Evaluation of salivary electrolytes during estrous cycle in Murrah buffaloes with reference to estrus detection

Indu Devi1, Pawan Singh1, Surerder Singh Lathwal1, A. Kumaresan2 and Kuldeep Dudi3

1. Livestock Production Management Section, ICAR - National Dairy Research Institute, Karnal - 132 001, Haryana, India; 2. Department of Animal Reproduction (Livestock Production Management), Livestock Research Centre, ICAR - National Dairy Research Institute, Karnal - 132 001, Haryana, India; 3. Animal Nutrition Group, National Dairy Development Board, SCF-80, Panchkula - 134 109, Haryana, India.

Corresponding author: Indu Devi, e-mail: indulathwal@gmail.com, PS: pawansinghdbas@gmail.com, SSL: lathwal314@gmail.com, AK: ogkumaresan@gmail.com, KD: dr.dudi.kuldeep@gmail.com

Received: 04-06-2016, Accepted: 14-09-2016, Published Online: 28-10-2016

doi: 10.14202/vetworld.2016.1157-1161 How to cite this article: Devi I, Singh P, Lathwal SS, Kumaresan A, Dudi K (2016) Evaluation of salivary electrolytes during estrous cycle in Murrah buffaloes with reference to estrus detection, Veterinary World, 9(10): 1157-1161.

Abstract

Aim: Timely estrus detection is one of the critical factors for increasing reproductive efficiency in buffaloes. In recent decades, saliva has become a more popular as a noninvasive source for determining physiological status of animals by various biochemical electrolytes. This study was designed to assess and correlate changes in different salivary minerals concentration (calcium, inorganic phosphorus, magnesium, sodium, potassium, and chloride) during different stages of the estrous cycle in Murrah buffaloes.

Materials and Methods: The saliva samples were collected during the different phases of the estrous cycle from 20 Murrah buffaloes in early morning hours and were assayed using respective minerals assay kits.

Results: The concentrations of calcium (8.76±0.08-12.11±0.11 mg/dl), inorganic phosphorus (6.56±0.13-14.72±4.50 mg/dl), magnesium (2.27±0.14-5.79±0.15 mg/dl), sodium (139.47±0.31-159.62±1.22 mmol/L), potassium (109.28±0.41-137.07±0.68 mmol/L) varied during the different phases of estrous cycle. The concentration of calcium, inorganic phosphorus, magnesium, sodium, potassium, and chloride in saliva were significantly (p<0.0001) higher during estrus phase compared to other phases of the estrous cycle. All these minerals were positively and significantly (p<0.0001) related to estradiol concentration while salivary concentrations of calcium, magnesium, sodium, and chloride showed a significant (p<0.0001) negative correlation with progesterone level in blood plasma.

Conclusion: These preliminary findings indicate that there are definite variations in salivary mineral and electrolyte concentrations during different phases of the estrus cycle. These results may be used as an aid for estrus detection/confirmation in buffaloes although validation of the results using a large number of animals is required.

Keywords: heat detection, noninvasive method, saliva electrolytes, silent heat.
women. The ferning is caused by NaCl, which cyclically increases under the influence of estrogen [8]. Progesterone reportedly has a natriuretic effect [9], and the increase in progesterone after ovulation is thought to be followed by a compensatory rise in aldosterone concentration and subsequent effects on levels of electrolytes such as sodium, potassium, and calcium. Magnesium has also been found to have its role in regulation of menstrual function, along with its role in basal metabolism that changes over the course of the menstrual cycle in women [10].

With this backdrop, this study has been envisaged to evaluate the proportional changes in salivary minerals such as calcium, magnesium, sodium, potassium, and inorganic phosphorus during different phases of estrous cycle in Murrah buffaloes.

**Materials and Methods**

**Ethical approval**

This study was duly approved by the Institutional Animal Ethics Committee, ICAR - National Dairy Research Institute, Karnal, Haryana, India.

**Experimental animals**

A total of 20 normally cyclic and healthy Murrah buffaloes (*Bubalus bubalis*) free from any anatomical disorder and/or reproductive disabilities and diseases maintained at the Livestock Research Centre, NDRI, Karnal, Haryana, India, were selected as an experimental animal in this investigation. These buffaloes were kept separately in a loose housing system within a premise of 240 m² covered and open area (as per BIS). The main purpose of the above said housing system was to provide enough space for their free movement and better observation of signs of estrus. The animals were fed *ad libitum* with conventional diet as per the normal feeding schedule being followed for buffaloes.

**Confirmation of estrus and sample collection**

The buffaloes were carefully monitored for estrus signs including vulva swelling and reddening, vaginal mucous discharge, and restlessness. In addition, the exhibition of male behavior toward estrus buffalo such as Flehmen reaction, vaginal licking and mounting was taken into account to confirm the estrus phase. Finally, the estrus was confirmed by rectal palpation by experienced veterinarian. Saliva and blood samples were collected continuously for 25 days between 6.00 and 8.00 a.m. (before feeding).

