Full Length Article

OESTRUS SPECIFIC PROTEINS FROM SALIVA AND CERVICO VAGINAL FLUIDS IN OESTRUS AND SILENT OESTRUS BUFFALOES

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ABSTRACT

Buffaloes have high productive potential but poor manifestations of oestrus signs and silent oestrus act as a major constraint in buffaloes affecting their reproduction. There are several detection tools used for detection and confirmation of oestrus in cattle but none of the methods have succeeded to overcome the problem of silent estrus in buffaloes. In recent years proteomics approach has gained much attention to identify proteins associated with various physiological and disease conditions in saliva and cervico vaginal fluid mostly in humans and up to certain extent in cattle and buffaloes. In this study twelve pluriparous Murrah buffaloes aged 4 to 7 years with good body condition were selected and divided into group I (regular oestrus), group II (silent oestrus). Each group carries six animals and saliva was collected at proestrus (day -3), oestrus (day 0) and diestrus stage (day 7). Cervico vaginal fluid (CVF) was collected only at oestrum stage. Protein profiling of saliva were studied by SDS - PAGE analysis. The SDS-PAGE analysis showed proteins of molecular weight 150, 110, 80, 40 kDa during proestrus; 150, 120, 70 and 50 kDa during oestrus; 250, 150, 90, 50 and 37 kDa during diestrus in group I buffaloes. Similarly, proteins of molecular weight 250, 150, 100, 80 and 25 kDa during proestrus; 150, 100, 75 and 50 kDa during oestrus and 250, 100,75 and 25 kDa during diestrus were observed in group II buffaloes. The SDS-PAGE analysis of CVF showed proteins of molecular weight of 100 and 75 kDa in group I buffaloes and 250 and 150 kDa in group II buffaloes. Oestrus specific proteins with different molecular weights were identified in terms of different bands size. However further study is warranted to find out their role during oestrus in regular and silent oestrus buffaloes for the development of specific diagnostic assay.

Key words: Oestrus, Silent oestrus, Cervico vaginal fluid, Saliva, Proteins

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INTRODUCTION

Expression of these oestrus behaviour signs is not very obvious or prominent in buffaloes (Takkar *et al.,* 1980) and mostly considered as poor estrus exhibitors. Therefore, the buffalo oestrus is denoted as silent heat (Danell *et al.,*1984). In the oestrus cycle of buffaloes, oestrus is a short period of time, and ovulation occurs 11 hours following its onset (Singh *et al.,* 2000). Volatile compounds are reportedly one of the reliable indicators to detect estrus (Achiraman and Archunan, 2006), the accuracy of the diagnostic kits are poor due to the shelf life and evaporative nature of the components. To overcome this problem, several attempts have been made to develop diagnostic assay for detection of oestrus in buffaloes, but none are very reliable or efficient (Karthikeyan *et al.,* 2013) and there is a need to identify the precise time of oestrus in buffaloes to enhance the success of artificial insemination.

Muthukumar *et al.* (2014) have identified 416 proteins in the cervico – vaginal fluid in buffaloes with many biological functions and with comprehensive differences between the oestrus and diestrus phases and 68 oestrus specific proteins and further documented the high expression of (Heat Shock Protein) HSP – 70, a stress responsive protein during the oestrus phase in buffalo.

CVF is composed of a mixture of oviductal, uterine, cervical and vaginal secretions and their production is influenced by health status, the microbiome and pregnancy (Sheldon *et al.,* 2009 and Zegels *et al.,* 2010).

Saliva, a biological fluid from different salivary glands have buffer, salts, nucleic acid, organic compounds and protein. (Lamkin and Oppenheim, 1993 and Levine, 1993). Saliva can be used as a diagnostic tool because it has an advantage of being non invasive in comparison to other body fluids. Totally 179 proteins were identified in all phases of oestrus cycle of which 23 proteins were found in all phases, 22 specific to proestrus, 17 specific to diestrus and 37 proteins oestrus specific (Muthukumar *et al.,* 2014) and further suggested that β - enolase and TLR - 4 (Toll Like Receptor – 4) exclusively expressed during oestrus can be taken as an indicator of oestrus in buffaloes.

