forward, downward, outward, and then upward like the curled horns of a ram. As in cows, the uterine horns are quite firm and erect during estrus, and the intercornual space is very distinct. Regarding the internal morphology of the uterus, about 125–150 ( $125.17 \pm 18.52$ ) caruncles can be observed as small, raised, button-like areas [27]. The ovary is oval-shaped and varied between 1 and 2 cm in diameter. A corpus luteum with a distinct neck is palpable in the ovary between days 8–14 of the cycle; thereafter, neck becomes indistinct



Segment of the genital tract	Measurements			Notes
	Length (cm)	Thickness (cm)	Width (cm)	
Vulva	14.33 ± 3.25 (ventral) 12.50 ± 3.73 (dorsal)	0.20 ± 0.01		
Vagina	21.83 ± 2.16	0.40 ± 0.06		
Cervix	6.00 ± 0.35	2.16 ± 0.18	1.08 ± 0.11	3.17 ± 0.37 annular rings
Uterine body	5.45 ± 0.70	0.49 ± 0.05	1.98 ± 0.41	
Left uterine horn	19.0 ± 2.0	0.43 ± 0.09	0.97 ± 0.32	
Right uterine horn	21.16 ± 2.11	0.47 ± 0.09	2.18 ± 0.30	
Left oviduct	33.42 ± 5.63			Diameter 0.30 ± 0.10 cm
Right oviduct	35.17 ± 5.90			Diameter 0.29 ± 0.09 cm
Left ovary	2.75 ± 0.15	1.58 ± 0.44	1.67 ± 0.42	Weight 4.44 ± 1.20 g
Right ovary	3.02 ± 0.29	1.68 ± 0.45	1.73 ± 0.24	Weight 3.82 ± 0.59 g

**Table 2.** Biometry of the female genital tract in Mithun cows (adapted from [27]).

and at about 21 days of the cycle only a scar of the CL is present [28]. The available data on the reproductive tract biometry is provided in **Table 2**.

## 6. Endocrinology of the estrous cycle

As it occurs in the cow, the progesterone (P4) concentration is the lowest at estrus and gradually increases until day 6 of cycle (day 0 represents the ovulation day) [29]. From day 7 onward, the concentration of progesterone raises sharply to reach a peak on day 11. After this day, progesterone concentrations decrease steadily to reach a minimum at the following day of estrus. Secretory pattern of FSH and LH has shown significant negative relationship with P4 during first and last 6 days of the cycle. A biphasic peak has been reported for both FSH and LH in Mithun cyclic females. Concentration of FSH and LH starts to increase from day 5 and day 6 before estrus, respectively. Then they attain their first peak on day 2–3 before estrus, and thereafter, their levels decrease, and the second peak is observed on the day of estrus. The concentrations of both gonadotropins decrease thereafter to basal level, those of FSH (on day 3 or 4 of the cycle) 1 or 2 days earlier than those of LH (on day 4 or 5 of the cycle) [29].

Dhali et al. [29] reported the existence of two kinds of patterns regarding the estrogen secretion through the estrous cycle of Mithun females. Some cows displayed only one peak of estradiol 17-β ( $18.92 \pm 3.76$  pg/ml) during the entire estrous cycle, on day 4 before estrus. In these females, the FSH peak reported before estrus was significantly lower compared to females

presenting two peaks of estradiol 17- $\beta$ . The first peak ( $20.32 \pm 3.61$  pg/ml) was recorded 4 days before estrus and the second peak ( $19.23 \pm 2.14$  pg/ml) on the day of estrus [29].

The mean length reported for the Mithun estrous cycle is  $21.2 \pm 0.3$  days (19–24 days). The emergence of the first follicular wave has been proposed to occur just after ovulation, while the FSH values are still elevated and the progesterone in low values [29]. The emergence of the second follicular wave would occur approximately at day 14 of the cycle, in association with the raising FSH concentrations that will originate the first FSH peak, along with increasing LH and E2 concentrations. The functional capacity of the CL starts decreasing from day 11 onward and circulating P4 concentration starts reducing. This decrease allows the rising of FSH and, around day 16, the selection of the dominant follicle from the second follicular wave. The deviation of ovulatory follicle was proposed to occur around day 18 of the cycle. The circulatory concentration of E2 starts to decrease with the regression of subordinate follicles. During the late stage of cycle, low concentration of P4 stimulates the rise in LH which helps in the final maturation of ovulatory follicle and triggers ovulation [29].