Once 2nd estrus was confirmed, the collected samples were categorized as proestrus (−3 to −1 days), estrus (0 day), metestrus (1-2 days), and diestrus (14-21 days) phases. Collected saliva samples were centrifuged at 3000 rpm for 15 min at 4°C to remove any feed particle, etc. The separated saliva samples were stored in cryovials at −20°C. The electrolytes were estimated at the Central Institute for Research on Buffaloes, Hisar (Haryana) using mineral specific assay kits. The blood sample was centrifuged at 4°C at the rate of 3000 rpm for 20 min to separate the plasma and stored at −20°C.

**Electrolytes and hormones estimation**

Calcium estimation was done by Calcium Kit (OCPC Method). Phosphorus estimation was done by Phosphorus kit (Molybdate U.V method). Sodium estimation was done by colorimetric method (Modified Maruna and Trinder’s method). Potassium estimation was done by colorimetric method based on Turbidimetric method. Magnesium estimation was done by Magnesium kit (Calmagite method) and chloride estimation was done by Chloride kit (Thiocyanate method). The hormones were estimated using E2 and P4 bovine ELISA test kits (Endocrine Technologies, Inc., Newark, CA).

**Statistical analysis**

The data thus generated were subjected to Statistical Analysis System software package to find out the significant difference in salivary electrolytes and plasma endocrine profile of Murrah buffaloes under different stages of estrous cycle.

**Results and Discussion**

The levels of saliva electrolytes such as calcium (12.11±0.11 mg/dl), inorganic phosphorus (14.72±4.5 mg/dl), magnesium (5.79±0.15 mg/dl), sodium (159.62±1.22 mmol/L), potassium (26.85±1.22 mmol/L), and chloride (137.07±0.68 mmol/L) were observed to be significantly (p<0.01) higher during estrus phase compared to all other phases of estrous cycle (Table-1 and Figure-1). Inorganic phosphorus and calcium gradually decreased from estrus to diestrus phase. Magnesium was found highest in estrus phase, but lowest in proestrus phase. During the estrus period, sodium, potassium and chloride increased significantly (p<0.01) as compared to metestrus and diestrus phases of the estrous cycle.

**Table-1: Mean±SE values of salivary electrolytes during different phases of estrous cycle in Murrah buffaloes.**

| Parameters                  | Phases of estrous cycle |
|-----------------------------|-------------------------|
|                             | Proestrus | Estrus | Metestrus | Diestrus |
| Calcium (mg/dl)             | 9.95±0.11^c | 12.11±0.11^a | 8.81±0.08^b | 8.76±0.08^c |
| Inorganic phosphorus (mg/dl)| 11.80±0.21^a | 14.72±4.5^a | 9.08±0.17^b | 6.56±0.13^b |
| Magnesium (mg/dl)           | 2.27±0.14^a | 5.79±0.15^a | 3.92±0.12^b | 3.28±0.5^b  |
| Sodium (mmol/L)             | 150.30±0.32^a | 159.62±1.22^a | 141.40±0.90^c | 139.47±0.31^c |
| Potassium (mmol/L)          | 22.16±0.33^a | 26.85±1.22^a | 15.29±0.34^a | 12.40±0.22^a |
| Chloride (mmol/L)           | 121.98±0.77^a | 137.07±0.68^b | 117.12±1.08^c | 109.28±0.41^d |

Values bearing different superscripts in a row differ significantly (p<0.01). SE=Standard error
The least square means of plasma estradiol and progesterone levels during proestrus, estrus, metestrus, and diestrus phases of estrous cycle were observed to be 29.46±0.65, 19.35±0.35, 26.68±0.19, and 5.43±0.13 pg/ml, respectively, and 1.01±0.90, 0.57±0.20, 1.25±0.30, and 2.59±0.12 ng/ml, respectively (Table-2). The level of blood progesterone hormone was significantly (p<0.01) lower in estrus phase as compared to other phases. A significant correlation was found between saliva electrolytes and estradiol (Table-3).

The cyclic hormonal changes can affect a variety of physiological and biochemical processes. There are very few reports on the changes in salivary calcium, inorganic phosphorus, magnesium, sodium, potassium, and chloride levels in various phases of the estrous cycle in buffaloes. This investigation revealed that salivary electrolytes considerably varied depending on the reproductive status of animals. Peak serum calcium concentration observed at day 0 (estrus phase) may be due in part to high serum concentration of estradiol recorded during this phase. Similar observations were reported [11] in mares during the estrus phase of the estrous cycle. In addition, the ability of estradiol to retain salt and to alter ion transport in various other epithelial cells could be partly responsible for high saliva calcium observed during the estrus phase. It is reported that estrogen causes increase in parathyroid activity [12] which leads to marked acceleration of calcium uptake [7] and decreases of its elimination from pigeon’s gut [13]. This increase in serum calcium level in estrus phase may be necessary to support the increased neuromuscular activity, and ovarian hormone synthesis and release associated with this phase of estrous cycle. Changes in progesterone concentrations are not correlated with the changes in salivary electrolytes during cycle, suggesting that this hormone does not account for the change between the preovulatory phase and the post-ovulatory phase.

