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SHORT COMMUNICATION

# Interrelationship between milk constituents, serum oestradiol and vaginal mucus indicators of oestrus in Egyptian buffaloes

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## Summary

The intensity of heat signs in buffaloes is generally low and the incidence of suboestrus varied from 15 to 73% (Buffalopedia). The objective of this study was to investigate the feasibility of monitoring the changes in some milk constituents, oestradiol levels and electrical conductivity of vaginal mucus during peri-oestrous period in prediction of the timing of oestrus in buffaloes. Twenty-one Egyptian buffaloes aged 3–9 year, 1st–6th lactations, were examined by oestrous detector and ultrasonographically for monitoring the ovarian and uterine activity for 7 days around the time of standing oestrus. Sodium, potassium, chloride and lactose were assayed in aqueous phase of milk; besides, oestradiol was estimated in serum. Current results declared highly significant acute changes in milk constituents at the time of oestrus characterized by peaking of chloride and sodium levels and lowering of potassium and lactose values. The alternation in milk composition when arranged in decreasing order of magnitude, sodium was the highest (77.78 ± 0.69%), followed by chloride (61.60 ± 1.52%) and potassium (−58.14 ± 10.89%). Concomitantly, milk lactose decreased by 26.07 ± 7.97% compared to baseline levels. Synchronously, vaginal electrical resistance (VER) showed a significant ($p < 0.01$) decrease, but serum oestradiol 17$\beta$ levels surged (59.93 ± 7.29 pg/ml) on day of oestrus. Serum oestradiol level was negatively correlated with VER ($r = -0.577$), potassium ($r = -0.661$), positively correlated with chloride ($r = 0.707$) and sodium ($r = 0.579$) and not correlated with lactose levels. These results for the first time suggested that the changes in constituents of milk during peri-oestrous period may be used as a practical non-invasive indicator for oestrous detection and prediction of ovulation in Egyptian buffaloes.

**Keywords** buffalo, oestrus, oestradiol, ionic constituents, milk, ovulation

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## Introduction

Development of accurate and practical methods for oestrous detection in buffaloes is critically needed. The success of oestrous detection by visual observation depends on both intensity of oestrous manifestation and frequency of observation, a finding that seemed to be more difficult in buffaloes than in cattle (El-Wishy et al., 1985; El-Wardani, 1990).

In buffalo, the presence of a teaser bull is helpful to identify buffaloes in heat; in this case, the standing oestrus is the most reliable sign referable to a next ovulation. Other heat detection aids utilized in buffalo include: pedometers; vaginal probes; pressure sensitive telemetry device. All the behavioural oestrous symptoms in buffaloes were observed during the period of oestrogen surge and ovulation occurred after 13.4 ± 1.0 h after end of total oestrogen surge (Mohan et al., 2010). Gupta and Purohit (2001) found a distinct relationship between vaginal electrical resistance (VER) and ovulation in buffaloes as they recorded a more pronounced decrease in VER from di-oestrus to oestrus that started to rise after ovulation. Using VER, an additional 36.6% of the buffaloes could be detected in oestrus proving that VER can be used successfully to predict the stage of oestrous cycle, ovarian status and ovulation.

To the best of the author's knowledge, there has been no study examined the changes in milk composition and the reproductive hormone interrelationship around the time of oestrus in buffaloes. In Holstein cows, sodium concentration in milk was estimated as

the only component that varies significantly during the 3 days around oestrus (days −1, 0 and +1) contrary to other milk component; however, none of these components appeared to be a practical indicator of oestrus (Cowan and Larson, 1979). In Jersey cows, Akdag et al. (2010) indicated that oestrus has a negative effect on milk yield without any remarkable effect on the milk composition. In goats, the changes in the composition of the aqueous phase of milk were noticed approximately four days preceding oestrus and characterized by an increase in sodium and chloride concentrations as well as a decrease in potassium and lactose, while fat, protein and immunoglobulin concentrations were not affected (Peaker and Linzell, 1974).

The aim of the present study aimed at monitoring the changes in some milk constituents (sodium, chloride, potassium and lactose), oestradiol levels and electrical conductivity of vaginal mucus during peri-oestrous period as predictors of the timing of heat onset in Egyptian buffaloes.

## Materials and methods

This study was conducted on a total number of 21 multiparous Egyptian buffaloes, 3–9 years old and 1–6 lactations, belonged to the Experimental Farm of Animal Production Department, Faculty of Agriculture, Minufiya University located in the Middle Nile Delta during the period from November 2010 to September 2011. All animals were fed a balanced ration consisting of concentrates as well as clover during winter–spring and crop residues during summer–autumn with free access to straw, mineral licks and water.

