

Case Study

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## Effect of Ovsynch Protocol and Mineral Supplementation on Fertility in Pubertal and Peripubertal Buffalo Heifers

Ravikumar Kaliannan\*, Selvaraju Mani and Prakash Subramaniyan

Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Namakkal -637 001, TANUVAS, Tamil Nadu, India

\*Corresponding author

### ABSTRACT

#### Keywords

Ovsynch, Pubertal, Peripubertal, Buffalo heifers

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Forty pubertal and peripubertal buffalo heifers divided into four groups. Groups I and III were supplemented with mineral mixture. Group II and III were treated with Ovsynch protocol consisted of 10 µg i.m. injection of GnRH on the day of start (d 0), 500 µg i.m. injection of PGF<sub>2</sub>α seven days later (d 7), another 10 µg i.m. injection of GnRH 48 h after PGF<sub>2</sub>α (d 9) and AI 16 to 18 h after second GnRH injection (d 10). Group IV was control. The ovulatory response and conception rates were 50.00 and 20.00; 60.00 and 30.00; 90.00 and 40.00 and 100.00 and 20.00 per cent in Group I to IV, respectively. It conclude that ovsynch protocol and mineral supplementation improved conception rate in pubertal and peripubertal buffalo heifers.

### Introduction

Buffalo usually attain puberty can be highly variable, ranging from 18 to 46 months.

The delay in puberty, consequently delays conception and results in low reproductive efficiency and lengthening of the non-productive life. Synchronization of estrus has been helpful in solving of these problems in buffalo heifers. Pursley *et al.*, (1995) reported that when estrus was synchronized with GnRH - PGF<sub>2</sub>α - GnRH protocol, ovulation occurred within 18 h period in all the treated animals. Although much work has been done

using ovsynch in synchronization of ovulation in cattle, information on their use in buffaloes especially in peripubertal and pubertal buffalo heifers is limited. Hence the present study was taken up

To study the efficacy of mineral supplementation on estrus induction and conception rates in peripubertal and pubertal buffalo heifers.

To study the efficacy of Ovsynch protocol with or without mineral supplementation on estrus induction and conception rates in peripubertal and pubertal buffalo heifers.

## Materials and Methods

Forty healthy graded Murrahpubertal and peripubertal buffalo heifers aged between 2 and 4 years were selected after thorough gynaeco-clinical examination and ultrasound examination of ovaries. Buffalo heifers were dewormed orally with combination of Albendazole (7.5 mg/kg body weight) and Ivermectin (200µg/kg body weight) bolus. Then randomly and equally divided into four experimental groups viz., Group I to III (Treatment groups) and Group IV (Control group). Groups I and III buffalo heifers given mineral mixture bolus orally at the dose rate of one bolus per day per buffalo for 10 days from the day of selection. The constituents of bolus of mineral mixture includes Copper -1.4 gm, Cobalt – 0.1112 gm, Iodine- 0.14 gm, Iron – 1.4 gm, Manganese.56 gm, Selenium 0.006 gm, Zinc-0.56 gm, Vitamin E -0.05 gm, live Live Yeast – q.s., respectively. Buffalo heifers of Group II and III were treated with Ovsynch protocol consisted of intramuscular injections of 10 µg of GnRH (2.5 ml, Buserelin acetate, Ovulanta<sup>®</sup>, Vet Mankind, New Delhi, India) on the day of start of synchronization of ovulation (d 0), 500 µg of PGF<sub>2</sub>α (2 ml, Cloprostenol, Pragma<sup>®</sup>, INTAS Pharmaceuticals, Ahmedabad, India) seven days later (d 7) and another 10 µg of GnRH (2<sup>nd</sup> GnRH) 48 hours after the PGF<sub>2</sub>α (d 9) and Timed Artificial Insemination (TAI) at 16 to 18 hours after the second GnRH injection (d 10) as described by (Pursley *et al.*, 1995). In group I and IV buffalo heifers Artificial Insemination was done during the observed estrus. The groups II and III buffalo heifers were closely observed for estrus signs during the period of treatment and during the 66 h after PGF<sub>2</sub>α administration. Buffalo heifers in the group I was observed for signs of estrus for 30 days from the completion of mineral mixture supplementation. Estrus response in percentage was calculated as number of animals expressed estrus divided by number

of animals treated in each group and multiplied by 100. Onset of estrus was calculated in hours, from the time of PGF<sub>2</sub>α administration to the time of first appearance of estrus signs in group II and III, whereas in group I, the onset of the estrus was recorded from the time of completion of mineral mixture supplementation to the first appearance of estrus signs. The intensity of estrus was studied using behavioral changes, physiological changes and gynaecological observations and it was scored as described by (Rao and Rao, 1981) with slight modifications. Ovulatory response was assessed by rectal examination and ultrasonography at 10 days after induced estrus in groups II and III and observed estrus in group I and IV. Conception rate was calculated as number of buffalo heifers conceived to TAI at induced estrus in groups II and III and at observed estrus in group I and IV divided by number of animals treated in each experimental group and multiplied by 100 and it was expressed in percentage. Pregnancy was confirmed by rectal palpation and ultra sound scanning at 60 days post insemination.