MATERIALS AND METHODS

The present study was carried out in Post Graduate Research Institute in Animal Sciences (PGRIAS), Kattupakkam, Potheri, Kanchipuram District. Buffaloes were maintained under semi intensive system with isomanagerial feeding and ad libitum water. After feeding and watering the buffaloes were allowed for grazing in the morning. The study was conducted from November 2019 to January 2021. The pleuriparous buffaloes aged 4 to 7 years with good body condition were selected for the present study. Experimental buffaloes were ruled out for pregnancy and reproductive abnormalities and selected buffaloes were observed continuously for two oestrus cycle length for identification of silent oestrus by rectal examination and transrectal ultrasonography. From the screened animals, twelve buffaloes were selected and randomly divided into two groups viz. group I (regular oestrus) and group II (silent oestrus).

Collection of samples

***Saliva*:** The experimental buffaloes were restrained and using a 20 ml syringe, around 1 to 10 ml of saliva was aspirated from the mouth (accumulated at lower jaw) from individual buffaloes. Immediately following collection, the samples were transported to laboratory in ice for further analysis. In order to precipitate the cellular debris, the collected saliva samples were centrifuged at 5000 rpm at 4°C for 5 minutes. The supernatant was taken in 2 ml eppendorf tube containing protease cocktail inhibitor (10µl/ml) and kept at -80°C until further study (Shashikumar *et al.,* 2018)

***Cervico vaginal fluid*:** Cervico vaginal fluid collection was carried out by rectal examination. Perineum was washed with water and wiped dry to remove faecal matter. Sterile Artificial Insemination (AI) sheath was inserted along with AI rod intravaginal guided per rectally and AI rod was then removed. A 20 ml syringe was fixed with AI sheath and CVF was aspirated slowly into the syringe via the sheath. The collected sample was transferred to the centrifuge tube, protease cocktail inhibitor was added at the rate of 10 µl/ml and transported to laboratory for further processing in ice pack. The collected samples were homogenized using glass homogenizer and centrifuged at 12000 rpm for 15 minutes at 4°C. The supernatant was collected and stored at -80°C until further analysis (Muthukumar *et al.,* 2014).

Sodium Dodecyl – sulphate Polyacrylamide gel procedure

Glass plates and spacers are assembled in gel casting apparatus. Components of the resolving gel was mixed and poured into the gel plates leaving level 2 cm above the stacking gel. A layer of distilled water was placed over the top of the resolving gel to level the resolving gel and to prevent oxidation and then allowed the resolving gel to solidify for 30 minutes at room temperature. Distilled water was drained from top of the resolving gel. The mixed stacking gel components was poured into the gel plates over the resolving gel. To the top, the spacers with comb was inserted and the gel was allowed to stand at least one hour at room temperature. Comb was then removed and the gel assembly was assembled into the Mini - Protean II apparatus. Freshly prepared 1x running buffer was added to the apparatus and prepared samples were loaded into the wells of the gel. The gel was electrophoresed until the dye reached the bottom of the gel and the gel was removed from the apparatus for staining with coomassie brilliant blue over night. After staining, destaining was performed and the gel was documented.

RESULTS AND DISCUSSION

Electrophoretic profile of salivary protein

The proteins of different molecular weight in the saliva during proestrus, oestrus and diestrus in group I of regular cycling buffaloes and group II of silent oestrus buffaloes are presented in Plates 1 to 6. The SDS-PAGE analysis showed proteins of molecular weight 150, 110, 85, 40 kDa during proestrus (Plate 1); 150, 120, 70 and 50 kDa during oestrus (Plate 2); 250, 150, 90, 50 and 37 kDa during diestrus (Plate 3) in group I buffaloes. Similarly, proteins of molecular weight 250, 150, 100, 80 and 25 kDa during proestrus (Plate 4); 150, 100, 75 and 50

