

## STUDY ON EFFICACY OF COSYNCH AND OVSYNCH PROTOCOLS ON FERTILITY IN REPEAT BREEDER BUFFALOES

**Srikant Biradar, M.K. Tandle\*, M.D. Suranagi, S.M. Usturge,  
N.A. Patil and Y. Hari Babu**

### ABSTRACT

The objectives of the present study were to compare efficacy of Cosynch and Ovsynch estrus synchronization protocols on fertility in repeat breeder buffaloes, to study vaginal electrical resistance in various stages of estrous cycle and post insemination until pregnancy diagnosis and its relation and to estimate the biochemical constituents in cervico-vaginal mucus and its correlation with vaginal electrical resistance and pregnancy rate. A total of 24 non-descript repeat breeder buffaloes below 10 years of age were divided equally into three groups and subjected to Cosynch, Ovsynch protocols and a control group and were timely inseminated with fertile semen. Cosynch and Ovsynch protocols exhibited estrus signs (100%) within 48 to 72 h after PGF $2\alpha$  injection. Out of 8 repeat breeder buffaloes 3 became pregnant (37.50%) in Cosynch and 4 buffaloes became pregnant (50.00%) in Ovsynch groups respectively.

In control group, out of eight repeat breeder buffaloes 2 became pregnant (25.00%) without any treatment. The VER values were lowest after 12 h from onset of estrus in all the repeat breeder buffaloes. The repeat breeder buffaloes with mean VER value of  $219.26\pm 4.64$  at the time of insemination confirmed to be pregnant

than compared to the non-pregnant buffaloes with mean VER values of  $239.30\pm 5.59$  at the time of insemination. The VER values may be used to know the pregnancy status in buffaloes on day 8 to 9 post A.I. ( $317.78\pm 13.21$  vs.  $309.30\pm 12.28$ ) in pregnant vs. non-pregnant buffaloes. The VER values rose from  $317.78\pm 13.21$  on day 8 to 9 continuously up to  $363.33\pm 7.99$  on day 45 in pregnant buffaloes whereas the same VER values  $309.30\pm 12.28$  on day 8 declined up to  $282.00\pm 12.39$  on day 45 in non-pregnant buffaloes.

**Keywords:** vaginal electrical resistance, buffaloes, repeat breeders, Cosynch, Ovsynch, estrus synchronization

### INTRODUCTION

One of the most important and commonly encountered sub fertile conditions in buffalo that plays a vital role in dairy economics is repeat breeding. The condition may occur due to defects in gametes, failure of gametic encounters, endocrine dysfunctions, infection, nutritional defects etc., which ultimately leads to either fertilization failure or early embryonic death (Ahmed *et al.*, 2010). Optimum fertility in repeat breeder buffaloes can be achieved by improving management nutrition and

application of advanced reproductive technologies (Dhami *et al.*, 2009).

During last two decades, considerable attention has been focused on reproductive endocrinology of buffaloes, with the aim of developing models to improve reproductive efficiency, particularly when using controlled breeding techniques. The utilization of schedules for estrus and ovulation synchronization could partly eliminate the aforementioned problems and contribute to the broader use of AI for improving reproductive and productive traits in buffalo species (Presicce *et al.*, 2005; Baruselli *et al.*, 2007).

Apart from the weak manifestation of behavioral estrus, scanty mucus discharge in the buffaloes substantially contributes towards the difficulty of visible detecting estrus. Automated systems of detection of estrus are being developed using electronic technology such as pedometry, radiotelemetry and vaginal electrical resistance probe. The use of a vaginal electrical resistance (VER) probe to predict oestrus and ovarian activity has been studied by Gupta and Purohit (2001) in Indian buffaloes. They proved that, VER can be used successfully to predict the stage of oestrus cycle, ovarian status and ovulation; insemination at low VER distinctly improved the conception rates in buffaloes. Studies on the properties of vaginal mucus have established that, lesser electrical resistance of vaginal mucus at estrus period. During oestrus there is a rise in the volume and ionic content of vaginal mucus, consequently the electrical resistance of vaginal mucus increases.

Platt *et al.* (1968) stated that, electrolytes of the cervical mucus were very important for promotion of sperm viability and transport. Biochemical examination of cervical mucus gives an idea about the reproductive changes during the different phases of the oestrus cycle and

suggests opportunities to increase the reproductive efficiency of buffaloes (Eltohamy *et al.*, 1990).