## 7. Reproductive traits

No breeding programs can be implemented unless the animal has sound reproductive traits. Earlier reports [30, 31] showed that Mithun has a fairly good reproductive performance. Mithun is a polyestrous animal. Females become sexually mature at 2–3 years of age. Mithun bulls become mature at 3–4 years of age. Mithun cows present a high reproductive efficiency, as indicated by the reported calving-related traits (calving interval:  $402.85 \pm 3.04$  days, gestation length:  $296.25 \pm 0.77$  days, birth weight:  $20.85 \pm 0.24$  kg) [32], which permit to obtain one calf in a year. Mithun productive life ranges from 16 to 18 years.

Different studies present slight differences among values respecting the reproductive traits in Mithun compared with local cattle (*Bos indicus*) and buffaloes. **Table 3** summarizes the available information. In general, Mithun requires a higher number of services than indigenous cows when artificial insemination is used, which could be associated to improper heat detection, lack of knowledge of the inseminator about the characteristic morphology of the reproductive organs in Mithun. Also, the concentration of spermatozoa per dose or incorrect insemination may be the cause of more services (artificial insemination) for conception in this animal [34]. The length of pregnancy is in general longer in Mithun than the European cows [4, 36], but it is shorter for Mithun  $\times$  cattle mating ( $281.7 \pm 1.2$  days) [34]. This suggests that purebred Mithun calves needs a little longer to mature than crossbred calves [34].

The average birth weights reported in male and female Mithun calves are presented in **Table 4**. In general, female calves born weighed lighter than the males [40]. These differences are maintained across pregnancies [34] although Haque et al. [39] only found significant effects of parity in the birth weight of female calves.

Seasonal fluctuation in the Mithun reproductive pattern has been described. A higher number of calving (33.3%) occurs during monsoon and autumn seasons followed by 16.7% calving rate was observed during summer and winter seasons [34], reflecting a higher conception rate



Reproductive traits	Mithun	Cattle [33]	Buffalo [33]
Type of estrous cycle	Polyestrous [4, 31, 34]	Polyestrous	Polyestrous
Age at first estrus	598.2 ± 168.4 d [34] 527.9 d [35] 22–39 mo [31]	15 (10–24) mo	21 (15–36) mo
Age at first conception	723 ± 169.9 d [34] 779.70 d [4] 644.8 d [35]		24–36 mo
Weight at first estrus (kg)	247.8 ± 35.1 [25, 34]		250–275
Length of estrous cycle (d)	21.9 ± 2.9 [34] 19–24 [31] 21.65 [4]	21 (14–29)	21 (18–22)
Duration of heat (h)	45.4 ± 12.2 [34] 10–36.9 [31] 42.68 [4]	18 (12–30)	21 (17–24)
Service per conception:	2.30 [4]	1.91	1.3/1.76
Natural breeding	1.4 [34]	1.27	1.15
Artificial Insemination	5.0 [34]	1.40	1.48
Gestation period (d)			
Purebred	296.1 ± 3.9 [34] 282–320 [31] 293.27 [4] (293–303) [36] 296.25 ± 0.77 d [32]	280 (278–293)	315 (305–330)
Mithun × cattle	281.7 ± 1.2 [34] 283 d [35]		
Age at first calving (d)	1014.4 ± 260.3 [34] 1192.50 [4]	30 (24–36) mo <i>B. indicus</i> : 44 mo Crossbred: 34 mo	42 (36–56) mo
Postpartum estrous (d)	96.2 ± 24.0 [34]	Uterine involution —45 d 1st Ovulation—30 (10–110)	35 (16–60) 75 (35–180)
Days open (d)	172 ± 83.5 [34] 203 [4]	55–85	35–185
Calving intervals	349–395 d [31] 402.85 ± 3.04 d [32]	13 (12–14) mo	18 (15–21) mo
Natural service	465 ± 80.5 d [34]		

Reproductive traits	Mithun	Cattle [33]	Buffalo [33]
Artificial insemination	838 ± 158.5 d [34]		
Home region	300–400 days [37]		
<i>Ex-situ</i>	553 days [38]		
Optimum time for service after the onset of heat	21–30 h [34]	Middle of estrus	
Lactation length			315 ± 28.28 d
Productive life			14.3 ± 2.46 y
Lifespan			21.0 ± 3.36 y
Lifetime number of calving			10.4 ± 1.30

The sources are referenced within square brackets.

h—hour; d—days; mo—months; y—year.

