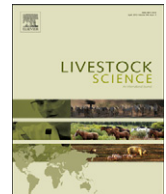




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Cervical mucus characteristics and peri-estrous hormone concentration in relation to ovulation time in Zebu (Sahiwal) cattle



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ARTICLE INFO

Article history:

Received 26 August 2011

Received in revised form

10 December 2012

Accepted 19 December 2012

Keywords:

Cervical mucus

Ovulation

Progesterone

LH

Zebu cattle

ABSTRACT

The present study was conducted to determine the association among cervical mucus characteristics, peri-estrous progesterone (P_4) and luteinizing hormone (LH) concentrations and ovulation time in Zebu cattle. In experiment 1 the relationship between cervical mucus characteristics and ovulation time was determined. Cervical mucus was collected from Sahiwal cows ($n=60$) before artificial insemination from mid-cervix and rheological and chemical properties were studied. Ovulation time was determined by ultrasound examination at 2 h intervals starting from the onset of estrus to ovulation. Cervical mucus was copious and thin in 70.2 and 63.2 percent of estrus periods, respectively. Cervical mucus from 57.9 percent of the estruses had a typical arborization pattern. The Means \pm SEM of pH, electrical conductivity and spinnbarkeit value of the mucus were 7.59 ± 0.06 , 15.21 ± 0.15 mS/cm and 11.06 ± 1.06 cm, respectively. Estrus to ovulation duration was significantly greater ($P < 0.05$) in cows that had 8–16 cm spinnbarkeit value of cervical mucus than cows with higher or lower values. In experiment 2 the relationship among peripheral P_4 , LH concentrations and ovulation time was determined. For this purpose blood samples were collected from 20 Sahiwal cows (10 natural estruses and 10 induced estruses) at 2 h intervals from the onset of estrus until ovulation. Peripheral P_4 and LH concentrations were measured using bovine-specific ELISA kits. The duration of estrus to LH-peak and LH-peak to ovulation was 2.2 ± 1.62 h and 29.45 ± 1.73 h in natural estrus and 3.3 ± 0.67 h and 32.75 ± 3.62 h in induced estrus, respectively. The peak LH value did not vary significantly in synchronised (15.26 ± 2.8 ng/mL) and natural estrus (12.67 ± 1.96 ng/mL). Plasma P_4 concentration on the day of estrus was significantly greater ($P < 0.05$) in the synchronised estruses (1.23 ± 0.23 ng/mL) compared to the natural estruses (0.65 ± 0.11 ng/mL). The estrus to ovulation interval was significantly ($P < 0.05$) greater when the P_4 concentration was > 1 ng/mL on the day of estrus (36.17 ± 0.73 h in natural and 37.57 ± 4.32 h in synchronised) compared to < 1 ng/mL (31.28 ± 1.74 h in natural and 28.83 ± 3.61 h in synchronised). Taken together, the spinnbarkeit value and arborization pattern of cervical mucus had strong relationship with plasma P_4 concentration and ovulation time indicating that the parameters could be used as a tool to decide optimal time of insemination in Sahiwal cattle.

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1. Introduction

In the bovine, expression of estrus and ovulation is the result of highly synchronized hormonal milieu during the peri-estrous period (Beg et al., 2003; Galina and Orihuela,

2007). During the luteal phase, high circulating progesterone (P_4) blocks the preovulatory luteinizing hormone (LH) surge thus ovulation and also expression of estrus behavior. When blood P_4 concentration starts to decline under the luteolytic effect of $PGF_{2\alpha}$, increases the pulse frequency of the pituitary gonadotrophic hormones (FSH and LH). As the Graafian follicle matures under the influence of these hormones during the last 3–5 days of estrous cycle, a large quantity of estradiol is synthesized and secreted. The estradiol, at a threshold concentration, causes the onset of behavioral estrus and triggers a surge of LH leading ovulation (Machado et al., 2008; Roelofs et al., 2010). Blood estradiol concentration reaches its highest concentration concurrent with the maximum estrus behavior score (Lyimo et al., 2000).