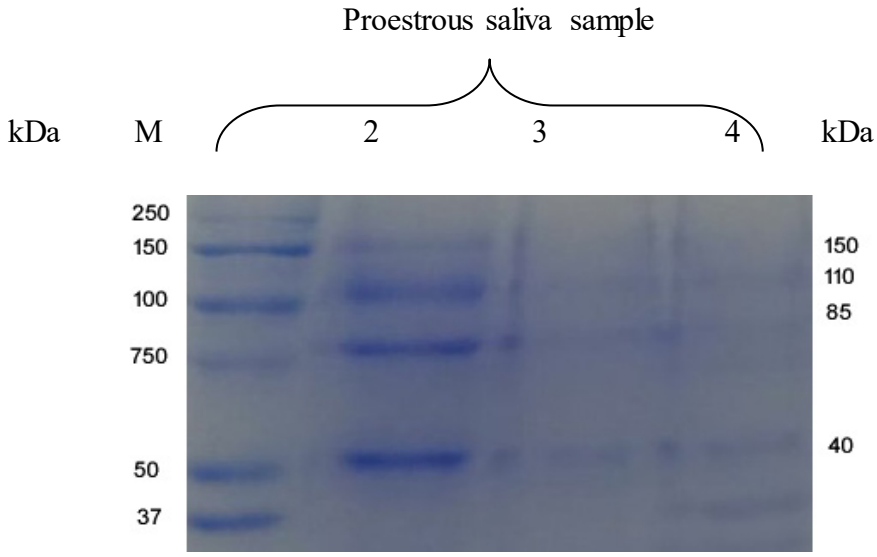

Plate 1. SDS - PAGE gel of salivary proteins during proestrus in group I buffaloes. M – Marker and L_2 – L_4 – Samples

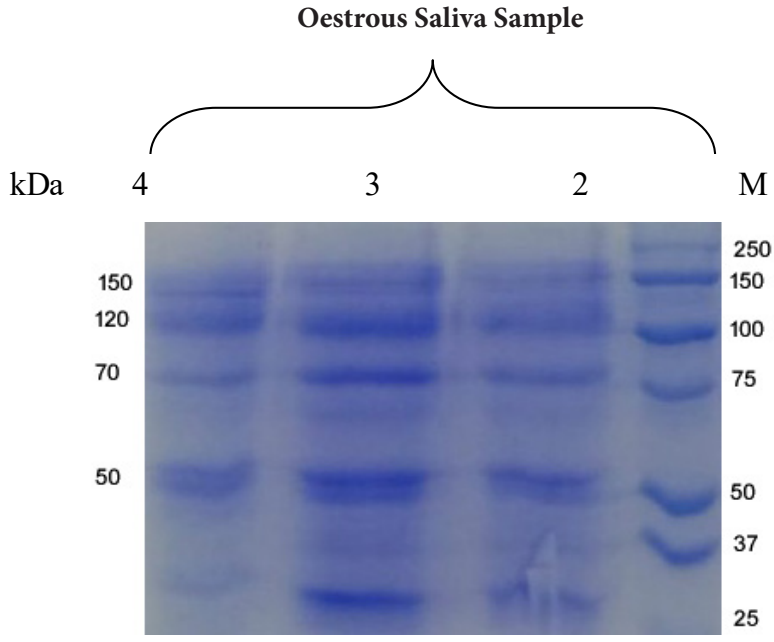

Plate 2. SDS - PAGE gel of salivary proteins during oestrus in group I buffaloes. M – Marker and L_2 – L_4 – Samples

