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Studies on Progesterone Concentration and Fertility Response in Postpartum Subestrus Buffaloes during Breeding and Low Breeding Seasons

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ABSTRACT

Background: The dairy and livestock sector plays a very important role in national economy of India by contributing close to one third of gross income of rural households and nearly half of gross income in case of those without land. Subestrus is the most prevalent, frustrating and challenging problem encountered in postpartum buffaloes. Postpartum subestrus is one of the most prevalent, frustrating and challenging reproductive problem encountered in rural resulting into prolonged inter-calving period, reduced milk production and thus greatly affecting the economy of our farming community.

Methods: The present study was carried out on Graded Murrah buffaloes during the period from September 2017 to August 2019 (includes breeding and low breeding season) which were maintained at farm and field. Treatment of sub-estrus lactating graded Murrah buffaloes (*Bubalus bubalis*) using Double $PGF_2\alpha$ (DPG) and Presynch-Ovsynch (POVS) their influence on fertility in farm and field condition during breeding and low breeding season was studied. Total cholesterol and progesterone concentration was recorded during different days/period of protocols. Further, overall per cent conception and pregnancy rates were recorded and compared between two protocols.

Result: Progesterone levels increased significantly on day 21 post AI in pregnant buffaloes, whereas decreased significantly in non-pregnant buffaloes both in farm and field during breeding and low breeding season. The overall per cent conception and pregnancy rates were higher in POVS group than DPG group at both farm and field as well as during breeding and low breeding seasons.

Key words: Breeding season, Buffalo, Conception rate, Progesterone, Total cholesterol.

INTRODUCTION

Indian buffaloes show better reproductive efficiency during winter compared to summer months which is attributed to environmental stress (Tailor *et al*., 1990). The stress and adverse environmental factors had direct effect on neuoro-endocrine set up which resulted in hyperprolactinemia, reduced pulsatile gonadotropin secretion, poor follicular maturation and poor estradiol production culminating in poor heat expression (subestrus) and anoestrous condition (Palta *et al*., 1997). Cholesterol the most important sterol which is synthesized from acetate and it is an essential precursor of all steroid hormones synthesized in the body. The high cholesterol levels in the cycling animals lead to increased ovarian activity resulting in increased secretion of steroids during estrus (Savalia *et al*., 2014 and Parmar *et al*., 2015). Progesterone is responsible for stimulation of cyclicity, follicular development and for maintenance of pregnancy and progesterone assay offers the possibility of determining the level of ovarian activity and its estimation can be used as a supportive diagnostic tool along with conventional rectal palpation.

Keeping in view the above facts and limited work reported on comparative efficacy of double prostaglandin and presynch-ovsynch protocols in subestrus buffaloes under farm and field pertaining to Andhra Pradesh climatic conditions (breeding and low breeding season).

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MATERIALS AND METHODS

The present study was carried out on lactating Graded Murrah buffaloes during the period from September 2017 to August 2019 (includes breeding and low breeding season) which were maintained at farm and villages (Table 1). Buffaloes with varied parity (1 to 6) and body condition score (BCS) that experienced normal parturition, aged between 6-10 years and had not exhibited estrus for the past 3 to 12 months or more were monitored and selected based on ovarian activity by per rectal examination, trans-rectal ultrasonography (TRUS) as subestrus.

Group 1 Sub-estrus buffaloes were administered with double dose of intramuscular injection of Cloprostenol sodium @ 500 µg on day 0 and day 11 followed by fixed time artificial insemination (FTAI) at 72 and 96 hours or/ and at observed estrus after the second dose of $PGF_2\alpha$ and Group 2 postpartum sub-estrus buffaloes which were subjected to Presynch-Ovsynch protocol which consisted of two intramuscular injections of Cloprostenol sodium @ 500 µg on day 0 and day 14, followed by GPG protocol, which was initiated after a gap of 12 days after the second $PGF_2\alpha$ of the Presynch. Fixed time artificial insemination was performed (FTAI) at 16-18 hours or at observed estrus after the second dose of GnRH.

The serum concentrations of cholesterol (CHOD-PAP method) recorded in different days/periods in postpartum sub-estrus buffaloes that were adopted with DPG and POVS protocols. The serum progesterone concentration was estimated by using ELISA technique with the help of progesterone kits (PROGESTERONE EIA, XEMA Co. Ltd, Russia). The Xema progesterone EIA is a 96 well plate, solid phase immunoassay, utilizing the competition enzyme immunoassay principle. The progesterone concentration was estimated on the day of initiation of treatment (day 0), on day 11 and day 18 after FTAI for Group 1 buffaloes.

Whereas for Group 2 on day -10, 0, 14, 26, 33, 35 and 53. Conception rate at induced estrus was calculated as the percentage of buffaloes that became pregnant at first insemination as confirmed by trans-rectal ultrasonography on day 28 and per-rectal palpation in between days 45 and 60 post insemination. The overall conception and pregnancy rates for all the treatment groups were recorded in buffaloes reared under farm and field conditions during breeding and low breeding seasons. Efficacy of protocols were compared based on conception and pregnancy rates following FTAI.