**Table 3.** Comparison of Mithun reproductive traits with those of indigenous cattle and buffaloes values are given as average; when available, range values are provided within brackets.

Gender	Average birth weight (Kg)			
	Overall	At 1st lactation	At 2nd lactation	At 3rd lactation
Male calves	24.3 ± 6.9 [39] 21.67 ± 0.15 [32]	19.7 ± 2.1 [34]	21.3 ± 1.5 [34]	23.0 ± 0.0 [34]
Female calves	20.20 ± 4.08 [39] 19.63 ± 0.21 [32]	15.6 ± 1.4 [34]	19.7 ± 2.6 [34]	21.3 ± 1.8 [34]
Nonspecified	19.1 ± 3.3 [35]			

The sources are referenced within square brackets.

**Table 4.** Birth weight in Mithun calves.

in winter (December–February). This could be explained by the availability of green fodder after summer in the period of monsoon, autumn, and winter which improve the body condition and health status of breeding animals [41]. It was also reported that a decreasing trend exists to a shorter postpartum anestrus in Mithun calving in autumn and monsoon seasons (87.3 and 94.2 days, respectively), compared to those calving in summer or winter (158.7 and 174.7 days, respectively) [34]. A retrospective study on the calving trends in semi-intensive farms showed the highest birth rate in September, December, and January, while the lowest rate was recorded in May and June [40].

On respect to the fertile period, it has been demonstrated that a 70.6% successful conception is obtained when the service (natural mounting) is provided at the middle of estrus, within 21–30 h of heat. If artificial insemination is used, a 100% successful conception can be obtained when the insemination occurs within 31–40 h of heat [34].

Correlations were established between the weights at first calving, pregnancy length, the length of the service period and calving interval for Mithun. Negative correlations were high

for the weight at first calving and the service period ( $-0.684$ ) or the calving interval ( $-0.577$ ), but low for the weight at first calving and the pregnancy length ( $-0.138$ ). Moderate positive correlations were found for the pregnancy length and the service period ( $0.475$ ) or the calving interval ( $0.514$ ). A positive high correlation was observed between the service period and calving interval ( $0.881$ ) [31]. Under extensive free-range rearing systems, a suitable practical method for selective breeding is the introduction of tested and superior bulls, in a proportion of 1 bull to 10 breeding females, together with the elimination of bull with poor performances (both productive and reproductive). Efforts should be made to replace breeding bulls preferably once in 5 years to avoid inbreeding depression. Under the semi-intensive system, the female can be detected in heat to be bred with superior bulls either through natural service or artificial insemination [37].

## 8. Estrus behavior and signs

The behavioral signs of estrus most frequently displayed by Mithun cows are accepting the mounting by a Mithun bull, standing to be mounted, and congestion of the vaginal mucosa and swelling of the vulva. In contrast to other bovines [42], estrous signs such as mucus discharge, restlessness, tail raising, frequent urination, and loss of appetite are less pronounced in Mithun cows. The length of estrus is longer in primiparous Mithun cows than in multiparous, due to an elongation of the mounting behavior, Flehmen reflex, and restlessness displayed by younger Mithun females. This allows the primiparous animals to be mounted for more times than the multiparous females. The relative incidences of the behavioral manifestations have been described in Mithun females, and provide a good indicator for distinguishing between primary and secondary estrus signs. Sniffing of the vulva by bulls and standing to be mounted by bulls/other herd mates was recorded in 91.30% each. The increase in the frequency of urination was observed in 82.61% of the female in estrus; alike allowing the bull chin resting on the rump. Only 65.22% of Mithun females in estrus displayed restlessness, which was pronounced in 8.70%, but not pronounced in 56.52% of the females. Bellowing was exhibited by 56.52% Mithun female [42], being frequent in 39.13% of the females, but infrequent in 17.39%. The homosexual behavior during estrus is less prominent in Mithun compared to cows. Seeking for the company of other animals (looking anxiously outside) was found in 56.52% of the Mithun females in estrus. Licking of the body of other animals during estrus was observed in 47.83%, but mounting herd mates during estrus was observed in only 26.09% of females. Reduced food intake or loss of appetite was observed in only 8.70% Mithun females in estrus [43].