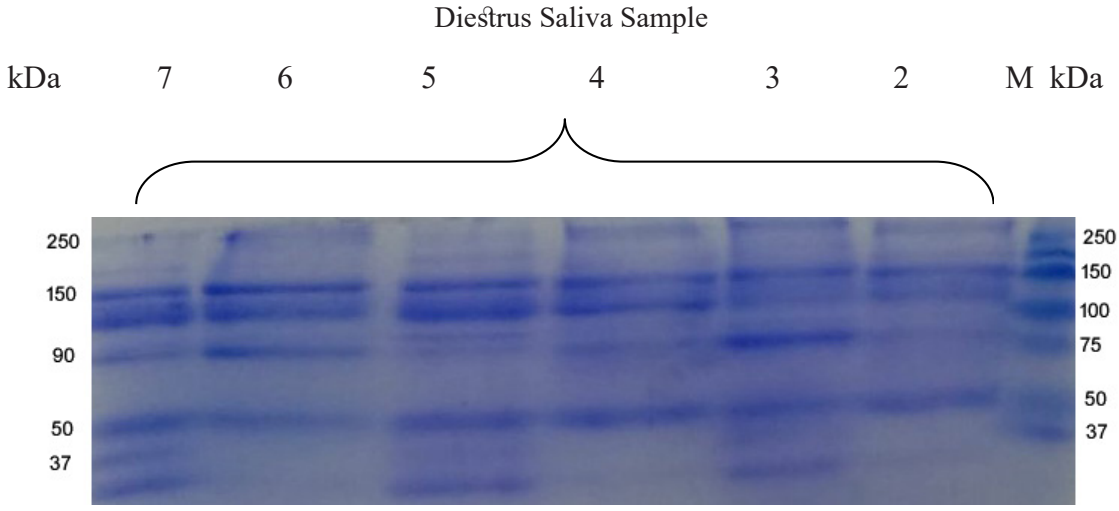

Plate 3. SDS - PAGE gel of salivary proteins during diestrus in group I buffaloes. M – Marker and L_2 – L_7 – Samples

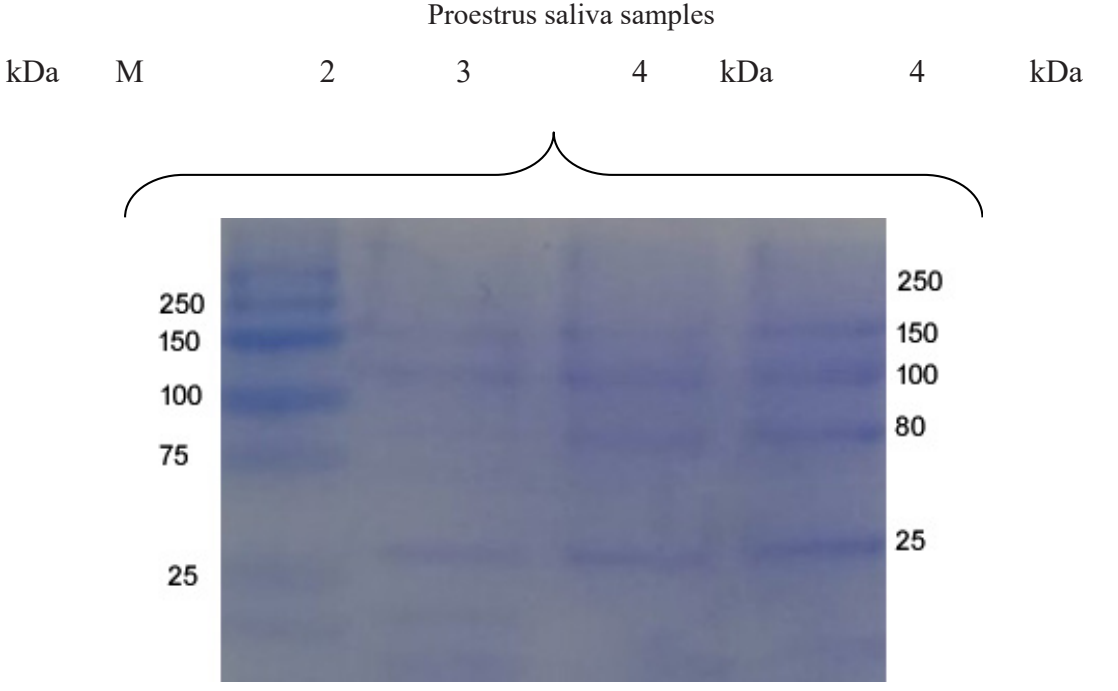

Plate 4. SDS - PAGE gel of salivary proteins during proestrus in group II buffaloes. M – Marker and L_2 – L_4 – Samples