There is paucity of literature on application of Cosynch and Ovsynch in repeat breeder buffaloes and in vaginal electrical resistance values, early detection of pregnancy using vaginal electrical resistance and biochemical constituents in cervico-vaginal mucus in repeat breeder buffaloes, so an attempt was made. The objectives of the present study were to compare efficacy of Cosynch and Ovsynch estrus synchronization protocols on fertility in repeat breeder buffaloes, to study vaginal electrical resistance in various stages of estrous cycle and post insemination until pregnancy diagnosis and its relation and to estimate the biochemical constituents in cervico-vaginal mucus and its correlation with vaginal electrical resistance and pregnancy rate.

## MATERIALS AND METHODS

A total of 24 non-descript repeat breeder buffaloes below 10 years of age were divided equally into three groups and subjected to Cosynch, Ovsynch protocols and a control group and were timely inseminated with fertile semen. The vaginal electrical resistance (VER) readings were measured daily by estrus detector probe from all the non-descript repeat breeder buffaloes from the day of GnRH injection until day 45 post insemination. The cervico-vaginal mucus from all the non-descript repeat breeder buffaloes were collected and subjected for estimation of calcium, total proteins, inorganic phosphorus, chloride and sodium.

## RESULTS AND DISCUSSION

### Efficacy of cosynch and ovsynch protocols

In the present study, on attempting fixed time insemination by Cosynch protocol, pregnancy rate was found to be 37.50%. The findings are almost similar as reported by Pursley *et al.* (1998) 37.00% in lactating dairy cows. Most field trials indicate only a small reduction in conception rates when Cosynch is compared to Ovsynch (Pursley *et al.*, 1998; Geary *et al.*, 2001). Estrus induction response in repeat breeder buffaloes treated with Ovsynch protocol in the present study is 100% within 48 to 72 hours of prostaglandin injection, which is similar to the results of similar to results of Chaikhun *et al.* (2010) wherein, by Ovsynch protocol in swamp buffaloes the induction of estrus was 100%. All the repeat breeder buffaloes of both treatment groups shown estrus within 48 to 72 h post PGF<sub>2α</sub> injection and these results are similar as observed by Gupta *et al.* (2008) wherein all the cows and buffaloes were in heat 72 h post PGF<sub>2α</sub> injection and cows or buffaloes in early and late stages of the cycle tend to exhibit heat within 48 to 72 h after PGF<sub>2α</sub> administration. In the present study, on attempting fixed time insemination by Ovsynch protocol, pregnancy rate was found to be 50.00%. The findings are almost similar as reported by Neglia *et al.* (2001) and Baruselli (2001) 48.80% in buffalo cows; De Rensis *et al.* (2005) 43.70% in Mediterranean Italian buffalo; Chaikhun *et al.* (2010) 51.40% in Swamp buffaloes cows and Baruselli *et al.* (2003) 51.00% in multiparous buffaloes. Higher conception rate has been documented, as 56.50% by Berber *et al.* (2002) in buffaloes. Low conception rates has also been documented by many research workers after conducting ovsynch protocol viz. 33.30%, 36.00% and 18% by Paul and Prakash (2005) in Murrah

buffalo; Neglia *et al.* (2003) in Italian cyclic buffalo.

### Vaginal electrical resistance values after PGF<sub>2α</sub> injection in non-descript repeat breeder buffaloes

The mean VER values in Cosynch and Ovsynch groups on the day (0 h) of PGF<sub>2α</sub> injections were 295±12.95 and 272±13.46 respectively. The fall in VER was rapid and occurred one day after PGF<sub>2α</sub> injection for all non-descript repeat breeder buffaloes and the mean VER values after 24 h PGF<sub>2α</sub> injection were 252.5±4.53 and 256.25±9.05 in Cosynch and Ovsynch groups respectively.