During a period of seven days centred around the oestrous onset (−3 ~0 and 0~ +3 days), detection of oestrus and prediction of ovulation were performed by daily observation, rectal palpation and ultrasonographic examination using a 6/8 MHz linear array transrectal transducer (Pie Medical 240 Vet®; Pie Medical Equipment B.V., Maastricht, Netherland) as well as monitoring the changes in the electrical conducting capacity of the vaginal mucus using Estrus Detector (Draminski ® Electronics in Agriculture, Owocowa, Polland). Buffaloes in oestrus (day 0) were characterized behaviourally by their acceptance of the male, frequent urination, bellowing, valval swelling and mucus discharge. Ultrasonographically, the onset of heat was verified by the existence of a large anechoic pre-ovulatory follicle (>1.5 cm) on the ovary parallel with the change in uterine echotexture (i.e. heterogeneous hypoechoic uterine wall) and an opened echogenic cervical canal on day 0.

Daily blood and milk samples were collected from all animals prior to each examination. Milk samples were collected and centrifuged consecutively at 3000 *g* at 4 °C for 15 min, and the aqueous phase of the milk was aspirated and stored at −20 °C until analysed for its lactose, sodium, chloride and potassium content. Lactose (%) was examined in milk by means of an automatic milk analyser (Lactoscan; Milkotronic, Bolgharia). Sodium and potassium were evaluated as ppm and converted to g/ml in digested milk samples using flame photometer. Chloride (g/ml) was analysed by 0.02 silver nitrate titration.

Blood samples (6 ml) were collected by jugular vein puncturing into clean dry test tubes, and sera were separated by centrifugation at 3000 *g* for 20 min within 2–4 h of collection and stored frozen at −20 °C until assayed for oestradiol using solid-phase ELISA kits (DRG International, Marburg, Germany).

Data were collected, normalized to day of oestrus (day 0 = day of oestrus), tabulated and statistically analysed to compute the relationship between oestradiol hormone and some milk components as well as changes in electrical conducting capacity of vaginal mucus. Daily mean values for each estimate were compared according to least square with one-factorial ANOVA using SPSS program (version 16). $p < 0.05$ was considered statistically significant.

## Results

As shown in Fig. 1, on the day of oestrus, there was a highly significant ($p < 0.0001$) increase in levels of chloride ($0.89 \pm 0.03$ g/l) and sodium ($0.67 \pm 0.020$ g/l) contrary to that detected in levels of potassium ($0.040 \pm 0.003$ g/l) and lactose ($2.99 \pm 0.020$ g/l) of buffalo milk as compared to their recorded baseline levels (0.41 g/l, 0.197, 0.09 g/l, 4.11 g/l respectively). Such changes in milk constituents were parallel with the behaviourally standing oestrus and changes in the electrical conducting capacity of the vaginal secretions, which showed a significant ($p < 0.01$) decline by $29.87 \pm 1.16\%$ ($202.86 \pm 11.84$ Ω) matched to the baseline recordings (294 Ω) on the day of oestrus (day 0). The existence of a large anechoic pre-ovulatory follicle (>1.5 cm) on the ovary was detected by the ultrasound examination, which came in parallel with the change in uterine echotexture (i.e. heterogeneous hypoechoic uterine wall) and an opened echogenic cervical canal as well as an increased serum levels of oestradiol 17$\beta$ ($59.93 \pm 7.29$ pg/ml vs. 33.7 pg/ml (baseline value)) on day 0 that declined abruptly thereafter.