Spermatozoa, upon deposition into the female reproductive tract travel through different environments to reach the site of fertilization. For natural service, ejaculated semen is deposited in the vagina and sperm enters the cervix where cervical mucus filters out spermatozoa with poor morphology and motility (Saurez and Pacey, 2006). Although this barrier is bypassed in artificial insemination (where frozen semen is deposited in the uterine body), characterization of cervical mucus is a critical determinant for the timing of insemination (López-Gatius et al., 1996). During the peri-estrous period, higher estrogen influences the genital tract, rendering it edematous, tonic and highly secretory, especially the cervical glands, leading to characteristic heavy mucus flow (Roelofs et al., 2010). An increase in water and electrolyte concentration in the cervical mucus, which changes the glycoprotein:water ratio leading to alteration in the rheological properties. Cervical mucus becomes increases, becomes more watery, translucent, less viscous and easier to traverse by spermatozoa during the follicular phase compared to the luteal phase of the cycle when mucus is scanty, viscous and unfavorable to sperm penetration (Rutllant et al., 2005).

Quantifying P_4 and LH concentrations during peri-estrus improves the accuracy of predicting of optimal time of insemination to ensure conception. However, measuring these hormones is laborious, costly, time consuming and requires sophisticated equipment and thus not suitable for field conditions. Circulating hormone concentrations during peri-estrus influences the estrus duration, ovulation time, cervical mucus characteristics and conception. Although the relationship among these parameters has been studied in *Bos taurus* cattle, very little work has been carried out on *Bos indicus* cattle. Therefore, the present study was designed to evaluate the relationship among ovulation time, peri-estrous blood hormone concentration (P_4 and LH) and cervical mucus characteristics in Sahiwal cattle.

2. Materials and methods

2.1. Experiment 1: Relationship between symptoms of estrus, cervical mucus characteristics, and ovulation time

2.1.1. Experimental animals

The present study was conducted at the Cattle Yard of the National Dairy Research Institute (NDRI), Karnal, Haryana.

Sahiwal cows ($n=60$; from 1st to 5th parity) were utilized to study the association between cervical mucus characteristics and ovulation time. The cows included in the study were free from anatomical and reproductive disorders and also free from diseases. Experimental animals were maintained in a loose housing system under group management practice. Dry and lactating animals were housed separately in paddocks with brick on edges flooring.

2.1.2. Cervical mucus characteristics

Cervical mucus was collected prior to artificial insemination by mid-cervical aspiration using sterile blue sheaths (IMV Technologies France, France) and a universal artificial insemination gun by the recto-vaginal method. A blue sheath was fitted in the universal artificial insemination gun to serve as a long syringe. The piston of the universal insemination gun acted as plunger and assisted in drawing out the cervical mucus. Immediately after collection, 5 mL cervical mucus was taken to the laboratory in a sterile test tube and tested for the following parameters:

- i. *Quantity*: quantity was assessed on the basis of quantity of mucus discharge observed which was classified as absent/scanty, moderate and copious.
- ii. *Appearance*: the mucus obtained was observed visually for appearance, which was classified as clear, cloudy and dirty (Deo and Roy, 1971).
- iii. *Consistency*: consistency of the mucus was assessed by placing 2–3 drops onto a grease free glass slide and inclining the slide to 45° . The movement of the mucus was observed and grouped into three different consistencies (Deo and Roy, 1971); thick, moderate, and thin.
- iv. *Spinnbarkeit value*: two to three drops of cervical mucus were placed on a grease free slide and another grease free glass slide was placed over it. The slides were slowly moved away from each other to stretch between two slides and the slide was moved until mucus broke. The distance between the two slides was measured just before the break of the mucus string with a cm scale mounted to a wall (Panigrahi, 1964). The spinnbarkeit values were grouped as 0–8 cm, 8–16 cm, 16–24 cm and > 24 cm.
- v. *Arborization pattern*: to analyze arborization pattern, 2–3 drops of cervical mucus were placed on grease free glass slide and spread uniformly, air dried and examined under low power objective ($10\times$) of microscope. Fern patterns were grouped into typical, atypical and nil categories.
- vi. *Electrical conductivity*: cervical mucus was lysed by vortexing (Spinix Corporation, CA, USA) until it became a free flowing liquid. Electrical conductivity (EC) was measured in the lysed cervical mucus using a pH-Conductivity Benchtop (Orion 4 star, Thermo Electron Corporation, USA). The electrical conductivity was recorded in mili-Siemens/cm unit (mS/cm) and values were classified as < 14 mS/cm, 14–16 mS/cm and > 16 mS/cm.
- vii. *pH*: pH was measured in the lysed samples using pH-Conductivity Benchtop (Orion 4 star, Thermo

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