

# Monitoring Vaginal Electrical Impedance in Bangladeshi Water Buffaloes During Postpartum Period

Nasrin Sultana Juyena<sup>1,\*</sup>, Mahmudul Hassan<sup>1</sup>, Habibur Rahman Mollah<sup>1</sup> and Shushanto Kumar Rabidas<sup>2</sup>

<sup>1</sup>Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

<sup>2</sup>Lal Teer Animal Breeding House, Lal Teer Livestock Development Limited, Bangladesh

**Abstract:** The achievement of early cyclicity and pregnancy after calving are of vital importance for sustainable buffalo farms, for which proper detection of oestrus and correct time of AI are necessary. Vaginal electrical impedance (VEI) observation using heat detector was used to identify estrus in buffalo cows during post-partum period. In the first group, 12 buffalo cows with post-partum >300 days were synchronized with OVSYNCH protocol and VEI was recorded at different stages of synchronization protocol. VEI values decreased significantly ( $P < 0.01$ ) during second administration of GnRH in both pregnant and non-pregnant buffalo cows and it was indicative for the presence of oestrus. Higher VEI values were observed at second administration of GnRH and AI in non-pregnant group than the pregnant group. In second group, 8 buffalo cows with a post-partum period 40 to 96 days were used to record VEI daily. Lower VEI values (260-350 $\Omega$ ) were observed during oestrus and then started to increase at higher level after oestrus. The day difference between two successive lower/higher values of "VEI waves" ranged from 17-25 days, which might indicate the oestrous cycle in buffalo cows. Moreover, physical signs such as colour of vulva, mucus were less intensive in buffalo cows of second experiment. The study suggests that the measurement of vaginal impedance could serve as an indicator of the oestrus in buffalo cows and daily impedance measurements are necessary to confirm the stages of the estrous cycle.

**Keywords:** Buffaloes, vaginal electrical impedance, oestrus, post-partum period.

## INTRODUCTION

Buffaloes act as a reliable 'living bank' to serve the immediate needs of the rural masses in several communities in Bangladesh. Despite its importance as a tool for economic growth and poverty alleviation in rural areas of Bangladesh, they are much neglected till now. Buffaloes of Bangladesh have been recognized to possess low reproductive performance [1]. Buffalo cows rarely show mounting behavior [2] and variable duration of estrus (6-64 h) making the prediction of time of ovulation difficult. Moreover, the period of postpartum anoestrus is usually longer in buffalo than in cattle under comparative management conditions [3]. Postpartum anoestrus is a major constrain for successful breeding program in dairy herd. This condition is very serious in buffalo cows due to covert sign of oestrus. Farm personnel can detect heat in about 20-35% of animals. It is stated that high percentage of buffalo cows show silent oestrus, the longer period between calving and postpartum cyclicity [4]. Silent oestrus is a common problem in buffaloes even under good management and non-stressful periods of the year [5]. Moreover, the different duration of oestrous (4 to 64 h) impedes the exact detection of ovulation and results in limited application of AI in buffaloes [6]. To overcome this problem oestrus

detector can be used to observe vaginal electrical impedance (VEI). Understanding and investigating VEI "waves" as a functional unit during the oestrous cycle, could facilitate the development of value indicating oestrus.

The Oestrous Detector monitors changes in electrical resistance of the vaginal mucus. Oestrous Detector is responsive to the hormonal changes that coincide with oestrus and is a much simpler and more efficient process [7]. Differences in VEI readings resulted from the oestrous cycle correspond to its phases, providing vital information for efficient and effective breeding. However, there is little study on VEI in water buffaloes in the world. Moreover, this is first study on VEI of buffaloes in Bangladesh. The present experiment was performed to study VEI at different stages of OVSYNCH synchronization protocol and to monitor VEI to detect oestrus and oestrous cycle in buffalo cows during post-partum anoestrus.

