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Determination of correct AI timing using rheological property of cervical mucus and their relation to conception rate: A review

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Abstract
Despite of enormous progress in understanding physiology of oestrous cycle and the advancement in the field of heat detection kits, the conception rate remained low in cows and buffaloes by artificial insemination (AI). Many factors like quality control of semen, storing of semen straws, handling by technician, nutritional and genetic status of animals etc. affects conception rate but the timing of AI remained the major contributor for low A.I. success rate. The differential secretion of female genital tract in cows and buffaloes can improve conception rate by aiding in determining the correct timing of artificial insemination (A.I.). The physical properties of secretion of cervical mucus during different phase of oestrous changes dramatically and can be exploited as a non-invasive tool for mid oestrous detection. This review focused on the physical characteristics of cervical discharge in different period of oestrous and quick microscopic observation of its spreading pattern and there effect on conception rate. This review will elicit the best possible combination of rheological property of cervical mucus for determining correct timing of A.I. to achieve high conception rate during oestrous.

Keywords: Rheological property, cervical mucus, AI timing, conception rate

Introduction

Prediction of accurate time of insemination is an important tool to increase the success rate of AI programme and reduce the repeat breeding cases. Circulating hormone concentrations of progesterone, oestrogen, LH during periestrus period modulates the oestrus duration, ovulation time, cervical mucus characteristics and conception. Improvement in the accuracy of predicting optimal time of insemination to ensure conception are being practised by estimating progesterone (P4) and LH concentrations during periestrus (Pursley *et al.*,1998) [20]. However, measuring these hormones is laborious, costly, time consuming and requires sophisticated equipment and thus impractical for field veterinarians (Lyek *et al*., 2013) [10]. Time has come to identify the alternative non-invasive, economical, quick technique like cervical mucus characterisation for determining the time of insemination. Cervical mucus is a viscoelastic secretion of constantly secreting mucus producing cells of the endo-cervix (Glover, 1960) [7].

Spermatozoa, upon deposition into the female reproductive tract travel through different environment store to reach the site of fertilization. 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 (Lo´ pez-Gatiusetal., 1996) [12]. During the periestrual period, higher oestrogen influences the genital tract, rendering it oedematous, tonic and highly secretory, especially the cervical glands, leading to characteristic heavy mucus flow (Roelofs *et al*., 2010) [21]. Whereas Tsiligianni *et al*. (2011) [24] reported the occurrence of steadily low progesterone concentrations during preovulatory period, trigger alterations of certain CM characteristics, while extremely high oestradiol concentration could prevent the occurrence of these alterations. An increase in water and electrolyte concentration in the cervical mucus, which changes the glycoprotein: water ratio leading to alteration in the rheological properties. Endo- cervix's changes and its secretions leads to change in glycosylation which affect the sperm transport and cervical barrier (Pluta *et al*., 2011) [19]. 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 unfavourable to sperm penetration (Rutllant *et al*., 2005) [22].

Rheological properties of cervical mucus

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

A. Quantity: The quantity parameter is assessed on the basis of quantity of mucus discharge and classify as absent/scanty, moderate and copious. Lyek *et al*. 2013 [10] reported copious cervical mucus discharge during oestrus periods in 70.2% and scanty in 4.9% of cows with a greater proportion of copious discharge in pluriparous cows (75.6%) than primiparous cows (56.2%). The cows with scanty cervical mucus discharge failed to conceive upon insemination.

B. Appearance: The cervical mucus is observe visually to categorise it into clear, cloudy and dirty category (Deo and Roy, 1971) [6]. Study by Lyek *et al*. 2013 [10] suggested the highest conception rate in cows with greater conception rate in the cows with mucous discharge of moderate consistency (45.4%) followed by clear (32.4%) and cloudy (25%) discharge. No cow was able to conceive discharging thick mucus. Lim *et al*. 2014 [11] reported the success rate of conception of 67.3% in transparent, 23.6% in turbid and 9.1% in dirty mucus samples. Conception rate in Murrah buffalo was recorded as 45.25% in clear mucus secretion as compared to only 14.28% in cloudy appearance (Verma *et al*., 2014) [25].

C. Consistency: The consistency of the mucus was assessed by placing 2–3 drops onto a grease free glass slide and inclining the slide to 45^{0}. The movement of the mucus was observed and grouped into three different consistencies (Deo and Roy, 1971) [6]; thick, moderate, and thin. Verma *et al*. 2014 [25] reported higher conception rate (47.54%) in moderate consistency of cervical mucus in buffalo. Lim *et al*. 2014 [11] compared the consistency of CM into thin vs. thick and revealed higher conception rate of 81.8% in thin than 18.2% in thick CM.

D. 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) [17]. High conception rates were observed when the cervical mucus had spinnbarkeit values between 8–16cm (37.5%) in Sahiwal (Lyek *et al*., 2013) [10], 15.3±0.3 cm in Kankrej (Modi *et al*., 2011) [15], 16-24 cm in buffalo with 48.64% conception rate (Verma *et al*., 2014) [25]. Tsiligianni *et al*. 2011 [24] also reported a fluctuation pattern of low SBK cm at beginning of oestrous that followed time windows of increase and decrease till end of oestrous in super ovulated cows.