During estrus, the vaginal mucosa of Mithun females was recorded as reddish pink (pronounced), pink (moderate), and pinkish (slight) in 34.78, 43.48, and 21.74%, respectively. Edema of vulva was recorded in 82.61% of female Mithuns in estrus, of which pronounced edema and slight edema were recorded in 21.74 and 60.87% of estrus females, respectively. Discharge of the vaginal mucus does not always occur spontaneously in Mithun females in estrus, and has only been reported in 78.26% of the females. In 39.13% animals, the mucus was not discharged spontaneously until rectal palpation was carried out. The quantity of genital mucus was scored as copious, scanty, and absent in 34.78, 43.48, and 21.74% of the females, respectively. The

color of the vaginal mucus varied between transparent (61.11%), steel bluish (22.22%), and whitish (16.67%). Also, the consistency of the vaginal mucus varied from thin (55.56%) to thick (44.44%). The occurrence of foam in the mouth was observed in 30.43% animals [44].

## 9. Pregnancy diagnosis and parturition

For pregnancy diagnosis, the transrectal criteria used in cows also applies in Mithun. The slip of the fetal membranes, distension of the uterine horn, and the presence of CL in the ovary are observed at the earliest by the 6th week of gestation. The pregnant uterus and ovary sink into the abdominal cavity after 3 months of pregnancy, and are not be palpated after 3 months of pregnancy, as described in cattle. The fremitus of the uterine artery is differentiable after 4 months and is much prominent or forceful from 6 months of gestation onwards. Enlargement of the middle uterine artery is observed after 5 months of pregnancy. Pregnancies in the right horn are more frequently observed (60%) in comparison with cattle [45].

The external signs of pregnancy in Mithun are similar to those of cattle, namely the abdominal distension, mammary gland development, and fetal ballottement. The abdominal distension, due to the gradual accumulation of voluminous uterine contents, can be detected after 5 months, but it is prominent only in more advanced stages of pregnancy. The udder development is noticed from 6 months onwards in Mithun heifers, or in the last 1–5 weeks of pregnancy in the case of multiparous cows. The teats are engorged at 12 h prepartum. Edema and relaxation of the vulva, derived from the progressive relaxation of pelvic ligament, is noticeable in the last few weeks of gestation. Three to four days before parturition, the vulvar lips become increasingly droopy and flaccid, along with a marked shrinking of the croup. In 74% of pregnancies, the fetus is balloted or its movement observed through abdominal wall after 6 months of pregnancy [45].

The process of the parturition is more or less similar in Mithun and in cattle. The pregnant mother becomes nervous and tries to leave the rest of the herd just before parturition. They go through the forest area to find a hidden place. They do not come back to the stall and stay in the forest [46]. In Mithun, most of the calving occurs during the night [47]. Signs of impending parturition in Mithun include restlessness, increased micturition at 2–5 min interval and increased walking. Mithun cows give birth in a laying position. **Table 5** summarizes the periparturition behavior in Mithun.

Mithun dams start licking the calf immediately after birth. The calf stands up within 22 min of birth, and first sucks colostrum within 30 min of birth [22]. The average birth weight of the calves, irrespective of sex is found to be 21 kg. The calves stood at their feet an average time of 40 min and the average time for first suckling is to be 50 min on day 1 [49]. During this time period, the dam threats any person or animal that closely approaches the baby. She browses and grazes around her newborn baby so that she can keep a sharp watching to her calf. After 1 or 2 days, she usually comes back to her herd. The calf brows and play around her mother, and feeds of milk 12–15 times in a day. At the 1st week, the Mithun mother is very careful about the safety of her offspring. After 1 week, she gradually returns to her normal and the calf was found moving freely in the herd [25, 34].

Parameters	Groups		
	Nagaland	Arunachal Pradesh	Mizoram
Duration of concentrate feeding (min)	200	199	84
Duration of tree leaves feeding (min)	96	458	538
Duration of drinking (min)	1.06	2.02	2.92
Duration of standing time (min)	807	971	936
Frequency of standing (no.)	12	8	11
Duration of lying time (min)	463.2	373	450
Frequency of lying down (min)	12	8	11
Frequency of defecation (no.)	11	8	8
Frequency of urination (no.)	8	6	8
Duration of parturition process (min)	39.38	63	12.05
Standing on its feet by calf after birth (min)	13.18	18	25.12
First suckling by calf after birth (min)	70.27	68	44.9
Voiding of meconium after birth (min)	28.92	56	1189.1
First urination after birth (min)	443.67	531.5	51.18

