Full Length Article

RELATIVE INTENSITY OF OESTRUS AND PROTEIN CONCENTRATION OF SALIVA AND CERVICO-VAGINAL FLUID IN BUFFALOES

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ABSTRACT

Accurate detection of oestrus is a key factor for breeding and successful conception in buffaloes which is a major constraint in buffaloes. Protein concentrations of saliva and cervico vaginal fluid (CVF) were determined during various stages in normal cycling, silent oestrus and anoestrus buffaloes. Eighteen pluriparous buffaloes were selected and divided into Group I (regular oestrus; n=6), Group II (silent oestrus; n=6) and Group III (anoestrus n=6) buffaloes. Saliva was collected at proestrus, oestrus and diestrus stages of Group 1 and II animals and on a random day in Group III animals. CVF were collected only at oestrus stage of Group I and Group II buffaloes. Intense, intermediate and no oestrus signs were noticed in Group I, II and III buffaloes respectively. Non-significantly higher protein concentration was recorded in saliva and CVF during oestrus stage in Group I and II buffaloes.

Keywords: Cervico-vaginal fluid, oestrus, protein concentration, saliva.

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INTRODUCTION

Buffaloes have high productive potential, but poor manifestations of oestrus signs and silent oestrus are the major constraints in buffaloes affecting their reproduction. Hence, accurate and efficient detection of oestrus is a key factor for right time of artificial insemination to achieve successful conception rate in buffaloes. There are several detection tools used for detection and confirmation of oestrus in cattle but none of the methods have been succeeded to overcome the silent oestrus problem in buffaloes. In the recent past, researchers have tried biomarkers from different biological secretion for the detection of oestrus in buffaloes and many biomarkers

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have been identified and isolated from urine, saliva and cervical vaginal fluids for the detection of oestrus in buffaloes.

However, the scientific information regarding the proteomics from saliva and cervico-vaginal fluids (CVF) during different stages of oestrous cycle and anestrous buffaloes are scanty (Shashikumar *et al.,* 2018)**.** The present study places on record the protein concentrations of saliva and CVF in normal cycling, silent oestrus and anoestrus buffaloes.

MATERIALS AND METHODS

A total of 18 pleuriparous buffaloes (4 -7 years) with good body condition maintained at Post Graduate Research Institute in Animal Sciences, Kattupakkam were utilized for the study. The buffaloes were randomly divided into three groups viz Group - I with regular oestrous cycle (n=6), Group - II with silent oestrus (n=6) and Group III with anoestrus n=6). The intensity of oestrus was studied using behavioral changes, physiological changes and gynaecological observations and it was scored as described by Rao and Rao (1981) with slight modifications.

The selected buffaloes were observed for intensity of oestrus by the changes of physiological, behavioural and gynaecological changes. The scoring was done based on the behavioural, physiological and gynaecological examination. The animals which scored 0 - 5 points on a scale out of 15 were animals with weak intensity of oestrus. The animals which scored 5 - 10 points were with intermediate signs and animals which scored 11 - 15 points were with intense in their oestrual signs.

Saliva was collected at proestrus (day -3), oestrus (day 0) and diestrus (day 7) stages in Group I and II buffaloes and on a random day in Group III animals. CVF was collected at oestrus stage in Group I and II buffaloes.

The experimental buffaloes were restrained and using a 20 ml syringe, around 1 to 10 ml of saliva was aspirated from the mouth. The collected saliva samples were centrifuged at 5000 rpm at 4^0c for 5 minutes and the supernatant was taken in 2 ml eppendorf tube containing protease cocktail inhibitor and kept at -80^0C until further study (Shashikumar *et al.,* 2018).

Cervico-vaginal fluid collection was carried out by per rectal examination. Sterile A.I sheath was inserted intravaginally along with A.I rod and 20 ml syringe was fixed with A.I sheath. Cervico-vaginal fluid was aspirated slowly into the syringe via the sheath. Protease cocktail inhibitor was added to the collected sample. Samples homogenized and centrifuged at 12000 rpm for 15 minutes at 4^0C and supernatant stored at – 80^0C (Muthukumar *et al.,* 2014).

