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Effect of Ovsynch protocol on conception in postpartum buffaloes during peak and low breeding seasons

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Abstract
High reproductive efficiency is a prerequisite for high life-time production in dairy animals. Synchronization of ovulation is a recent reproductive tool to augment fertility in bovines. However, the success rate is highly variable in postpartum buffaloes during peak (October-March) and low (April-September) breeding seasons. Hence, the present study was designed to investigate the impact ovsynch protocol on reproductive performance of buffaloes during peak and low breeding seasons. A total number of 40 graded Murrah buffaloes were randomly and equally divided into two experimental groups (10 buffaloes each) *viz*. Groups I (Treatment group) and Group II (Control group) during peak breeding season (PBS; between October and March) and low breeding season (LBS; between April and September). Experimental buffaloes were treated with Ovsynch protocol (10 µg of GnRH on day 0, 500 µg of $PGF_2\alpha$ on day 7 and another 10 µg of GnRH 48 hours after (day 9) the $PGF_2\alpha$) and timed artificial insemination at 16 to 18 hours after (day 10) the second GnRH injection. Control group buffaloes were observed for the signs of estrus for 30 days from the time of selection and artificial insemination was done during the observed estrus during PBS and LBS, respectively. All the buffaloes during PBS and LBS exhibited estrus (100 per cent) following the synchronization of ovulation protocols. The percentages of ovulatory response following synchronization of ovulation on day 10 post AI in buffaloes were 80.00 and 40.00 per cent, in groups I and II, respectively during PBS and the values in LBS were 60.00 and 30.00 per cent, respectively. During PBS, the conception rates observed were 50.00 and 30.00 per cent in group I and II, respectively and the values in LBS were 30.00 and 20.00 per cent, respectively. It is concluded that, Ovsynch protocol is found to be effective in both PBS and LBS.

Keywords: Ovsynch, conception, buffalo, season

Introduction
The domestic water buffalo (*Bubalus bubalis*) is an important livestock resource in many countries of Asia, the Mediterranean region and Latin America. The buffalo has been traditionally regarded as a poor breeder due to having poor fertility in the majority of conditions under which they are raised (Barile, 2005) [3]. This is manifested mainly as inherent late maturity, long postpartum anestrus intervals, poor oestrus expression in summer, distinct seasonal reproductive patterns, poor conception rates (CR), poor estrus detection and prolonged inter-calving intervals.
In South Asia, buffaloes are predominantly of the river type and contribute mainly for milk, meat, manure, draught power and fuel to rural farmers. The pattern of estrus in buffalo is different from that of cattle since majority of buffaloes exhibit estrus from October to March when ambient temperature is low and Temperature–Humidity Index (THI) value is less than 70 (Singh *et al.*, 2011) [24].
In addition to ambient temperature, humidity and solar radiation profoundly affect expression of reproductive rhythm in buffaloes and cows (Upadhyay *et al.*, 2009) [25]. The months of October–March were considered as the peak breeding season and the reminder of the year as the low breeding season (Manjunatha *et al.*, 2009) [15] in buffaloes. Genetic improvement of buffaloes for increasing milk yield can be achieved by application of artificial insemination (A.I.). Successful results of A.I. depend on accurate detection of estrus. However, buffalos rarely show mounting behavior (Baruselli *et al.*, 2003) [5] and experience poor estrus expression and variable duration (6–64 h) of estrus (Ohashi, 1994) [18] making the prediction of time of ovulation difficult. Synchronization of estrus has been helpful in solving of these problems and can remove the necessity of estrus detection thereby making it possible to provide AI in the predicted time.

However, evaluating the exact time of ovulation is a condition for efficient synchronization of estrus and if this could be achieved, then a major advancement in reproductive management in buffaloes would occur. Hence synchronization of ovulation is practiced to augment fertility in buffaloes (Arun and Amarna, 2020 [2] and Selvaraju *et al.*, 2021) [23]. However, the success rates of above said treatment is highly variable in postpartum buffaloes during peak (October-March) and low (April-September) breeding seasons. Hence, the present study was designed to investigate the impact ovsynch protocol on reproductive performance of buffaloes during peak and low breeding seasons.

Materials and Methods

An experiment was conducted in buffaloes brought to the Large Animal Gynaecology Unit of Teaching Veterinary Clinical Complex (TVCC), Veterinary College and Research Institute, Namakkal and Veterinary Dispensaries and Tamil Nadu Co-operative Milk Producer's Federation (TCMPF) Co-operative Societies located in and around Namakkal district of Tamil Nadu for this study. A total number of 40 healthy graded Murrah buffaloes in their postpartum period of more than 90 days and aged between $2^{nd}$and 5^{th} parity were utilized for this study. The selected buffaloes were subjected to thorough gynaeco-clinical examination before commencing the experimen**t.** All the selected buffaloes were randomly and equally divided into two experimental groups *viz*. Group I (Treatment groups) and Group II (Control group) during peak breeding season (PBS; Between October and March) and low breeding season (LBS; Between April and September). Each group comprised of 10 buffaloes.