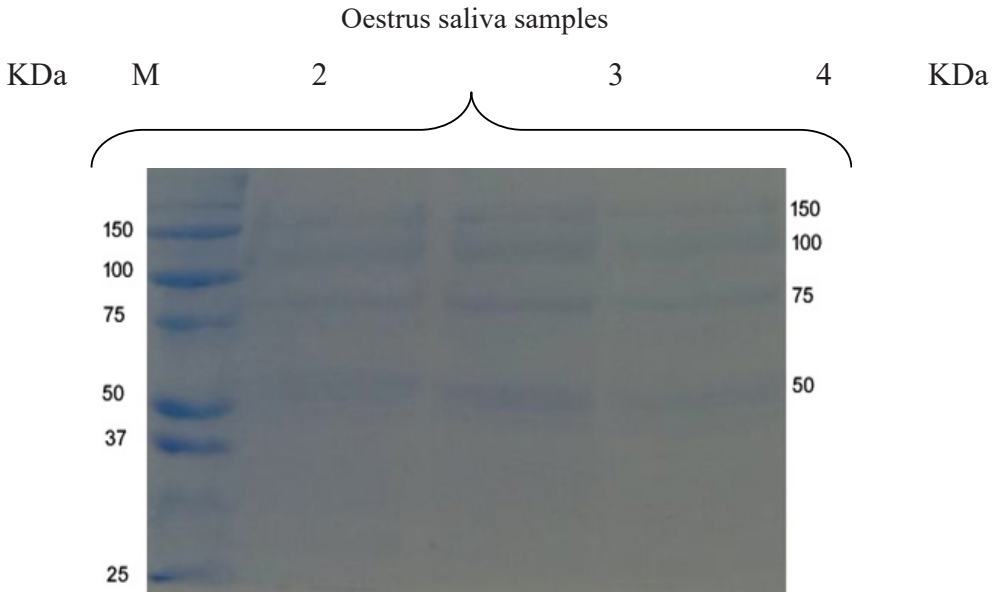

Plate 5. SDS - PAGE gel of salivary proteins during oestrus in group II buffaloes. M – Marker and L_2 – L_4 – Samples

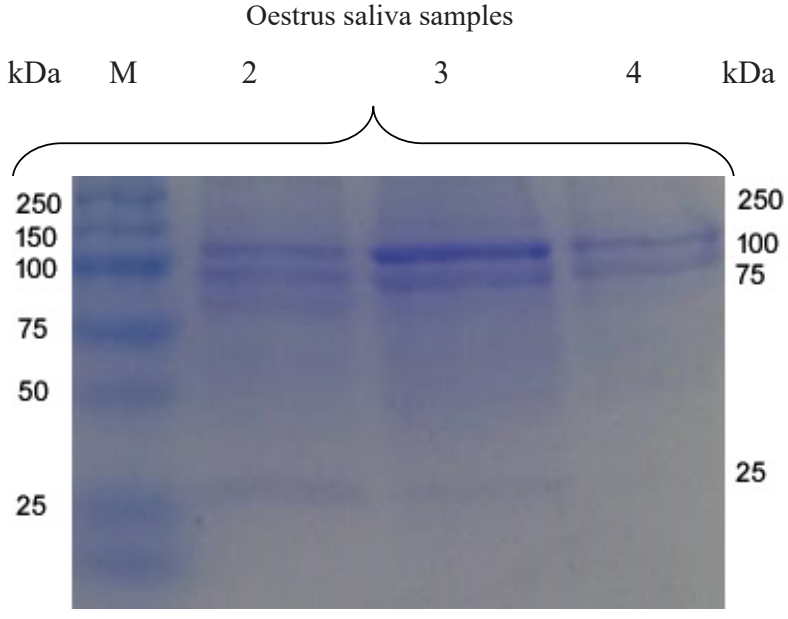

Plate 6. SDS - PAGE gel of salivary proteins during diestrus in group II buffaloes. – Marker and L_2 – L_4 – Samples