E. Arborization pattern: The arborisation pattern studied by placing 2–3 drops of cervical mucus on grease free glass slide and spread uniformly, air dried and examined under low power objective (10X) of micro-scope. Arborification of cervical mucus indicates crystallization in branched arborescent patterns resembling fern fronds or palm leaves and fractal like crystallisation (Cortes *et al*., 2012) [4]. A fractal is a structure comprised of smaller parts that resemble the whole in a smaller scale (Mandelbrot, 1993) [14] and possess attributes such as roughness, irregular shape at every level, high degree of organization, self-similarity, lacunarity, and a characteristic fractal dimension (FD) (Losa, 2009) [13]. The crystallization pattern is referred as typical when it possess high level of symmetry and comprises four well-defined axes (stems) projecting from the same central point. The axes are arranged perpendicularly, forming angles of approximately 90°, and are delimiting four well-defined quadrants. From each axis, branching's (venations) of variable length originate, forming pine-like, arboriform structures (Cortes *et al*., 2012) [4]. Alena *et al*. (2008) [2] reported that cervical mucus crystallization (CMC) affected results of cervical mucus survival test, the highest motility of sperms after the 60 and 90 minutes was assumed in the case of club moss – ferny (14.80% and 7.96%) and ferny-like crystallization (13.82% and 8.47%). Cortes *et al*. 2014 [4] also reported occurrence of fern pattern as a star-like morphology with six well-defined straight axes (hexagonal symmetry), emerging from the same central point and forming 60º angles. Fern patterns are group into typical, atypical and nil categories. Conception rate was 38.2% in cows inseminated when the cervical mucus had typical arborisation pattern with zero success in cervical mucus of no pattern (Lyek *et al*., 2013) [10]. Modi *et al*. 2011 [15] also concluded that Kankrej cows showing typical fern pattern during oestrous period are regular breeder whereas as atypical fern pattern mostly indicative of repeat breeder. Verma *et al*. 2014 [25] reported higher conception rate in typical fern pattern in comparison to atypical and nil pattern (54.9% vs. 20% and 0%) in Murrah buffalo.

F. 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 (Orion4star, Thermo Electron Corporation, USA). The electrical conductivity was recorded in mili-Siemens/cm unit (mS/cm) and values were classified as 0-14 mS/cm, 14–16mS/cm and >16 mS/cm. High conception rates were observed when the cervical mucus had spinbarkeit values between 8–16 cm (37.5%), conductivity <16 mS/cm (35.29%) and pH 4.8 (44.44%) (Lyek *et al*., 2013) [10].

F. pH: The pH of cervical mucus usually measure in the lysed samples using pH-meter. Higher conception rate (44.44%) were recorded in Sahiwal cattle with cervical mucus pH >8 (Lyek *et al*., 2013) [10], 42.18% in Murrah buffalo with pH range of 7.0-7.5 (Verma *et al*., 2014) [25]. Modi *et al*. 2012 [15] reported that regular Kankrej breeder cows possess alkaline mean pH of cervical mucus in comparison to repeat breeder (8.39 vs 6.19).

Cervical mucus characteristics at the time of service/artificial insemination influences conception in cows. Changes in the periestrual concentrations of hormones influence the characteristics of cervical mucus.

The appearance of cervical mucus indicates the sexual health status of the animal (Tsiligiannietal, 2001) [23]. Generally the cervical mucus is clear and transparent (such as the albumen portion of a hen's egg); any deviation from this might indicated is orders in the genital tract (Deo and Roy, 1971) [6]. Greater conception rates were observed in cows inseminated when the cervical mucus was clear, which might be due to better reproductive status and proper stage of insemination (Lyek *et al.*, 2013) [10]. Cervical mucus during the oestrus period changes to a thin and transparent fluid showing non-Newtonian properties. Conception rate was greater in cows when cervical mucus had non-Newtonian behaviour at the time of insemination (Murugavel and Lopez-Gatius, 2009) [16]. The low progesterone profiles in cows had typical fern pattern of cervical mucus (Kumaresan *et al*., 2001) [9]. Conception rate was also influenced by the arborisation pattern since typical arborisation pattern is associated with proper balance in the hormone concentration, which facilitates conception. Lyek *et al*. 2013 [10] also found high conception rate in cows with typical arborisation pattern and no conception was recorded in cows without an arborisation pattern.

Under the influence of oestrogen there is an increase in muco-proteins, sodium chloride and water content of cervical mucus which alter the pH of the cervical mucus during periestrus. Alkaline pH of cervical mucus is favourable for sperm motility however it has been reported that with increase in pH, the viability and sperm penetration is adversely affected (Pattabiraman *et al*., 1967) [18].

During oestrus the electrolyte concentration tends to be greater under the influence of oestrogen and there by contributing to greater electrical conductivity. This high concentration of electrolytes in mucus is required to activate spermatozoa and uterine motility and the physical state of the mucus facilitates contact of ovum and spermatozoa. The average EC of cervical mucus (15.2170.15 mS/cm;range12.34–16.91) observed by Lyek *et al*. 2013 [10] which is in agreement with Bishnoi *et al*. (1983) [3]. Positive correlation between the electric conductivity of cervical secretions and conception rate in cows were observed (Bishnoi *et al*., 1983) [3].

During oestrus the cervical mucus has the capacity to be drawn into threads which is attributable to the presence of large molecules in the mucus and likely depends thereby delay the oestrus to LH peak duration (Bage *et al*., 2002; Gustafsson *et al*., 1986) [2, 8]. Animals with prolonged oestrus to ovulation interval or LH peak to ovulation interval might have had delayed LH peak compared to those ovulated in a normal time.

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