Buffaloes of group I was treated with Ovsynch protocol as described by Pursley *et al.* (1995). The Ovsynch protocol consisted of intramuscular injections of 10 µg of on the day of start of synchronization of ovulation (d 0), 500 µg of $PGF_{2}\alpha$ seven days later (d 7) and another 10 µg of GnRH (2^{nd} GnRH) 48 hours after the $PGF_{2}\alpha$(d 9) and Timed Artificial Insemination (TAI) at16 to 18 hours after the second GnRH injection (d 10).

Buffaloes belonging to groups I was bred by Timed Artificial Insemination (TAI) at 16-18 hours after the last GnRH injection and at observed estrus in group II with good quality frozen thawed semen. Those animals which failed to conceive and returned to estrus following TAI at induced estrus (in the experimental groups) or observed estrus in group II were subjected to Artificial Insemination in the subsequent estrus.

Ovulatory response was assessed by rectal examination at 10 days after induced estrus in groups I and observed estrus in group II by the palpation of the corpus luteum in any one of the ovaries in each buffalo and it was further confirmed by ultrasonography.

Conception rate was calculated as number of buffaloes conceived to TAI at induced estrus in groups I and at observed estrus in group II (First service conception rate) and subsequent estrus (Second service conception rate) 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 ultrasound scanning at 45 days post insemination.

Blood samples were collected from jugular vein with the help of sterile 18 Gauge needle from all experimental animals. Blood collection was done in the clotting activator vaccutainer in groups I, at the time of (i) selection of postpartum buffaloes (ii) initiation of Ovsynch treatment (d 0) (iii) $PGF_{2}\alpha$ injection (d 7) (iv) TAI (d 10) and (v) 10 days following TAI. In group II, the blood was collected at the time of (i) selection of animals (ii) AI and (iii) 10 days following AI. The serum samples were separated and stored at -20 °C until analysis steroid hormones (progesterone and estradiol-17β). Serum samples were analysed in duplicate for progesterone and estradiol-17β by radioimmunoassay (RIA) technique.

Result and Discussion

The percentages of ovulatory response following synchronization of ovulation on day 10 post AI in buffaloes were 80.00 and 40.00 per cent, in groups I and II, respectively during PBS and the values in LBS were 60.00 and 30.00 per cent, respectively. In group I the increased ovulatory response was recorded in both the seasons as observed by ultrasonography in this study. It clearly proved that Ovsynch programme is capable of inducing ovulations in buffaloes during PBS and LBS. Similar finding was reported in buffaloes by Ali and Fahmy (2007) [1] and in cows by Pursley *et al.* (1995) [20]. Low ovulatory responses of 60-80 per cent (Berber *et al.*, 2002) [6] in buffaloes and cows (Mialot *et al.*, 2003) [16] were reported following Ovsynch programs. However, very low ovulatory response of 55 per cent has been reported in cows (Santos *et al.*, 2002) [22]. Ravikumar (2003) [21] observed 54.54 per cent ovulation rate in postpartum anestrus buffaloes treated with Ovsynch treatment. The higher ovulatory response in the present study might be due to the prompt intervention at early to mid-luteal stages with large follicles at the time of initiation of Ovsynch protocol a described by Vasconcelos *et al.* (1999) [26].

In group I (Ovsynch), the overall first and second service conception rate obtained in buffaloes in this study was 50.00 and 30.00 per cent, during PBS and LBS. In control group, the overall percentage of first and second service conception rate was 30.00 and 20.00 in PBS and LBS. It clearly indicated that Ovsynch programme increased the conception rates in buffaloes than control. The conception rates of 33.33 (Paul and Prakash, 2005) [19], 60.00 (Baruselli *et al.*, 2001) [4] and 55.60 to 64.20 per cent (Berber *et al.*, 2002) [6] were reported in buffaloes following Ovsynch protocols during the breeding season. In cows, varying conception rates of 20 to 27 (DeJarnette *et al.*, 2001) [10], 30 to 38 (Cartmill *et al.*, 2001) [7], 47 (Fricke and Wiltbank, 1999) [12], 55 (Pursley *et al.*, 1995) [20] and 62.5 (Li Jun Feng *et al.*, 2003) [14] were recorded in different studies. However, Velladurai (2013) [27] observed the highest conception rate of 87.50 per cent in RFM affected cows treated with Ovsynch protocol. The increased pregnancy rates in the buffaloes of group I might be related to the start of the protocol during early to mid diestrus as indicated by serum progesterone profiles and ovarian cyclicity in this study. Initiating the Ovsynch protocol during early to mid diestrus (day 5 to 12) produced greater pregnancy rates than when initiated on other days of the estrous cycle (Vasconcelos *et al.*, 1999) [26]. When the Ovsynch protocol was started between day 5 and 12 of the cycle, the pregnancy rates were greater because of greater incidences of ovulation after the first GnRH injection (Moreira *et al.*, 2001) [17]. El-Zarkouny *et* *al.* (2004) [11] stated that Ovsynch protocol increased the pregnancy rate by 10-12 per cent when implemented during favorable stage of the estrous cycle in cows.