The protein concentration of saliva and CVF were measured by Bradford method. Protein standards were prepared by dissolving 2 mg of bovine serum albumin (BSA) in 1ml of distilled water to make 2 mg/ml of protein. This protein solution was further diluted serially with phosphate buffer saline (PBS) to 1mg/ml and 0.5 mg/ml and up to 0.03125 mg/ml. The 96 well flat bottom polystyrene plate was used for the estimation of protein concentration in the saliva and CVF samples. BSA standard (20 µl) was added in each well from each dilution in duplicates and 200 µl of

Bradford reagent (Bio-Rad, USA) was added into each well. The final volume in each well was made to 220 µl. The plate was incubated in room temperature for 30 to 45 minutes and then the absorbance was measured at 595 nm in the ELISA reader (Bio-Rad, USA) and standard curve for BSA was generated based on the readings.

All the collected data were analyzed statistically by the method described by Snedecor and Cochran (1989). One way ANOVA followed by Tukey's test used for statistical analysis.

RESULTS AND DISCUSSION

Intensity of oestrus

The major behavioral signs of oestrus in buffaloes included restlessness, bellowing and frequent discharge of small quantities of urine. However, vocalization (bellowing), tail raising and homosexual behavior are shown by only a small proportion of buffaloes (Kanai and Shimizu, 1983; Gamit *et al.,* 2015).

In Group I, 16.66 per cent (1/6), 16.66 per cent (1/6) and 66.68 per cent (4/6) animals had shown weak, intermediate and intense oestrus respectively which was in agreement with previous reports.

In Group - II of silent oestrus animals, 16.66 per cent (1/6), 83.34 per cent (5/6) and 0 per cent (0/6) animals evinced weak, intermediate (vulval oedema, cervical relaxation and uterus tonicity) and intense oestrus signs, respectively and none of the animals in Group III showed oestrual signs.

Occurrence of ovulation without behavioral manifestation of oestrus is known as a silent oestrus or sub-oestrus and the reasons for sub oestrus are poorly known (Warriach *et al.,* 2008). The incidence of silent oestrus is higher during the postpartum and summer months (Awasthi *et al.,* 1998; Lohan *et al.,* 2004). Altered levels of pituitary hormone like FSH and LH (Janakiraman *et al.,* 1980; Razdan *et al.,* 1981), thyroid hormones (Gupta and Dhoble, 1988), ovarian hormones (Jain, 1988), heat stress (Jainudeen, 1986), nutrition (Schallenberger and Prokopp, 1995) and slower growth rate of follicles associated with lesser secretion of oestradiol (Das and Khan, 2010) are some of the reasons cited for altered oestrual behaviour.

Protein concentration from saliva and CVF

Edgar (1992) stated that the secretion and components of saliva changes in response to a wide range of different chemical and mechanical stimuli.

The salivary protein concentration (ug/ul) in group I and II buffaloes are 0.48 ± 0.01, 0.56 ± 0.03 and 0.48 ± 0.02, 0.50 ± 0.04, 0.54 ± 0.05 and 0.41 ± 0.01 during proestrous, oestrus and diestrous of cycle, respectively. The results of present study was in agreement with Shashikumar *et al.* (2018) who have reported the protein concentration in saliva varied from 0.26 to 2.23 µg/µl at different stages of the oestrous cycle in buffaloes. In Group III of anoestrus animals the mean protein concentration in saliva was 0.45 ± 0.08. There was no significant difference in the protein concentrations between anoestrus and the other groups of animals.

The protein concentration (µg/µl) of CVF during oestrus is 0.35 ± 0.18 and 0.55 ± 0.07 in regular and silent oestrus buffaloes, respectively. The protein concentrations from regular and silent oestrus buffaloes were not significantly different even though there was a slightly higher concentration of protein in silent oestrus buffaloes.

Alagendran *et al.* (2013) stated that the protein levels were maximum during ovulation and minimum during post ovulatory phase of the cycle in humans. Changes in components of CVF during oestrous cycle are under the hormonal control because hormonal fluctuation could influence the biochemical properties of the cervix (Prasad *et al.,* 1981: Dasari *et al.*, 2007).

Detection of oestrus is a problem in buffalo and identification of oestrus specific proteins will help to develop a protein based kit (Muthukumar *et al.,* 2014).

It is concluded from the present study, the intensity of oestrus is mostly intense in Group I animals and intermediate in Group II animals. Protein concentration was higher in oestrus stage compared to other stages in both groups.

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