**Table 5.** Peri-parturition behavior of Mithun cow and calf [48].

Events of parturition/intervals	Time lapse (h)
Stage I (stage of cervical dilatation)	
Between the onset of labor to exposition of the water bag	156.17 ± 46.71
Stage II (stage of fetal expulsion)	
Between the exposition of the water bag to forelimbs exposure	25.83 ± 8.94
Between the exposition of the forelimbs to exposition of muzzle	12.16 ± 4.54
Between the exposition of the muzzle to exposition of the head	21.75 ± 9.58
Between the exposition of the head to exposition of the shoulder	11.08 ± 4.54
Between the exposition of shoulder and back	0.68 ± 0.39
Duration of the complete expulsion of the fetus	71.52 ± 18.82
Stage II (stage of expulsion of the fetal membranes expulsion)	
Interval between the expulsion the fetus and fetal membranes	199.65 ± 54.75
Total time for completion of the parturition process	427.33 ± 74.48

**Table 6.** Relative length of the events occurring in the parturition of Mithuns (adapted from [47]).

The process of parturition is divided into three stages as in the cattle: stage I (stage of cervical dilatation), stage II (stage of fetal expulsion), and stage III (stage of the expulsion of the fetal membranes) (**Table 6**).

The fetal membranes of Mithun present an average weight of  $2.51 \pm 0.51$  kg, and are  $194.08 \pm 24.97$  cm in length and  $40.67 \pm 2.72$  cm in width [47]. The average number of cotyledons

in Mithun is  $60.08 \pm 5.81$ , distributed as follows:  $75.08 \pm 7.15$  cotyledons in the gravid horn and  $32.50 \pm 6.68$  in the nongravid horn [47].

Dystocia is common cause for perinatal calf mortality in bovine species. In Mithun, the fetuses are generally found in anterior presentation at calving, and dystocia due to fetomaternal disproportion is rare. When due to maternal causes, abnormal misshaped or small pelvic canal leads predispose the cow to dystocia or calving difficulty [50]. The transverse and vertical diameter of the pelvic inlet and the pelvic area are smaller in Mithun than the cattle. Other parameters such as the height between the hip joint and the croup, or the vertical diameter of the pelvic outlet is larger in Mithun compared to indigenous cattle. Besides, Mithun possess a more muscled hind quarter than the indigenous or dairy cattle [51]. Studies on Mithun pelvimetry would be helpful in establishing breeding programs, where a calving easiness could be a useful trait for selection of breeding animals. Also, a complete soundness breeding exam before the female introduction into reproduction would help to reduce the incidence of dystocia, to cull out the females presenting unsuitable pelvis conformation.

## 10. Infertility problems in Mithun

Problems associated with reproduction have rarely been observed in Mithun. However, cases such as anestrus, metritis, dystocia, placental retention, and placentophagy or postpartum anestrus have been reported in Mithun [26, 51, 52]. Similarly, the most common reproductive disorders in semi-intensive Mithun farm of Bangladesh include metritis (16.7%), irregular heat (25.0%), anestrus (8.3%), repeat breeder syndrome (8.3%), abortion (16.7%), cervicitis (25%), and calf mortality (24%) [38].

## 11. Concerns on the sustainability of Mithun populations

Due to the use of continuous inbreeding practices, in part because of the habitat fragmentation and the unavailability of certified sires in its dispersal area and the farmer preferences for a few dominant bulls, a gradual decrease in size, production, and reproductive efficiency has been reported in Mithun. The decline in the population size aggravates the inbreeding depression, and further reduces the breed's reproductive and productive fitness [53, 54], as well as its survivability [54]. When the genetic variation is reduced within a population, its vigor and ability to adapt to environmental changes are also reduced and may compromise the survival of the breed or population. Therefore, urgent action is needed to conserve this precious mountain bovine.

The fragmentation of the Mithun habitat relates with the traditional rearing system, based on free-ranging herds that only allow the grazing of a limited number of animals, in a particular hill pocket, without migration to other locations. Besides, human interactions and manipulation of biodiversity also affect the habitats of Mithuns, forcing them to migrate deeper into the forests, particularly in the further east, toward the border of the country. Deforestation, poaching, and illegal hunting aggravate the reasons for extinction. Due to deforestation and human settlement,



as well as the conversion of the forest land for agricultural and horticultural activities, the total forest area available and its holding capacity for rearing Mithun cattle has been reduced. Mithun prefers high altitude, hilly terrains of high slopes, and dense forest, as it protects against the direct sunlight and high rainfall, where plenty of fodder trees and shrubs are available. But these areas are reducing due to global warming or climatic changes, which decreases the rainfall and consequently the amount of vegetation or fodder to be used by Mithuns.