## MATERIALS AND METHODS

Twenty Bangladeshi water buffaloes with post-partum anoestrus were used. Buffaloes were divided into two groups. In group 1, twelve water buffalo cows with postpartum period more than 365 days (according to owner complain) were selected from Kanihari village of Trishal upazila, Mymensingh, Bangladesh and synchronized during September 2013. The body condition score of the buffalo cows varied from 3.0 to

\*Address correspondence to this author at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; Tel: +8801759674267; Fax: +8809161510; E-mail: juyenahabib@yahoo.com

4.0 (1.0 to 5.0 scale) and they were free from any detectable reproductive problems. The age of the buffaloes ranged from 5 to 12 years and their parity ranged between 1 and 4. The Ovsynch protocol was administered regardless of the stage of oestrous cycle in animals. Each animal was treated with hormones at 5.00 PM afternoon. For oestrous induction, GnRH analogue-Gonadorelin (5ml Ovurelin inj., Bomac Laboratories Ltd., New Zealand) was administered at D0. PGF<sub>2</sub> -Cloprostenol(2ml Ovuprost inj., Bomac Laboratories Ltd., New Zealand) at D 7, which was followed by injection of GnRH (2.5ml Ovurelin inj.) at D9. All hormones were injected intramuscularly in hip region. At 20-22 hours after second gonadorelin injection, AI of buffaloes was carried out by a trained AI technician using frozen semen from an Italian Mediterranean bull of known high fertility. The frozen semen was imported from Italy (COFA, Agricultural Cooperative Society, Orezola – 26048, Tidolo sigh of Cremona, Italy). Post-thaw sperm motility was 50-60%. Fixed time AI was done without considering the onset of prominent oestrus signs. However, some oestrous signs were observed such as, reddish vestibules, swollen vulva with marked edema in vulva lips and clear mucus hanging from the vulva. VEI was measured with Draminski<sup>®</sup> probe after administration of PG2 $\alpha$ , after administration of second dose of GnRH and before AI for proper detection of oestrous condition in buffaloes. Pregnancy diagnosis was performed by observing embryos with transrectalul trasonography using real-time B-mode ultrasound (Tringa Linear VET<sup>®</sup>, Esaote Pie Medical, Genova, Italy) equipped with a 7.5 MHz linear probe, between Day 40-45 after insemination.

In group-2, eight buffalo cows with postpartum period from 40 to 96 days were selected from a private farm "LalTeer Animal breeding House", of LalTeer Livestock Development Limited at Bhaluka, Mymensingh, Bangladesh. The impedance was measured once daily up to 37 days. Prior to using the probe of the estrous detector was washed in 1% potassium per manganate (PPM) solution. Then the

probe was inserted into the vagina and was placed aside either left or right to take reading from the vaginal wall. The reading was visible on the screen. Reading was taken three times and the average of three reading was noted. Care was taken to insert the probe at same length each time. To maintain the same length a mark was given on the probe by a marker.

### Statistical Analysis

All data of Group-1 were analyzed in GLM (General Linear Model) in statistical software SPSS 12. Difference in values were measured by two ways ANOVA (Analysis of Variance), where treatment for synchronization protocols were considered as independent variable and values of VEI and pregnancy rate were considered as dependent variables. Differences were considered significant at  $P < 0.05$ . Values were presented as means and S.E.M. For Group-2 descriptive analysis was performed.

### RESULTS

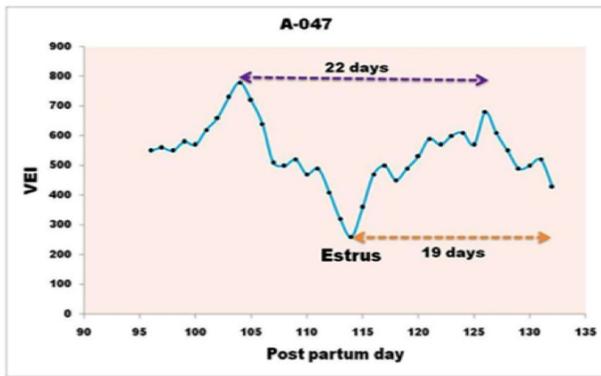
This is the first study on VEI of buffalo cows by heat detector in Bangladesh. VEI values recorded at different stages of the oestrus synchronization protocol are presented in Table 1. It was observed that VEI values decreased very significantly ( $P < 0.01$ ) during second administration of GnRH in both pregnant and non-pregnant buffalo cows. Whereas, VEI values increased significantly ( $P < 0.05$ ) in pregnant animals during AI. On the other hand, VEI values slightly increased at the time of AI non-pregnant (Table 1). Moreover, VEI recorded before administration PGF2 $\alpha$  was comparatively higher in pregnant buffaloes ( $403.5 \pm 18.2 \Omega$ ) in comparison to non-pregnant buffaloes ( $310.1 \pm 15.7 \Omega$ ). In contrast, higher VEI values recorded during second administration of GnRH and AI in non-pregnant buffaloes compared to pregnant buffaloes.