kDaduring oestrus (Plate 5) and 250, 100,75 and 25 kDa during diestrus (Plate 6) were observed in SDS - PAGE analysis in group II silent oestrus buffaloes.

Proteins of different molecular weight were present during the different stages of the cycle, all the proteins present were not the same in the different stages and in the different groups in the study. During oestrus period in group I and II, proteins of molecular weights 150, 120, 70, 50 were found in group I and molecular weights of 150, 100, 75 and 50 were present in group II. Proteins with molecular weights of 150 and 50 kDa were found in both the groups during oestrus and 100 and 75 kDa proteins were found only in the silent oestrus buffaloes of group II during the oestrus period. Similarly not only the protein with molecular weight 250 kDa was found in the saliva of regular cycling buffaloes during the diestrus stage of group I but was also found in the proestrus and diestrus stage of the group II buffaloes with silent oestrus. The protein with molecular weight 100 kDa was found in all the stages of the cycle in group II buffaloes with silent oestrus. The molecular weight of the proteins in the saliva ranged from 37 to 250 kDa in regular cycling buffaloes and 25 to 250 kDa in the silent oestrus buffaloes. The protein with the molecular weight 150 kDa was found in all stages of the oestrus cycle in the regular cycling buffaloes and 100 kDa was found in all the stages of the silent oestrus buffaloes. In the present study 150, 100, 75, 50 kDa proteins in oestrus stage of silent oestrus buffaloes warrantes further study to find the role of the specific proteins in silent oestrus which may be helpful for the development of diagnostic assay.

Electrophoretic profile of cervico vaginal fluid protein

The protein bands of different molecular weight in the cervico vaginal fluid during oestrus in group I of regular cycling buffaloes and silent oestrus of group II buffaloes are presented in Plate 7 and 8. The SDS-PAGE analysis showed proteins of molecular weights of 100 and 75 kDa in group I buffaloes during oestrus (Plate 7) and 250 and 150 kDa during silent oestrus in group II buffaloes (Plate 8)

In the present study, the appearance of protein band 100 and 75 kDa is similar to the results reported by Muthukumar *et al.* (2014). Further, they have identified 100, 75 kDa proteins band as α -actinin and heat shock protein, respectively. The role of 250 and 150 kDa protein in silent oestrus buffaloes was unknown. However, further study is warranted on the role of proteins during oestrus in buffaloes.

CONCLUSION

It could be concluded from the present study that, the protein concentration in the saliva and cervico vaginal fluid reflect the physiological status of the buffaloes. Salivary proteins with molecular weights of 150, 120, 110, 90, 70, 50, 40 and 37 kDa during regular oestrus, 250, 100, 75 and 25 kDa in silent oestrus were recorded and CVF proteins 100 and 75 kDa in regular oestrus and 250 and 150 kDa in silent oestrus were recorded in