In group I (Ovsynch) during PBS, the mean serum progesterone levels (ng/ml) at the time of selection, d 0, d 7, d 10 and 10 days post AI were 1.18±0.09, 1.26±0.10, 1.67±0.09, 0.50±0.07 and 2.43±0.10. The corresponding values during LBS were 1.07±0.12, 1.21±0.12, 1.41±0.08, 0.30±0.03 and 2.13±0.05. The mean serum progesterone levels (ng/ml) at the time of selection of buffaloes, at the time of AI and 10 days post AI in PBS were 1.06±0.15, 0.52±0.13 and 4.72±0.32 ng/ml, respectively and in LBS were 1.13±0.14, 0.28±0.04 and 2.97±0.13 ng/ml in group II, respectively.

Mean (±SE) serum progesterone concentrations in different phases of treatment in experimental and control group did not vary between PBS and LBS and the results suggested that season did not influence the secretary pattern of progesterone before, during and after synchronization of ovulation protocols in buffaloes. Group (I & II) in PBS and LBS had mean serum progesterone concentration > 1ng/ml at the time of selection indicating that all the selected buffaloes were cyclical. In groups I during PBS and LBS, the serum levels of progesterone were elevated on d 7. At the time of first AI (d 10) in group I during PBS and LBS had <1 ng/ml of mean serum progesterone levels indicating prompt luteolysis following $PGF_2\alpha$ injection on day 7 of the trial. The mean serum progesterone concentrations on day 10 post AI in both the groups of PBS and LBS reached more than 2.13±0.05 ng/ml with a maximum concentrations of 4.72±0.32 ng/ml and it indicated the prompt ovulatory responses following treatment in all the experimental groups during PBS and LBS.

In Ovsynch treated group, there was an elevated progesterone level from d 0 to d 7 in both the seasons. The buffaloes in the above said group might have been in the random stages of estrous cycle at the time of first GnRH injection as indicated by serum progesterone in this study and described earlier by Cerri *et al.* (2009) [8] in cows. In all the groups of PBS and LBS level of progesterone ranged below 0.57 ± 0.06 ng/ml in the blood on d 10 (induced estrus). This finding was in accordance with the report of Ganesh (2013) [13]. The reduction in mean serum progesterone concentration below 0.5 ng/ml in this experiment during induced estrus indicated that all the treatment protocols followed for synchronization of ovulation in this study effectively controlled the estrus and it might be the reason for improved conception in treated groups of PBS and LBS. Duchens *et al.* (1995) [9] suggested that elevated progesterone level at estrus might lead to asynchrony between onset of estrus and ovulation and consequently cause failure of conception.

During PBS, the mean serum estradiol-17β (pg/ml) levels in groups I at the time of selection, d 0, d 7, d 10 and 10 days post AI were 15.30±1.01, 15.67±1.39, 16.09±0.76, 32.23±1.35 and 17.51±1.83; and during LBS the levels were 14.92±1.43, 15.39±1.69, 15.66±0.78, 29.20±1.73 and 15.88±1.53. The mean serum estradiol-17β level (pg/ml) at the time of selection of buffaloes, at the time of AI and 10 days post AI in group II were 15.81±1.82, 37.61±1.82 and 16.35±1.55 pg/ml, respectively during PBS and they were 14.67±2.01, 28.70±1.85 and 15.74±1.82 pg/ml, respectively in LBS. At the time of selection of buffaloes in PBS and LBS, both the groups had more than 13 pg/ml of serum estradiol-17β indicating the follicular activity in all the selected buffaloes. Between PBS and LBS, no statistical difference in the mean serum estradiol-17β concentrations was observed in both the groups on all the days of estradiol -17β concentrations. However, the levels of serum estradiol -17β was higher in both the groups of PBS than the respective group of LBS on all the days of estimation including the time of AI and 10 days post AI and it indicated the seasonal influence on the secretion of estradiol-17β in buffaloes.

In the current investigation, serum estradiol-17β was found to be lower in both the groups of LBS than PBS. Upadhyay *et al.* (2009) [25] stated that low estradiol-17β level on the day of estrus during summer in buffaloes might be a factor for poor expression of estrus.

Conclusion

From this study, it concluded that Ovsynch protocol alone improved the fertility in postpartum buffaloes during PBS and LBS.

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