Typical changes in VEI values of individual buffalo cow during study period are presented in Figures 1-8.

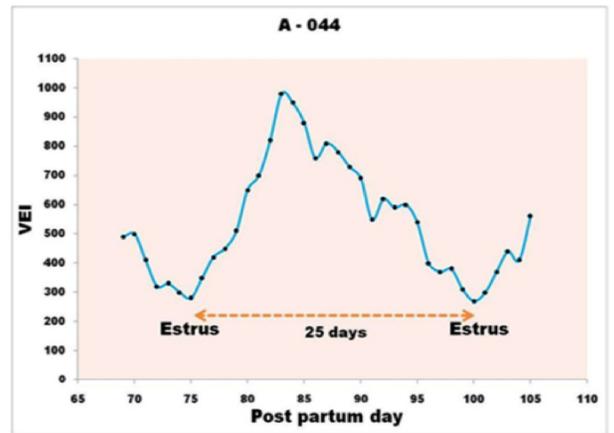
**Table 1: Values (mean  $\pm$  SEM) of Vaginal Electrical Impedance (VEI-mm  $\Omega$ ) in Pregnant and Non-Pregnant Buffaloes in Group-1**

Treatment	Pregnant (58.3%)	Non pregnant (41.7%)	P values
Administration of PGF	403.5 $\pm$ 18.2 <sup>***a</sup>	310 $\pm$ 15.7 <sup>a</sup>	0.002
Administration of GnRH	180.5 $\pm$ 7.2 <sup>b</sup>	216.1 $\pm$ 10.3 <sup>**b</sup>	0.004
During AI	200 $\pm$ 19.2 <sup>c</sup>	221 $\pm$ 11.7 <sup>*b</sup>	0.04

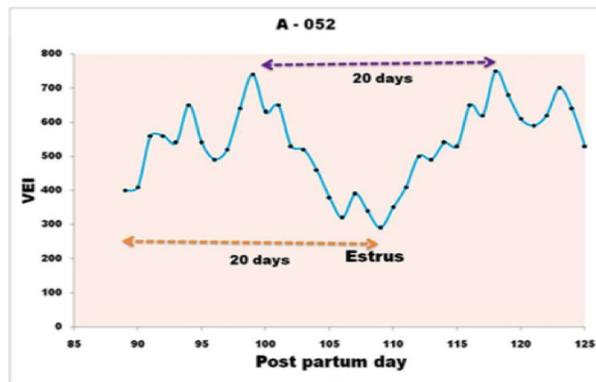
Symbol indicates significant difference between columns; a,b,c indicate significant difference within rows. \*significant at  $P < 0.05$ , \*\* significant at  $P < 0.01$ .