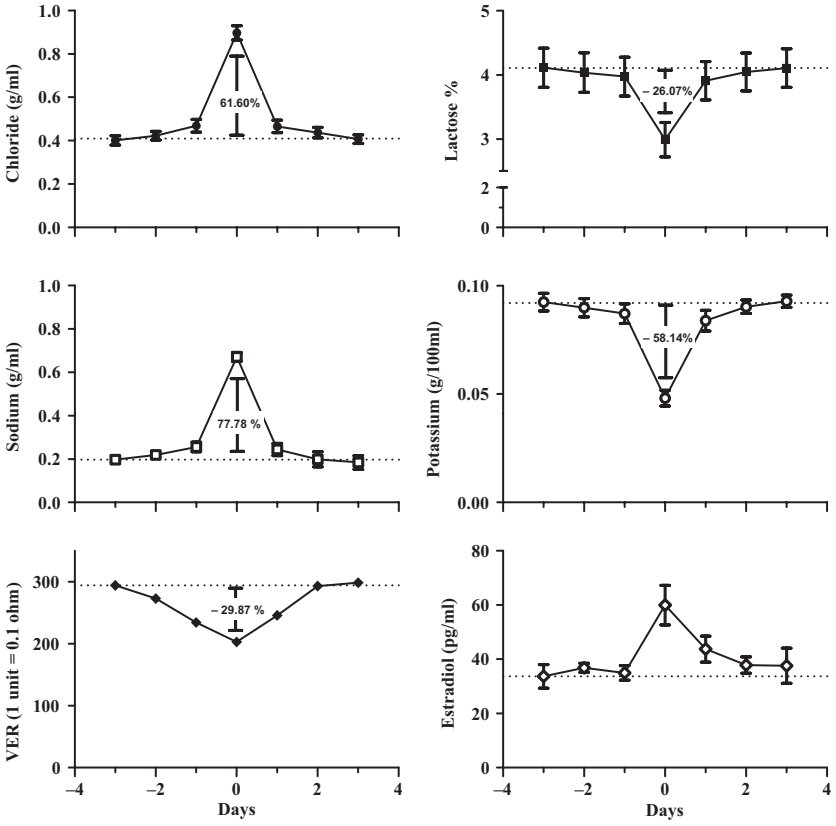


**Fig. 1** Changes in ionic milk constituents, serum oestradiol levels and vaginal electrical resistance (VER) during the period around oestrous period in Egyptian buffaloes. Levels of chloride (●), sodium (□), lactose (■) and potassium (○) were measured in the aqueous phase of milk. Oestradiol (◇) was measured in serum. VER (♦) indicated VER measured with Estrous Detector. Data were normalized to the day of oestrus (day 0). The percentage referred to the degree of acute change compared to basal line (horizontal dotted line).

Pearson correlation analysis (Table 1) demonstrated significant negative correlation between serum oestradiol and VER and potassium concentration ($r = -0.577$ and $-0.661$, $p < 0.01$, respectively), but positive correlation with chloride and sodium ($r = 0.707$ and $r = 0.579$, $p < 0.005$, respectively) and no correlation with lactose levels measured in milk. Sodium and chloride levels in milk were positively correlated ($r = 0.893$). Chloride was negatively correlated with lactose and potassium ($r = 0.302$; $p < 0.05$, and $r = -0.697$; $p < 0.01$ respectively).

**Table 1** Correlation between changes in ionic milk constituents, serum oestradiol levels and vaginal electrical resistance (VER) measured during peri-oestrous period in Egyptian buffaloes

| | Chloride | Lactose | Sodium | Potassium | Oestradiol |
|---|---|---|---|---|---|
| VER | −0.736** | 0.379** | −0.744** | 0.678** | −0.577** |
| Chloride | | −0.302* | 0.893** | −0.697** | 0.707** |
| Lactose | | | −0.446** | 0.626** | −0.232 |
| Sodium | | | | −0.845** | 0.579** |
| Potassium | | | | | −0.661** |

*,**Correlation (Pearson correlation) is significant at the 0.05 and 0.01 level (2-tailed) respectively. VER, vaginal mucus electrical conductivity measured with Estrus Detector.

## Discussion

The single most important problem limiting high reproductive efficiency in the national dairy herd is the poor detection of oestrus (Senger, 1994). Development of accurate and practical methods of oestrous detection in buffaloes is critically needed. The success of oestrous detection by visual observation depends on both intensity of oestrous manifestation and frequency of observation. In the current study, it has been suggested that the changes in some milk constituents, for example, sodium, potassium, chloride and lactose can be used to predict the timing of ovulation in Egyptian buffaloes. Generally, it has been found that detection of the oestrous behavioural pattern is more difficult in buffaloes (El-Wardani, 1990) than in cattle (El-Wishy et al., 1985).

The current study indicated the presence of a significantly higher chloride and sodium as well as lower potassium and lactose concentrations in milk of buffaloes at the onset of heat. This finding might suggest the presence of a close relationship between these milk components particularly sodium, oestradiol level and the final follicular growth on the ovary and buffaloes' behaviour at time of oestrous onset. At approximately 6 days before ovulation, the size of the pre-ovulatory follicle begins to increase, with the largest follicle growth just before ovulation (Bomsel-Helmreich et al., 1979). Similarly, oestradiol levels, which are markers for follicle growth (Baird and Fraser, 1974), begin to increase in both follicular fluid and plasma at this stage. Based on this incidence, the first change in milk composition seems to have a relationship to the events associated with the final stage of folliculogenesis. Similarly, some studies indicated that the milk sodium concentration was the only component that varied significantly during the 3 days around oestrus (Cowan and Larson, 1979). Intensive studies on the secretion of milk during established lactation in goats indicated the occurrence of a change in lactose and ionic composition of milk approximately four days prior to oestrus (Peaker and Linzell, 1974) due to the formation of a paracellular pathway (i.e. leaky 'tight' junctions between the mammary epithelial cells) under the influence of the hormones associated with the oestrous cycle (Peaker, 1976).

Results in the current study showed that oestradiol hormone correlated negatively with VER, lactose and potassium concentration and correlated positively with chloride and sodium. The negative correlations between the concentrations of lactose and the ions in milk during this time suggest that these constituents are transported from the secretary cells into milk by the transcellular pathway (Peaker, 1976) that perhaps controlled by ovarian steroid hormones.

In conclusion, estimation of some milk constituents (sodium, chloride, potassium and lactose) guided with measuring the vaginal electrical conducting capacity can be used for screening purposes of heat detection and prediction of the timing of ovulation in the Egyptian buffaloes that is likely to be meaningful in animals suffering from silent heat.

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