## Results and Discussion

All the pubertal and peripubertal buffalo heifers belong to ovsynch, ovsynch plus mineral mixture and control groups exhibited 100 per cent estrus. Similar finding was reported by (Velladurai *et al.*, 2015) in crossbred cows and (Biradar *et al.*, 2014) in repeat breeder buffaloes following Ovsynch treatment. Mineral mixture supplementation in group I buffalo heifers in this study showed 50 per cent estrus response and (Velladurai *et al.*, 2015) observed 62.50 per cent estrus response in mineral supplemented postpartum cross bred cows. This might be due to follicle size more than 10 mm at the time of AI in buffalo heifers as described by (Atanasov *et al.*, 2011) in Bulgarian Murrah buffaloes.

In the present study the overall mean time taken for the onset of estrus was  $129.6 \pm 55.56$ ,  $45.1 \pm 0.89$  and  $42.8 \pm 0.61$  h in mineral mixture, ovsynch and ovsynch plus mineral mixture groups, respectively. There was non-significant difference with regards to onset of estrus among ovsynch and ovsynch plus mineral mixture groups. (Ravikumar *et al.*, 2007) reported that the interval to onset of estrus after  $\text{PGF}_2\alpha$  was  $48.8 \pm 7.74$  and  $52.67 \pm 4.18$  h in ovsynch-subestrus and ovsynch anestrus treated postpartum buffaloes and in crossbred cows, it was  $49.04 \pm 3.17$  h (3).

The mean ( $\pm$ SE) duration of induced estrus in mineral mixture, ovsynch and ovsynch plus mineral mixture groups and control group were  $26.8 \pm 1.59$ ,  $23.7 \pm 1.27$ ,  $26.3 \pm 0.72$  and  $27.3 \pm 1.18$  hours, respectively. These findings were in concurrence with the reports of (Ravikumar *et al.*, 2007) in buffaloes and (Velladurai *et al.*, 2015) in cows.

The number and percentages of pubertal and peripubertal buffalo heifers showed intense, intermediate and weak estrus intensities were 4 (40.00), 1 (10.00) and 0 (0); 2 (20.00), 6 (60.00) and 2 (20.00); 5 (50.00), 4 (40.00) and 1 (10.00) and 5 (50.00), 5 (50.00) and 0 (0), in mineral mixture, ovsynch, ovsynch plus mineral mixture groups and control group, respectively. In this investigation, mineral mixture supplementation in ovsynch treated buffalo heifers (group III) resulted in higher estrus expression rates in pubertal and peripubertal buffalo heifers. Minerals act as co-enzymes for the production of reproductive hormones especially in steroidogenesis (Pandey *et al.*, 2007). Calcium plays a vital role in steroidogenesis by influencing delivery or utilization of cholesterol by mitochondria or by stimulating the conversion of pregnenolone to progesterone. GnRH stimulation of the pituitary gland to increase LH secretion involved calcium dependent mechanism

(Hurley and Doane, 1989). Even marginal deficiency of phosphorus was sufficient to cause disturbances in pituitary-ovarian axis without manifesting deficiency syndrome (Arosh *et al.*, 1998). These reasons might explain the increased rate of intense estrus intensity in mineral mixture treated pubertal and peripubertal buffalo heifers.

The percentages of ovulatory response following day 10 post AI in pubertal and peripubertal buffalo heifers were 50.00, 60.00, 90.00 and 100.00 per cent, in mineral mixture, ovsynch, ovsynch plus mineral mixture and control group, respectively. (Velladurai *et al.*, 2015) observed similar ovulatory response in mineral mixture treated (50 per cent) and ovsynch plus mineral mixture (100 per cent) treated postpartum cross bred cows. (Ravikumar *et al.*, 2007) recorded 83.33 and 54.54 per cent ovulatory response in ovsynch treated postpartum subestrus and anestrus buffaloes, respectively. 72 per cent ovulatory response was observed by (Hoque *et al.*, 2014) in postpartum cyclical buffaloes in ovsynch treatment. The reduced ovulatory response observed in mineral mixture group (50.00 percent) could be due to the sequelae of suppressed hypothalamic GnRH and pituitary LH secretion which depressed the ovarian function and in ovsynch treatment for getting good ovulatory response it requires suitable follicular development at the time of initiation of ovsynch and prevention of premature luteolysis (Pursley *et al.*, 1997). This result clearly proved that mineral mixture supplementation definitely improved the ovulation rates in pubertal and peripubertal buffalo heifers when they were combined with Ovsynch program in buffalo heifers. The percentages of conception rate in pubertal and peripubertal buffalo heifers were 20.00, 30.00, 40.00 and 20 per cent, in mineral mixture, ovsynch, ovsynch plus mineral mixture and control group, respectively. (Velladurai *et al.*, 2015)

observed 37.50 and 25.00 per cent first service conception in mineral mixture and ovsynch plus mineral mixture treated postpartum RFM affected crossbred cows. Similar conception rate were reported by Ravikumar *et al.*, (2007) 33.33 per cent and Hoque *et al.*, (2014) 28 per cent in postpartum cyclic buffaloes treated with ovsynch protocol.

The results of the present study conclude that ovsynch protocol and mineral supplementation improved conception rate in pubertal and peripubertal buffalo heifers.

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