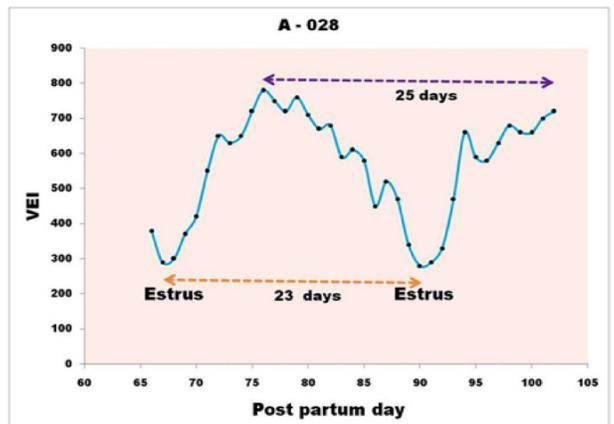
**Figure 1:** VEI was recorded from 96 days postpartum in buffalo No-047 and VEI was 550 Ω. AT 104 postpartum it increased to 780 Ω and then started to decrease and reached at lowest value 260 Ω at 114 postpartum when oestrus was observed. Then again increased to 680 Ω at day 126. Day difference between two highest peaks was 22 days which might indicate oestrous cycle of this buffalo.



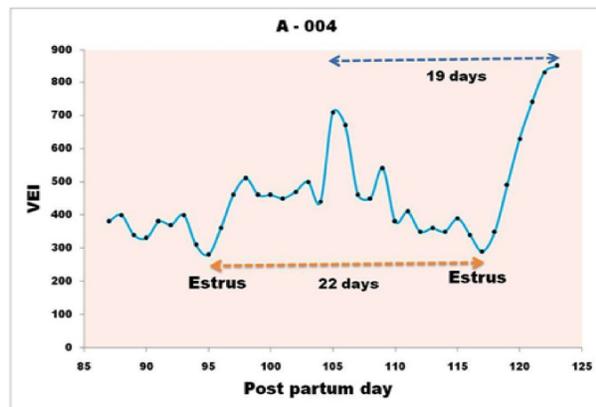
**Figure 4:** VEI was recorded from 69 days postpartum in buffalo No-044 and VEI was 490 Ω. AT 75 postpartum it decreased to 280 Ω and then started to increase and reached highest peak 980 Ω at 83 postpartum. Then VEI value decreased to 270 Ω at day 100. Day difference between two lowest peaks was 25.



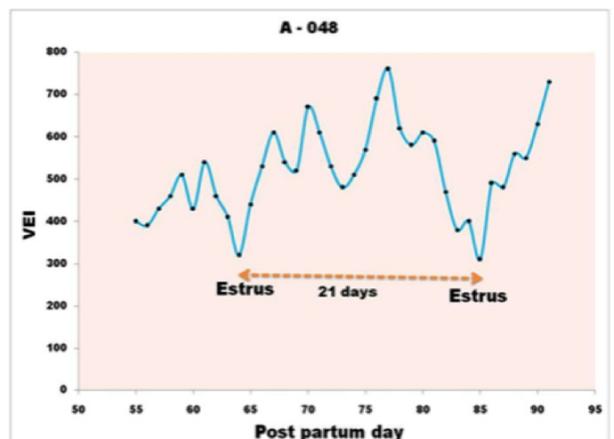
**Figure 2:** VEI was recorded from 89 days postpartum in buffalo No-052 and VEI was 400 Ω. AT 99 postpartum it increased to 740 Ω and then started to decrease and reached at lowest value 290 Ω at 109 postpartum when buffalo was in oestrus. Then again increased to 750 Ω at day 118. Day difference between two highest peaks was 20 days.



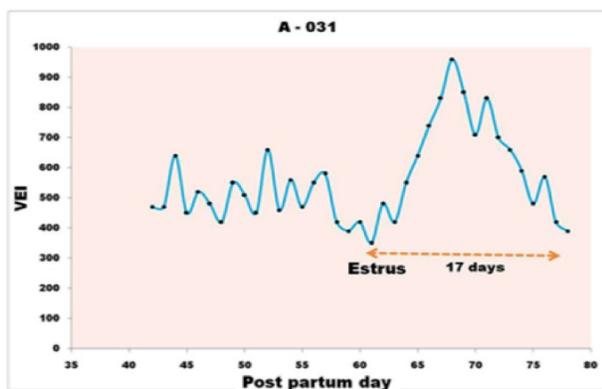
**Figure 5:** VEI was recorded from 66 days postpartum in buffalo No-028 and VEI was 380 Ω. AT 67 postpartum it decreased to 290 Ω when buffalo was in heat. The second lower VEI value was 280 Ω observed at day. Day difference between two lower values was 23.



