RESEARCH PAPER | Veterinary | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Pregnancy diagnosis by seed germination inhibition test in mithun (*Bos frontalis*) cows

KEYWORDS: Seed germination inhibition test, mithun, pregnancy diagnosis

P. Perumal

Scientist, Animal Reproduction Laboratory, National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland – 797 106

ABSTRACT *The present study was undertaken to assess the seed germination inhibition test on pregnancy diagnosis in mithun cows. Total of 14 mithun were selected to the study and divided into positive group (pregnant cows; n=7) and negative group (non- pregnant; n=7). The urine was collected through manual method from the pregnant and non- pregnant mithun cows. The urine from the both groups was diluted with distilled water at the ratio of 1:4. Fifteen good quality germinatable paddy seed were taken in sterile petri dishes and 15 ml of diluted urine was added. For each cow the test was conducted with a replica of six tests in six petri dishes. Paddy seeds with water only were also carried out as the control group. Seed germination inhibition percentage and shoot length were measured from three groups after three days. The result revealed that there was significant difference between the three groups of mithun cows in seed germination inhibition percentage and shoot length. It was concluded that the seed germination inhibition technique is useful to detect pregnancy in mithun cows as a simple, non- invasive and economical method. Future, estimation of hormones such as progesterone, estrogen and correlate with seed germination inhibition percentage and shoot length at different trimester of pregnancy is warranted to confirm the present findings.*

Introduction

Mithun (*Bos frontalis*) is a semi-wild free–range, rare bovine species present in the North-Eastern Hill (NEH) region of India. It is believed to have originated more than 8000 years ago from wild Indian gaur (*Bos gaurus*) (Simoons 1984). The animal has an important place in the social, cultural, religious and economic life of the tribal population particularly in the states of Arunachal Pradesh, Nagaland, Manipur and Mizoram. Recent statistics indicates that the mithun population is decreasing gradually due to lack of suitable breeding management, increase in intensive inbreeding practices, declining land area for grazing and lack of suitable breeding and feeding management in NEH region. Greater efforts are required from all quarters to preserve the mithun population to enhance the socio-economic status of this region. Since mithuns are semi wild animal and not fully domesticated, natural breeding is practiced in this species with accompanied limitations like disease transmission. Thus, use of suitable, simple and reliable detection of pregnancy diagnosis is utmost essential.

Attempt to increase reproductive efficiency primarily depends upon early and accurate method of diagnosis of pregnancy. Diagnosis of pregnancy soon after breeding is important for the early identification of non- pregnant animals so that production time loss as a result of infertility may be decreased by appropriate treatment or culling. An essential feature of the establishment of pregnancy in bovine species is the prolongation of luteal function. Pregnancy can be detected by various methods such as rectal palpation, ultrasonagraphic and radiographic analysis, progesterone assay, rosette inhibition test and detection of pregnancy specific proteins, etc (Wani et al. 2003). But these techniques needs laboratories facilities, professional experience and are invasive and expensive methods to detect pregnancy. These techniques are difficult to use in areas such as north eastern jungle areas, where mithuns are living in jungle in most of the times. So alternatively, simple, non- invasive and non- expensive pregnancy diagnosis test should be identified and utilized. Egyptian pregnancy test for detection of human pregnancy was utilized in cattle by differentiation of seed germination (Veena and Narendranath 1993, Narayana Swamy et al. 2010) and based on the results they suggested that this test could be used as a simple test to diagnose pregnancy in mithun.

The seed germination inhibition test is simple, non- expensive, non- invasive, non – skilful test and test the pregnancy anywhere and any place inside the jungle in the NEH regions. Mithuns are following natural breeding in the jungle and grazing fields and therefore the occurrence of pregnancy goes unnoticed by the farmers. Moreover restraint of mithun is difficult and need chute to collect blood or other samples to do pregnancy test. Therefore, a study was conducted to validate the application of seed germination inhibition test for detection of pregnancy in mithun cows.

Materials and Methods

Fourteen apparently healthy mithun cows of approximately 3 to 6 yr of age were selected with good body condition (score 5-6), maintained under uniform feeding, housing and lighting conditions in NRC on Mithun, Jharnapani, Nagaland, India. Each experimental animal was fed in this experiment as per the farm schedule. Each experimental animal was daily offered ad libitum drinking water, 30 kg mixed jungle forages (18.40% dry matter and 10.20% crude protein) and 4 kg concentrates (87.10% dry matter and 14.50% crude protein) fortified with mineral mixture and salt. The experimental animals were grouped into positive group (n =7), in which pregnant animals were taken, negative group (n=7) where non – pregnant animals were selected and control group where instead of urine distilled water was used. The pregnancy was confirmed by rectal palpation method. The urine samples were collected through manual method between 7.00 to 9.00 AM and used for testing same day. In the laboratory, the urine was diluted at the ratio of 1:4 with distilled water. In each sterile Petri dish fifteen paddy seeds were taken on the blotting paper and 15 ml of diluted urine was added. For each cow, the test was conducted with a replica of six tests in six Petri dishes. The Petri dishes were covered with the trays to avoid evaporation, wherein there was little air movement at the bottom of the inverted trays and they were undisturbed for three days. After three days, the seeds were examined for germination inhibition percentage and shoot length in positive, negative and control groups as suggested by Veena and Narendranath (1993).

$$\text{Germination inhibition percentage} = \frac{\text{No. of seeds not germinated in Petri dishes}}{\text{Total No. of seeds taken in Petri dishes}} \times 100$$

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

The results were analysed statistically using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL). Differences with values of $P < 0.05$ were considered to be statistically significant.

Result and Discussion

Mean seed germination inhibition percentage and shoot length of positive, negative and control group were presented in the table -1. The result of this study revealed that there was a significant ($p< 0.05$) difference between the three experimental groups in the two criteria of the seed germination. Highest mean seed germination inhibition percentage and lowest shoot length was observed in positive pregnant mithun cows and reciprocal values were observed in control group. At the same time the pregnancy was confirmed by rectal palpation. Similar reports were also reported in cattle (Veena and Narendranath 1993, Narayana Swamy et al. 2010). Moreover, combination with simple pregnancy diagnostic kits with this type traditional method of pregnancy diagnosis will be very helpful to detect pregnancy diagnosis in species like mithun in jungle area of NEH regions. It was concluded that the seed germination inhibition technique is useful to detect pregnancy in mithun cows as a simple, non- invasive and economical method. Future, estimation of hormones such as progesterone, estrogen and correlate with seed germination inhibition percentage and shoot length at different trimester of pregnancy is warranted to confirm the present findings.

Table1: Seed germination inhibition percentage and shoot length (cm) of paddy seeds in pregnant and non – pregnant mithun cows

Groups	Germination Inhibition Percentage	Shoot Length (cm)
Control Group	19.57 ± 1.54^{a}	5.91 ± 0.86^{a}
Negative Group (Non-Pregnant)	24.02 ± 1.80^{b}	3.67 ± 0.73^{b}
Positive Group (Pregnant)	78.91 ± 2.09^{c}	0.53 ± 0.52^{c}

Within column means with different letters (a, b, c) differ significantly ($P < 0.05$)

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