The statistical analysis shows that, in Cosynch group there is a significant difference between the 0 h to 24 h, 48 h and 72 h, but no significant difference observed at 24 h to 48 h and also in 48 h and 72 h. In Ovsynch group, there is significant difference observed between 0 h and 72 h however, there was no significant difference observed between 24 h, 48 h and 72 h. Mean VER was highest at PGF<sub>2α</sub> injection and significantly declined at estrus and within day differences (12 h interval) in VER were not significant. There is paucity of references on the present topic. The lowest VER values were recorded at 72 h in both Cosynch group and Ovsynch group with mean values of 218±4.79 and 220±10.85 respectively. These results are similar to results reported by Gupta and Purohit (2001) in buffaloes, wherein the VER around estrus after prostaglandin injection show a slight fall after 24 h but steep fall in next 24 h and with lowest VER synchronous to estrus and also similar to the results reported by Cevik *et al.* (2010) in his study he found that, the vaginal electrical resistance measurements in Ovsynch group were lower (P<0.05) at day 10 than during 7<sup>th</sup> day.

### **Vaginal electrical resistance values during estrus period at 6 h interval in all repeat breeder buffaloes**

The mean VER values were gradually decreased from onset of estrus to 12 h from onset of estrus in all the three groups. Then, later VER values gradually increased up to 30 h after onset of estrus in Cosynch, Ovsynch and control groups. The VER values were lowest after 12 h from onset of estrus in all the non-descript repeat breeder buffaloes of all the groups. The statistical analysis shown that, in Cosynch group there was a significant difference in VER values between 30 h with 0 h, 6 h, 12 h and 18 h and also in VER values between 24 h with 6 h and 12 h and between 0 h and 12 h. In contrast, there was similar trend in VER values in Ovsynch and control groups but statistical evidence shows that, difference was non-significant. There is paucity of references on the present topic.

### **Vaginal electrical resistance values during different stages of estrus cycle in non-descript repeat breeder buffaloes**

The mean VER values in proestrus stage were  $273.75 \pm 08.33$ ,  $264.37 \pm 10.62$  and  $285.62 \pm 08.78$  and these values were decreased in estrus stage to  $223.12 \pm 02.30$ ,  $235.00 \pm 07.44$  and  $238.75 \pm 03.75$  in Cosynch, Ovsynch and control groups respectively. Further, the mean VER values in metestrus stage were  $290.00 \pm 08.60$ ,  $285 \pm 12.25$  and  $266.75 \pm 10.32$  and these values were raised in diestrus stage to  $326.63 \pm 11.54$ ,  $311.82 \pm 11.41$  and  $314.99 \pm 12.67$  in Cosynch, Ovsynch and control groups respectively. In Cosynch group, there was significant difference in mean VER values between proestrus, estrus and diestrus stages, however, there was no significant difference between proestrus and metestrus stages in non-descript repeat breeder

buffaloes. In Ovsynch group, there was significant difference between the proestrus to diestrus, estrus and diestrus, however, there was no significant difference noted between metestrus to diestrus stage, metestrus to proestrus and proestrus to estrus stage. In control group, there was significant difference between proestrus and estrus and also between estrus to diestrus. However, no significant difference observed between proestrus and metestrus and proestrus to diestrus and metestrus to estrus stages. There is paucity of references on the present topic.

### **Vaginal electrical resistance values from day 0 to 45 post insemination in pregnant and non-pregnant buffaloes**

The VER values were lowest on day of estrus ( $219.26 \pm 4.64$  vs.  $239.30 \pm 5.59$ ) in pregnant vs. non-pregnant non-descript repeat breeder buffaloes and these values started to raise day by day up to 21 days in pregnant buffaloes and up to day 7 in non-pregnant buffaloes. The VER values shown a decline from day 19 to 23 post insemination for non-pregnant buffaloes compared with VER values of pregnant buffaloes which increased during the same period. The VER values from day 19 to 23 were significantly higher for pregnant buffaloes compared to non-pregnant buffaloes. However, there were constant VER values from day 24 to 45 in pregnant buffaloes whereas, in non-pregnant buffaloes there was again decrease in trend of VER values from day 41 to 45 post insemination. The non-descript repeat breeder buffaloes with mean VER value of  $219.26 \pm 4.64$  at the time of insemination confirmed to be pregnant than compared to the non-pregnant buffaloes with mean VER values of  $239.30 \pm 5.59$  at the time of insemination.