Another factor that is contributing to the reduction in Mithun numbers is the incidence of infectious diseases, like tuberculosis, para-tuberculosis, brucellosis, foot and mouth disease (FMD), infectious bovine rhinotracheitis (IBR), and bovine viral diarrhea [55]. The Mithun habitat itself has an important role in the persistence of zoonotic and non-zoonotic diseases within the area. Still, it has been suggested that Mithun in home tract may be more resistant to diseases, compared with *ex-situ* animals [32].

Mithun is grazing with local cattle in the common forest area which has increased the percentage of crossbreeding of Mithun with local zebu cattle [26]. This fact also results in the loss of unique species characteristics of Mithun and may also reflect the failure to correctly respond to behavioral disorders associated with Mithun's confinement and captivity. These are important drawbacks in the preservation of Mithun. Recently, regular mass Mithun slaughtering for meat purpose has further threatened the population density and the size of this population in the near future. Combining health/breeding risks with the possibility of excessive export for slaughter and the natural habitat destruction, the regime's commercialization schemes may endanger the Mithuns rather than increase their numbers, and therefore need to be monitored on the name of the species conservation. The mass slaughter of Mithun specimens of excellent quality, mature, characteristic, and big specimens during ceremonies or festival led to loss of good quality germplasm of Mithun, and leaving behind most poor quality or uncharacteristic specimens to be used for a breeding purpose which will impoverish the genetic quality of Mithun progeny [56].

Therefore, considerable and significant efforts need to be taken from all the stakeholders' quarters, including government, policy makers, agriculture and veterinary departments, researchers, breeders, nongovernment organizations, research institutions and universities, Mithun farmers to prepare proper planning or program to conserve the Mithun in its original form in their habitat.

## 12. Breeding policy for Mithun

To design and implement a sound breeding policy to improve the production performance of Mithun, a free-range animal, constitutes a real challenge. Indeed, farmers rear this animal under semi-intensive/extensive free range grazing conditions in its natural habitat. The Mithun herds move around the jungle throughout the year and bred naturally with the herd bulls. Under such managerial practices (with low input to maximize the gains) the Mithun improvement for meat and milk purposes through artificial insemination is not an easy task. In this production system, the farmers are not able to monitor their animals for the onset of estrus and take help of superior bulls at their own choice for breeding. Hence, a

practical approach toward Mithun breeding for better production is probably the introduction of superior male into the herd/farms.

With this purpose, in each state of the northeastern hilly regions (NEHR) recording Mithun populations, Mithun breeding farms should be established, where superior males and females could be maintained under good managerial condition. Separate intensive selection for meat and milk purposes should be the prime objective of these breeding farms. All the essential parameters regarding this rare genetic resource must be taken into consideration at the time of selection for propagation, preservation, and conservation of this valuable species. Simultaneously, superior males generated out of the nuclear stock should be provided to the farmers at the female:male ratio of 10:1. The state Mithun breeding farms will guarantee a continuous supply of superior males, separately selected for meat or milk, according to the farmer's choice. The introduction of artificial insemination using semen collected from superior bull may be an alternative to the improvement of Mithuns reared under intensive systems, in Mithun breeding farms, but it needs to be standardized to explore the possibility for field application. This would foster the development and application of diverse techniques of assisted reproduction that will be revised in the following sections.

### **13. Biotechnology development in Mithun reproduction**

#### **13.1. Standardization of semen preservation and artificial insemination in Mithun**

The improvement of the reproductive and productive performance of Mithun demands the females to be breed with semen of superior genetic bulls. This can be easily achieved through the artificial insemination (AI) with preserved semen. Different methods of semen collection and preservation were standardized for the Mithun. Mithun semen was successfully preserved at 4°C (liquid refrigerated semen), for approximately 2 days. It was also successfully cryopreserved in liquid nitrogen using a Tris-egg yolk-glycerol or citrate-egg yolk-glycerol extender with 5% glycerol concentration. Sperm quality was improved (approximately 23–25% increase in progressive motility and count of live sperm with intact acrosome) by adding 5% glycerol in split doses instead of in a single dose. It was also observed that Tris-egg yolk-glycerol extender was better than citrate-egg yolk-glycerol's for cryopreservation of Mithun semen. Inseminations with both frozen and liquid semen successfully produced calves through AI both at farm and field levels [56].