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

| Hormones       | Phases of estrous cycle | Proestrus   | Estrus      | Metestrus   | Diestrus    |
|----------------|-------------------------|-------------|-------------|-------------|-------------|
| Estrogen (pg/ml) |                         | 29.46±0.65ª | 19.35±0.35ª | 6.67±0.19ª  | 5.43±0.13ª  |
| Progesterone (ng/ml) |                     | 1.01±0.90ª  | 0.56±0.20ª  | 1.24±0.30ª  | 2.58±0.12ª  |

Values bearing different superscripts in a row differ significantly (p<0.01). SE=Standard error

**Table-3**: Pearson’s correlation coefficients of saliva electrolytes and blood plasma hormones.

| Hormones | Calcium | Phosphorus | Magnesium | Sodium | Potassium | Chloride |
|----------|---------|------------|-----------|--------|-----------|---------|
| Estrogen | 0.67ª   | 0.63ª      | 0.62ª     | 0.49ª  | 0.59ª     | 0.70ª   |
| Progesterone | −0.50ª | −0.23      | −0.46ª    | −0.37ª | −0.23     | −0.57ª  |

Values bearing superscript “A” are significantly correlated (p<0.0001)
In a similar research carried in dogs, level of salivary magnesium (Mg) has been reported to be highest during estrus and least during proestrus phase [14]. The significant (p<0.01) increase in salivary sodium, potassium, and chloride levels was seen during proestrus and estrus phases compared to postovulatory phases (diestrus phase). Possible cause for this change in sodium concentration includes the increased concentrations of antidiuretic hormone in the postovulatory phase [15], or of other steroid hormones. The possible reason for increased levels of these minerals might be their positive correlation with estrogen hormone as compared to progesterone. The fact that the change in plasma sodium is not associated with changes in weight or in concentration of urea, creatinine or albumin suggests that total body water and intravascular volume remain constant. Thus, it appears that sodium is lost in excess of water in the period of ovulation. It is known that saliva ferning depends principally on the electrolyte concentrations (especially NaCl, KCl, CaCl2) and chemo-physical properties of the mucins it contains (sialic acid) [16,17]. The estrogens increase the water content in mid-cycle and determine the most favorable condition, optimal proportion of water and optimal amounts of salts and sialomucin [18].

The level of estrogen hormone in blood was significantly (p<0.01) higher during proestrus phase than other phases of the estrous cycle in Murrah buffaloes. These hormonal findings were found in agreement with previous studies [19]. Peripheral P4 concentrations are significantly (p<0.01) higher during proestrus phase than other phases of the estrous cycle in Murrah buffaloes. These hormonal findings were found in agreement with previous studies [19].

Finally, along with the prevailing known factors affecting estrus expression and mechanisms for heat detection [22,23] new initiatives need to be taken and further research in this area should focus on identifying the biochemical mechanisms triggering changes in electrolyte metabolism and in mucin expression in saliva, all of which could explain the observed variations in electrolytes patterns. It is also of importance to elucidate the mechanism through which changes in sex steroid levels influence the different electrolytes observed among healthy buffaloes and in those suffering from reproductive disorders.

**Conclusion**

This study showed possibility of quantifying the salivary electrolytes such as sodium, potassium, calcium, magnesium, and inorganic phosphorus levels in saliva. As the increased activity of minerals tended to coincide significantly with the increase in plasma hormones like estradiol, it may be possible to use saliva samples for differentiation of stages of estrous cycle. Further, this study may open new ways to provide a non-invasive biological fluid to understand the relation of widely prevailing mineral deficiency in dairy animals and the level of various reproductive hormones in body.

**Authors’ Contributions**

Research work was done by ID. The experiment was designed and supervised by PS. SSL assisted ID in all technical support and data recording and KD assisted in literature collection and data analysis. PS and SSL provided valuable suggestion regarding design of experiment and data analysis. ID and KD compiled the results. KD and AK assisted in manuscript preparation. All authors have read and approved the final manuscript.

**Acknowledgments**

The research was supported by grants under bioacoustics tools for monitoring of dairy animals project, funded (Project no. 1000411) by Department of Biotechnology, Ministry of Science and Technology, New Delhi, Government of India.

**Competing Interests**

The authors declare that they have no competing interests.