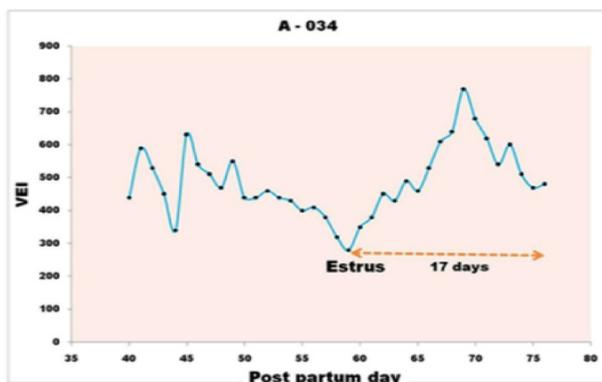
**Figure 3:** VEI was recorded from 87 days postpartum in buffalo No-004 and VEI was 380 Ω. AT 95 postpartum it decreased to 280 Ω when buffalo was in oestrus and then started to increase and reached highest peak 710 Ω at 105 postpartum. Then VEI value reached to 290 Ω at day 117. Day difference between two lowest values was 22.



**Figure 6:** shows VEI values recorded from 55 days postpartum in buffalo No-048 and VEI was 400 Ω. Two lower values were 320 Ω and 310 Ω. Day difference between two lower values was 21.



**Figure 7:** VEI was recorded from 42 days postpartum in buffalo No-031 and VEI was 470  $\Omega$ . AT day 61 postpartum it decreased to 350  $\Omega$  when buffalo was in heat. Oestrus was observed at day 78 when lower value was 390  $\Omega$ . Day difference between lower values was 17.



**Figure 8:** VEI was recorded from 40 days postpartum in buffalo No-034 and VEI was 440  $\Omega$ . AT day 59 postpartum it decreased to 280  $\Omega$  and then started to increase and reached highest peak 770  $\Omega$  at day 69 postpartum. Then again fallen down at day 76 to 480  $\Omega$ . Day difference between two lower values was 17.

In general, lower VEI values were observed during oestrus and then increased to a high level after oestrus. Resistance remained high near the next heat and then it dropped again. This is worthy to mention that daily VEI values had been observed daily up to 37 days to record the minimum level of resistance and the moment when a distinct rise in resistance occurred. The day difference between two successive lower/higher peaks in VEI waves ranged from 17-25 days which might indicate the oestrous cycle in buffalo cows. Two lower values were observed in buffalo No. 004, 044, 028, 048, 031 and 034, whereas, higher two peaks were considered in buffalo No. 047 and 052. Lower VEI values (260-350 $\Omega$ ) recorded in Group-2 supposed to be indicative of oestrus and was higher when we compared to VEI observed at the administration of second GnRH in the Group-1. Individual variation was existed. In addition, the

intensity of heat occurred was also recorded. Physical signs i.e. changes in vulva colour and mucus were less marked in buffaloes of Group-2.