#### **13.2. Estrus synchronization**

Protocols for estrus synchronization and timed AI has been developed for the Mithun, including those using prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) alone, the Ovsynch protocol (GnRH-PGF<sub>2α</sub>-GnRH), and progesterone-based controlled intra-vaginal drug releasing device (CIDR) [56].

In the synchronization protocol based on prostaglandins, two injections of PGF<sub>2α</sub> 11-day apart are given to cyclic Mithun cows after confirming the existence of a mature corpus luteum. Estrus signs following the injection of PGF<sub>2α</sub> and indicate the Mithun cows responded to this treatment and insemination can be done. The time from onset of estrus to ovulation is

$27.7 \pm 0.61$  h, with a range of 26–31 h, in PGF2 $\alpha$ -treated compared with  $26.9 \pm 0.31$  h, ranging from 26–29 h in untreated cows [56].

The Ovsynch protocol was proved useful for estrus synchronization of cyclic Mithun cows irrespective of the day of the estrous cycle at the beginning of the treatment. This protocol had a respond very good response from Mithun cows; a 75% conception rate was obtained when using this protocol [56].

CIDR is a very useful approach to synchronization of estrus in cyclic as well as postpartum anestrus Mithun cows. It results in more prominent physical as well as behavioral signs of estrus in either cyclic or anoestrous animals compared with nontreated cows in heat. Very interestingly, the use of CIDR at 45–50 days postpartum (dpp) induced estrus at 53–58 dpp, when the uterine involution was completed. In natural conditions, Mithun cows exhibit the first postpartum estrus at  $102 \pm 19.6$  days postpartum. CIDR is therefore useful and advantageous for the expression of clear physical and behavioral signs of heat, which facilitates heat detection, as well as a higher productive lifespan of at least 50 days [56].

### 13.3. Multiple ovulation and embryo transfer (MOET)

Mithun is an important livestock species in NEH region of India. The use of artificial insemination programs permits half improvement in genetic makeup through introduction of superior germplasm. However, embryo transfer technology (ETT) may be a useful method for a rapid improvement and multiplication of superior quality germplasm in animals of any species. The technique helps the breeders to produce genetically superior animals within a short period. MOET is not only to be used in genetic improvement of Mithun, i.e., *in situ* conservation, but also used in *ex situ* conservation of Mithun. MOET method can prevent extinction of Mithun in near future [56].

The superovulation/multiple ovulation and embryo transfer technology (ETT) has been successfully standardized for the Mithun species. The first Mithun calf, BHARAT, obtained through multiple ovulation and embryo transfer technology born in March 27, 2012, and the second calf (PRITHVI), born on May 11, 2012, at ICAR-National Research Centre on Mithun (Medziphema, Nagaland, India). Cryopreservation of Mithun embryos has also been standardized. MOHAN, the first Mithun calf, was born in May 12, 2012 from transfer of a 100-day-old cryopreserved embryo, also at ICAR-National Research Centre on Mithun. The standardization of the embryo transfer protocol in Mithun will help in the conservation and propagation of quality germplasm in all the Mithun-inhabited areas of NEH region [56].

## 14. Conclusion

Mithun is a meat (mainly) animal of Himalayan foothills of Southeast Asia. It can survive in varying environmental conditions and converts crop residue into high quality protein meat. Mithun is a pride and prestigious member of the tribal community. Mithun is a social, friendly, and intelligent animal. Mithun is a very fertile animal and can produce one calf per year. However, its population is fluctuating due to many reasons, although it not yet endangered. In modern

biotechnology era, we need to protect and conserve its germplasm in Mithun home region. Therefore, all the quarters from government to Mithun growers need to take appropriate policy, decision, and action to preserve the Mithun. In ICAR-NRC on Mithun, India, the work of conservation of Mithun in semi-intensive system has been started in last century and successfully implemented in the institute level. Now the implementation was successful in field level by adaptation of the latest nutrition, reproductive, productive, and health management program such as in other bovine and bubaline species. In the reproduction side, artificial insemination, estrus synchronization coupled with timed AI, and embryo transfer technology will help to go a long way to achieve the target of propagating quality germplasm in the farmer's field.

## Conflict of interest

The author declares that there is no conflict of interest involved in the present work.

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