The VER values ( $323.33 \pm 11.55$ ) started

raising from day 9 in pregnant buffaloes till day 45 ( $363.33 \pm 7.99$ ) post insemination whereas the VER values ( $302.70 \pm 12.17$ ) shown decline from day 9 in non-pregnant buffaloes till day 45 ( $282.00 \pm 12.39$ ) post insemination. The VER values may be used to know the pregnancy status in buffaloes on day 8 ( $317.78 \pm 13.21$  vs.  $309.30 \pm 12.28$ ) in pregnant vs. non-pregnant buffaloes. The VER values raised from  $317.78 \pm 13.21$  on day 8 continuously up to  $363.33 \pm 7.99$  on day 45 in pregnant buffaloes whereas the same VER values  $309.30 \pm 12.28$  on day 8 declined up to  $282.00 \pm 12.39$  on day 45 in non-pregnant buffaloes. In the present study, buffaloes with mean VER values  $219.26 \pm 4.64$  at the time of insemination confirmed to be pregnant than compared to the non-pregnant buffaloes with mean VER of  $239.30 \pm 5.59$  at the time of insemination. These results are in agreement with the findings of Rofsdal (1974), Rohloff and Patel (1976), Korber (1985) and Meena *et al.* (2001). Gupta and Purohit, (2001) proved that VER can be used successfully to predict the stage of oestrous cycle, ovarian status and ovulation; insemination at a low VER distinctly improves the conception rate in buffaloes (81.48 vs. 16.66% with 26 and 40 ohms respectively).

#### **Correlation between biochemical constituents of cervico-vaginal mucus on the day of estrus in pregnant and non-pregnant buffaloes**

The biochemical constituents of cervico-vaginal mucus collected on day of estrus viz. total proteins, inorganic phosphorus, calcium, chloride and sodium in the non-descript repeat breeder buffaloes were pooled and divided into pregnant and non-pregnant. The level of phosphorus, chloride and sodium in cervico-vaginal mucus samples on the day of estrus and later confirmed pregnant were  $15.77 \pm 3.28$  mg/dL,  $141.62 \pm 9.23$

mEq/L and  $322.00 \pm 3.98$  mEq/L, however, the same biochemical constituents were  $09.28 \pm 0.71$  mg/dL,  $116.68 \pm 7.19$  mEq/L and  $304.31 \pm 3.05$  mEq/L respectively in buffaloes which did not settle for pregnancy. There was significant difference between the buffaloes which became pregnant and non-pregnant with respect to phosphorus, chloride and sodium content in the cervico-vaginal mucus samples collected on the day of estrus with higher concentration in pregnant than non-pregnant buffaloes. However, the concentration of total proteins and calcium in the cervico-vaginal mucus samples collected on the day of estrus were higher in buffaloes which became pregnant than non-pregnant buffaloes but the difference was statistically non-significant. There is paucity of references on the present topic.

#### **Correlation between biochemical constituents of cervico-vaginal mucus and vaginal electrical resistance on the of estrus**

There is a positive correlation between concentrations of total proteins, phosphorus and calcium in cervico-vaginal mucus and VER values on the day of estrus in non-descript repeat breeder buffaloes of Cosynch group and there was negative correlation between concentration of chloride and sodium with VER values but in Ovsynch group there was a negative correlation between VER values and total proteins, phosphorus, calcium and sodium but there was positive correlation between the VER values and chloride concentration. In the non-descript repeat breeder buffaloes of control group, there was a positive correlation between the VER values with total proteins, phosphorus and calcium but there was a negative correlation between the VER values with chloride and sodium concentration. After pooling all the values, there was a positive correlation between the VER

values with total proteins and calcium but negative correlation exists between VER values and phosphorus, chloride and sodium concentration in cervico-vaginal mucus at estrus in non-descript repeat breeder buffaloes. There is paucity of references on the present topic.

From the present study, following conclusions were drawn: Ovsynch protocol is more efficacious in settling pregnancy than Cosynch protocol in non-descript repeat breeder buffaloes.

Estrus detection programme by using estrous detector probe may strengthen the artificial insemination and controlled breeding programme in non-descript repeat breeder buffaloes. Insemination at a low vaginal electrical resistance values improves pregnancy rate in non-descript repeat breeder buffaloes. VER values in buffaloes may play a role in early pregnancy diagnosis (day 8 to 9) post insemination at field/farm level but multiple measurements may still be required for increased precision. Higher concentration of total proteins, phosphorus, calcium, chloride and sodium concentration in cervico-vaginal mucus on the day of estrus in non-descript repeat breeder buffaloes shows more chances of getting pregnant.

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