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Evaluation of salivary biomolecules as an effective indicator in accurate and precised identification of estrus in Murrah buffaloes for timed AI

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Buffaloes play a major role in milk production, meat industries and other agricultural activities like draught production in India. India is having 108.7 million buffaloes and their contribution in total milk production of India is about 51% (DAHD&F 2013–14). The estrus in buffaloes is described as "silent or unobserved heat" (Suthar and Dhami 2010), because they express signs of heat only in coolest period of a day and most probably in early morning hours when no attendant have a keen eye on them. At present, veterinary practitioners and farmers who have large herds are facing a major problem in buffalo reproduction, particularly regarding with the accurate detection of estrus. Eventually, there is an increasing need to develop techniques to identify estrus to enhance the success rate of artificial insemination in buffaloes. The changes in various biomolecules in saliva are having a correlation with the changing hormonal patterns of progesterone and estradiol in serum during the menstrual cycle of a woman (Gandara *et al*. 2007). Multiple salivary constituents were studied in humans in the past to determine their relation to the menstrual cycle and the time of ovulation. They include sialic acid (Calamera *et al*. 1986), carbohydrates (Al-Khafagy *et al*. 2012) and proteins (Alagendran *et al*. 2013). Hence, the aim of the present study was to estimate the quantitative changes in the salivary biomolecules of buffaloes with reference to the different stages of estrous cycle to improve the reproductive success in buffalo herds by detection of heat with better accuracy and to develop a biomarker for the effective detection of estrus in buffaloes.

Cyclic healthy Murrah buffaloes (20) free from any anatomical and reproductive disabilities maintained at LRC, NDRI, Karnal, were selected as experimental animals. These buffaloes were kept separately in a loose housing system having covered and open area (as per BIS) and were fed as per the normal feeding schedule being followed for buffaloes at LRC. The present study was duly approved by the Institutional Animal Ethics Committee. Estrus detection and confirmation was done as per Thakur *et al*. (2013). Saliva was collected between 6 and 8 AM (before feeding). For collection of salivary samples the first post-partum heat was missed and then the buffaloes were observed for next heat from 17 days onward after the onset of first heat in these animals. Sampling saliva and blood was also started regularly from 17 days after the onset of first heat in these animals. Once estrus was confirmed, the samples were categorized as proestrus (–3 to -1 days), estrus (0 day), metestrus (1 to 2 days) and diestrus (14 to 21 days) phases. Collected saliva samples were centrifuged at 3,000 rpm for 15 min at 4°C and were stored in cryovials at –20°C. The blood samples were also collected and centrifuged at 4°C at the rate of 3,000 rpm to separate the plasma and stored at –20°C. The biochemical parameters were estimated by using respective standard assay kits at Central Institute for Research on Buffaloes (CIRB), Hisar, Haryana. Total carbohydrate estimation was done by total carbohydrate colorimetric assay kit, total protein estimation by total protein kit (Biuret mehod), total lipid estimation by lipid quantification kit, albumin estimation by albumin kit (BCG Method) and mucin 5β, hormones E_2 and P_4 were estimated by movine ELISA kits. Electrical conductivity was recorded in the pH-conductivity bench top and pH concentration was recorded by pH bench meter tutor. The significance of differences among different stages of estrous cycle was analysed by using one way analysis of variance technique followed by the post hoc Duncan's multiple test using statistical analysis system (SAS) software package.