## DISCUSSION

Heat detection technique based on animal behaviour can produce both false positives and false negatives [8]. Most detection methods are not suitable in herd practice in Bangladesh. Detection method should combine robustness, cost and herd feasibility. For this reason, the experiments were performed to study VEI with heat detector probe to monitor oestrus and oestrus cycle in buffalo cows during post-partum. In Group-1, it was observed that VEI decreased very significantly ( $P < 0.01$ ) during second administration of GnRH and increased during AI. Variation existed in VEI values of pregnant and non-pregnant buffaloes recorded at different stages of synchronization protocol. It is well known that oestrus occurs 24-48 hrs after intramuscular injection of PGF<sub>2</sub> in cattle as well as buffaloes. In OVSYNCH protocol, second dose of GnRH was administered 48 hrs after injection of PGF and Lower VEI recorded this stage indicated presence of oestrus. Presence of swollen and reddish vulva and mucus strand supports this finding. There is no precise report available on VEI values of buffaloes. Not only that, this is the first study on VEI with two electrodes technique in Bangladesh. We observed that VEI values ranged from 260  $\Omega$  to 350  $\Omega$  when buffalo was in heat. Research shows that cervical mucus increases in amount and becomes more thinner with elastic during the follicular phase of the oestrous cycle as reported in ewes [9]. During oestrus, oestrogen level rises leading to increased production of adrenocorticotrophic and aldosterone hormones. These hormones increase the levels of NaCl in the vaginal mucous resulting in a significantly lower level of electrical resistance than usual [10]. However, Gupta and Purohit [11] have revealed that vaginal impedance is lowest during the follicular phase and highest during the luteal phase of the oestrous. An inverse relationship exists between vaginal impedance and serum oestradiol in the follicular phase [12]. Moreover, we observed higher VEI values recorded during second administration of GnRH and AI in non-pregnant group compared to pregnant group. There is paucity of data regarding VEI values in indigenous water buffaloes in Bangladesh. Gupta and Purohit [11] reported that AI at a low VEI distinctly improved the conception rate in buffaloes.

Typical changes in VEI of individual buffalo cows were observed daily up to 37 days to record the

minimum level of resistance and the moment when a distinct rise in resistance occurred. It supports the statement of Aizinbud *et al.* [13] who stated that daily impedance measurements are necessary to confirm the stages of the oestrous cycle. The difference between two successive lower and/or higher VEI values ranged from 17-25 days, which might indicate the oestrous cycle length in buffalo cows. Result corresponds well with the finding of Jainudeen and Hafez [3] who reported that the duration of the oestrous cycle in buffalo ranged from 17 to 26 days with a mean of around 21 days. Lower VEI values recorded in Group-2 supposed to be indicative of oestrus and was higher when compared to VEI observed at the time administration of second GnRH in Group -1. It is important to point that buffaloes were milked twice per day during the study period. Lactation may increase circulating progesterone concentrations [14] that, in turn, may decrease GnRH-induced LH secretion [15] producing the decreasing ovulation efficiency.

Heat Detector monitors changes in electrical resistance of the vaginal mucus. Measurements are taken simply by properly inserting the probe in the vagina and taking the reading. No prior technical knowledge on the part of the operator is required. Estrous Detector responsive to the hormonal changes and can replace the more costly procedure of progesterone-based pregnancy detection [7]. The impedance technique is one of the tools that could be used for non-invasive monitoring of events occurring in cyclic animals [8]. Electrical impedance is measured by introducing a small alternating electrical current in the limited area of the cervix uteri, vagina, vaginal vestibule or vulva and measuring on its surface the electrical potential difference [11,16]. This is worthy to consider some physical factors such as, instability of the contact between the vaginal mucosa and electrodes due to the changes in the tone of the vagina, the entrapment of air in the vagina during insertion of an electrode-bearing probe or unequal hand pressure during taking impedance measure [17].

Externally detectable physical changes around the time of oestrus include swelling of the vulva and reddening of the vestibular mucosa. Mucus secreted from the cervix during oestrus is less copious in buffalo than in cattle. The concentration of oestradiol-17 $\beta$  in blood during the follicular phase of the oestrous cycle also appears to be relatively less in buffalo than that in cattle [18] and this has been suggested as a possible reason for the lesser intensity of oestrus exhibited by buffaloes. Considering these perspective, the

measurement of vaginal impedance could serve as an indicator of the follicular and luteal status in cyclic buffaloes.

In this study, the vaginal length was not measured where detector was placed. There might be differences among VEI values taken from different anatomical site of female reproductive tract in buffalo, which need to focus in future. Moreover, hormonal profiles (oestrogen and progesterone level) were not measured to conclude if there is any relationship between VEI and hormones is existed. Moreover, future study should be directed to identify factors that may affect the variability of impedance values in the vagina during the oestrous cycle in buffalo cows. Further study is in progress to detect definite range of VEI, which would allow us to improve our ability to predict heat more precisely by impedance techniques in buffaloes.

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