**References**

1. Srivastava, A.K. and Kumaresan, A. (2014) Scope of buffaloes in Indian dairy industry. *Asian Buffalo Mag.*, 1: 16-27.
2. Bulletin of World Dairy Situation. (2013). Available from: www.ukidf.org/documents/WorldDairySituation2013.pdf. Accessed on 12-10-2016.
3. Available from: www.icar.org.in/Vision%202050%20NRCM,%20Hyderabad.pdf. Accessed on 13-10-2016.
4. Department of Animal Husbandry, Dairying and Fisheries (2012). Government of India, 19th Livestock Census. Available from: dahd.nic.in/sites/default/files/19%20%20Livestock%202012.pdf. Accessed on 12-10-2016.
5. Thakur, K.S.R., Kumar, N., Kumar, P., Chaurasia, S. and Patel, N.B. (2013) Heat detection techniques in cattle and buffalo. *Vet. World*, 6(6): 363-369.
6. Dadlani, A.G., Chandwani, S., Desai, C.A. and Pandya, K.D. (1982) Serum electrolytes during various phases of menstrual cycle. *Indian J. Phys. Pharmacol.*, 26: 302-306.
7. Brommage, R., Binaeuca, C. and Carrie, A.L. (1993) Ovulation associated increase in intestinal calcium absorption during the rat estrous cycle is blunted by ovariectomy. *Biol. Reprod.*, 49(3): 544-548.
8. Alagendran, S., Archuman, G. and Achiranman, S. (2007) Prediction of ovulation in women through the occurrence of salivary fern prototype. *ICEAI J. Life Sci.*, 1: 7-15.
9. Landau, R.L. and Lugibih, K. (1958) Inhibition of the sodium-retaining influence of aldosterone by progesterone. *J. Clin. Endocrin. Metab.*, 18: 1237-1245.
10. Patricia, A.D. (1987) Magnesium and zinc status during menstrual cycle. *Am. J. Obstet. Gynecol.*, 157: 964-968.
11. Ali, F., Lodhi, L.A., Qureshi, Z.I., Samad, H.A. and Shahid, R.U. (2004) Some serum biochemical constituents of mares during different phases of reproductive cycle. *Pak. Vet. J.*, 24(3): 147-152.
12. Pitkin, R.M., Reynolds, W.A., Williams, G.A. and Hargis, G.K. (1978) Calcium-regulating hormones during the menstrual cycle. *J Clin. Endocrin. Metab.*, 47: 626-632.
13. Silverberg, M. and Silverberg, R. (1956) Steroid hormones and bone. In: Bourne, G.H., editor. Biochemistry and Physiology of Bone. Academic Press, New York. p632-644.
14. Johnson, A.J., James, J.O., Baumber, J.S. and Schneider, E.G. (1970) Effect of estrogen and progesterone on electrolyte...
15. Mikkelsen, W.M., Dodge, H.J., Valkenburg, H. and Himes, S. (1965) The distribution of serum uric acid values in a population unselected as to gout and hyperuricaemia. *Am. J. Med.*, 39: 242-251.

16. Alagendran, S., Devi, C.A., Karthikeyan, K., Anulmozhi, N. and Pushpa, N. (2009) Evaluation of thyroid profile in human saliva with special reference to ovulation. *Res. J. Med. Sci.*, 4: 441-445.

17. Pattanasuttinont, S., Sereepapong, W. and Suwajanakorn, S. (2007) The salivary ferning test and ovulation in clo-miphene citrate-stimulated cycles. *Med. Assoc. Thai.*, 90: 876-883.

18. Oster, G. and Yang, S.L. (1972) Cyclic variation of sialic acid content in saliva. *Am. J. Obstet. Gynecol.*, 114: 190-193.

19. Yilmaz, O., Yazici, E., Kahraman, A., Ozenc, E. and Ucar, M. (2014) The relationship between ovarian follicle population and follicle size during different stages of estrous cycle in Anatolian Water buffaloes (*Bubalus bubalis*). *Rev. Méd. Vét.*, 165(3-4): 111-115.

20. Mondal, S., Suresh, K.P. and Nandi, S. (2010) Endocrine profile of oestrous cycle in buffaloes: A meta-analysis. *Asian Aust. J. Anim.*, 23(2): 169-174.

21. Pahwa, G.S. and Pandey, R.S. (1983) Gonadal steroid hormone concentration in blood plasma and milk of primiparous and multiparous pregnant and non pregnant buffaloes. *Theriogenology*, 19: 491-505.

22. Rao, T.K.S., Kumar, N., Kumar, P., Chaurasia, S. and Patel, N.B. (2013) Heat detection techniques in cattle and buffalo. *Vet. World*, 6(6): 363-369.

23. Suthar, V.S. and Dhami, A.J. (2010) Estrus detection methods in Buffalo. *Vet. World*, 3(2): 93-96.

**********