The levels of all biomolecules were significantly ($P<0.01$) higher during estrus phase compared to all other phase (Table 1). The high level of proteins in estrus may be required to function as carriers for the ligands and convey the chemical signals. Our results are in agreement with the findings of others (Suthanthirakannan and Rameshkumar 2014) in cows. High concentration of proteins in the saliva of estrus buffaloes may be associated with the statement of Beynon and Hurst (2004), who reported that the excretion

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Table 1. Means ± SE values of salivary biomolecules during different phases of estrous cycle in Murrah buffaloes

Parameter	Phases of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Total carbohydrate (mg/ml)	18.334± 0.35^{B}	21.13± 0.40^{A}	14.309± 0.34^{D}	17.28± 0.18^{BC}
Total protein (mg/ml)	50.42± 0.43^{B}	53.86± 0.34^{A}	46.21± 0.30^{D}	48.11± 0.17^{C}
Albumin (g/dl)	0.451± 0.02^{B}	0.653± 0.05^{A}	0.501± 0.03^{AB}	0.453± 0.01^{B}
Mucin 5β (mmol/l)	19.79± 0.22^{A}	20.21± 0.23^{A}	18.26± 0.28^{B}	17.60± 0.20^{B}
Total lipid (mg/ml)	20.62± 0.68^{C}	51.31± 3.85^{A}	31.74± 0.90^{B}	18.93± 0.29^{BC}
Electrical conductivity (ms/cm)	9.40± 0.40^{B}	10.12± 0.48^{A}	8.19± 0.45^{B}	9.35±
Saliva pH	8.78± 0.05^{A}	8.66± 0.06^{A}	8.83± 0.06^{A}	8.86± 0.04^{A}

Values bearing different superscripts in a row differ significantly (P<0.01).

Table 2. Means ± SE values for blood plasma hormones levels during different phases of estrous cycle in Murrah buffaloes

Hormone	Phases of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Estrogen (pg/ml)	29.46± 0.65^{A}	19.35± 0.35^{B}	6.67± 0.19^{C}	5.43± 0.13^{D}
Progesterone (ng/ml)	1.01± 0.90^{B}	0.56± 0.20^{C}	1.24± 0.30^{B}	2.58± 0.12^{A}

Values bearing different superscripts in a row differ significantly (P< 0.01).

of major urinary proteins in urine during estrus can acts as pheromone carrier. Kannan and Archunan (2001) indicated that lipids play a crucial role in the sexual attraction in rat and had got specific role in olfactory communication (Rameshkumar and Archunan 2006). Increased content of mucin 5β in saliva of estrus buffaloes is found in agreement with findings of Alagendran *et al*. (2009) and responsible for salivary fern pattern which principally depends on the chemico-physical properties of the mucins. The measurement of electrical impedance can be used for monitoring of various reproductive events in female mammals. In present investigation, electrical conductivity was found highest during estrus phase of buffaloes and no related review was found. It might be because of the effect of estrogen on electrolytes, as concentrations of different salts determine the electrical conductivity of saliva. The pH of saliva of buffalo was within the alkaline range which was similar to the findings for cow saliva (8.2–8.5) as reported by Bailey and Balch (1961). No significant (P>0.05) difference was observed in pH of saliva during different phases of estrous cycle due to non-significant relationship between steroid hormones and salivary pH. The hormonal findings (Table 2) were found in agreement with previous studies (Glencross and Pope 1989, Mondal 2010). The high concentration of estrogen during proestrus and estrus may influence the production of estrus-specific signals. The level of blood progesterone hormone was significantly (P<0.01) lower in estrus phase as compared to other phases of estrous cycle in Murrah buffaloes. Peripheral P_4 concentrations have been found within normal range and no significant association was found between P4 and salivary biomolecules.

SUMMARY

The present study was designed to estimate changes in salivary pH, electrical conductivity (EC) and its various constituent biomolecules like total carbohydrates, total proteins, albumin, mucin 5β and total lipids, during different phases of estrous cycle. In present investigation, the levels of all biomolecules like total CHO, total proteins, albumin, mucin 5β, total lipids and EC were observed to be significantly (P<0.01) higher during estrus phase compared to all other phases. No significant difference was found in pH of saliva during different phases of estrous cycle. These changes may be helpful in explaining the relationship between characteristics of saliva and ovarian hormones viz. preovulatory estrogen surge. Thus, variation in salivary composition during different phases of estrous cycle may be helpful in accessing the specific phase of estrous cycle and carrying out the timed AI programme more precisely.

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