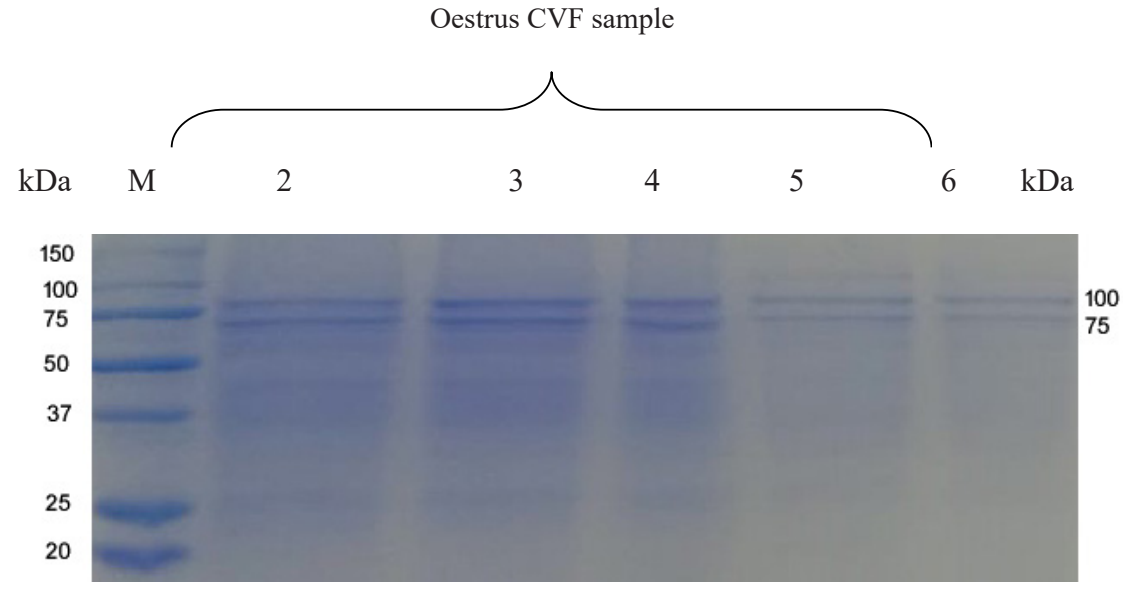

Plate 7. SDS - PAGE gel of CVF proteins during oestrus in group I buffaloes. M – Marker and L_{2} – L_{6} Samples

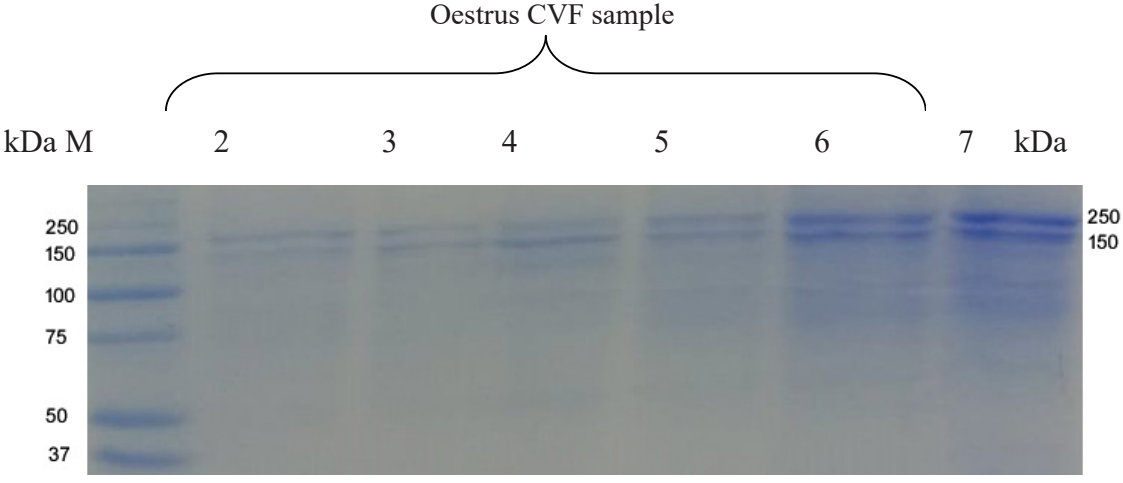

Plate 8. SDS - PAGE gel of CVF proteins during oestrus in group II buffaloes. M – Marker and L_{2} – L_{7} Samples

this study. Further study with other high end technologies like mass spectrometry is needed to develop specific markers to detect silent oestrus.

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