RESULTS AND DISCUSSION

Cholesterol concentration

There was no significant difference in serum total cholesterol concentration between days of the treatment with DPG protocol. The values varied non-significantly in pregnant and non-pregnant buffaloes on day 28 post AI in both farm (99.23±2.1 vs 97.76±1.45) and field (92.44±3.46 vs 86.21±2.65) condition. Similarly, the values of total cholesterol concentration varied non-significantly in pregnant and non-pregnant buffaloes on day 28 post AI in farm during breeding season (101.29±2.7 vs 98.76±1.48) and low breeding season (98.22±1.9 vs 96.72±1.43), while the corresponding values in field during breeding season was 99.47±1.49 vs 88.23±1.67 and during low breeding season was 93.41±1.43 vs 84.20±1.61.

In the present study the mean cholesterol concentration (mg/dl) recorded in DPG group was in agreement with the findings of Khasatiya *et al*. (2016). On the contrary, the findings of Phani (2017) revealed a higher mean cholesterol (mg/dl) concentration in buffaloes during the low breeding season under farm conditions. Prajapati *et al*. (2011) recorded a higher mean cholesterol (mg/dl) concentration in buffaloes treated with double PG protocol reared under farm conditions during early breeding (188.44±12.66), breeding (184.93±10.15) and low breeding (179.32±12.92) seasons.

There was no significant difference in serum total cholesterol concentration between days of the treatment with POVS protocol. The values varied non-significantly in pregnant and non-pregnant buffaloes on day 28 post AI in both farm (88.12±2.12 vs 86.12±2.13) and field (84.22±1.13 vs 79.12±2.13) condition. Similarly, the values of total cholesterol concentration varied non-significantly in pregnant and non-pregnant buffaloes on day 28 post AI in farm during breeding season (90.54±0.64 vs 92.12±0.87) and low breeding season (87.5±0.87 vs 88.12±2.91). However, the variations in the mean cholesterol concentration (mg/dl) might be due to the differences in season of study, nutritional and health status of the selected buffaloes.

Progesterone concentration (ng/ml)

The mean P_4 levels on day -10, 0 and 11 were more than 1 ng/ml. Thereafter the mean P_4 levels dropped steeply on day of AI. These levels again increased significantly on day 21 post AI in pregnant buffaloes whereas decreased significantly in non-pregnant buffaloes both in farm and field. Significant difference was observed in P_4 level between pregnant and non-pregnant buffaloes on day 21 both in farm and field. Similarly, P_4 levels increased significantly on day 21 post AI in pregnant buffaloes, whereas decreased significantly in non-pregnant buffaloes both in farm and field during breeding and low breeding season.

In the present study, the mean progesterone level during different periods of treatments in DPG group was in agreement with the findings of Phani (2017) who recorded the mean P_4 levels (ng/ml) at day (-10), 0, 11 and on the day of AI was 1.34±0.15, 1.56±0.14, 1.67±0.52 and 0.66±0.05 ng/ml, respectively in buffaloes reared under farm conditions during the low breeding season, while Pandey *et al*. (2011) recorded the mean P_4 levels (ng/ml) on the day of AI and 21 days post AI as 0.33±0.10 and 2.1±0.20, respectively in pregnant buffaloes and 0.35±0.10 and

Table 1: Classification based on high vs low breeding season in farm and field.

Seasons	Total animals screened in farm and field	True Anestrus
Breeding season (HB) (September to February)	Farm	12
	Field	38
Low breeding season (LB)(March to August)	Farm	14
	Field	42

1.1±0.30, respectively in non-pregnant buffaloes reared under farm conditions.

The data show that the mean serum progesterone concentrations on day -10 (>1 ng/ml) were decreased to basal values on day 0 and increased on day 14 (>1 ng/ml). The P_4 levels on day 26 (initiation of ovsynch protocol) were 1.01 ±0.08 and 0.96 ±0.14 in pregnant buffaloes and levels increased significantly on day 33. Thereafter the mean progesterone level dropped suddenly on day 9 and day of AI. These levels again increased significantly on day 21 post AI in pregnant buffaloes whereas it decreased significantly in non-pregnant buffaloes both in farm and field. There was no significant difference between breeding and low breeding in pregnant buffaloes in both farm and field conditions.

In the present study, the mean progesterone level during different periods of treatments in POVS group was in agreement with the findings of Colazo *et al*. (2013) who recorded the mean P4 levels (ng/ml) as 0.4±0.09 in buffaloes of farm conditions during the breeding season, while, Hoque *et al*. (2014) observed the mean progesterone (P_4) levels on day 0, day 12, day 22 post AI as 0.64±0.36, 1.47±0.33 and 2.14±0.13 ng/ml, respectively in buffaloes that ovulated and became pregnant under field conditions.

Conception rate

Comparatively the per cent conception rates at induced/ observed estrus, 2nd and 3rd service were higher in POVS group (42.85 vs 39.13; 14.28 vs 8.69 and 7.14 vs 2.1) as compared to DPG group, respectively in farm condition. While, the per cent conception rate at induced/observed estrus, 2nd and 3rd service were higher in POVS group (32.60 vs 32.16; 15.21 vs 11.18 and 6.52 vs 3.14) as compared to DPG group, respectively in field condition.

In the present study the overall conception rate in DPG group of buffaloes under farm conditions during the breeding season was on par with the observations of Rahman *et al*. (2012) and Honparkhe *et al*. (2008) who reported the conception rate as 64.70 and 65.6 per cent, respectively, whereas the present findings are in contrary with findings of Esposito *et al*. (2019) and Yendraliza *et al*. (2019) who recorded a higher conception rate (75.00 and 70.00%, respectively). On the contrary, DPG group buffaloes of the present reared under farm conditions during the low breeding season showed lowered conception rate when compared to the previous findings of Phani (2017) who recorded a higher conception rate (86.66%).

In the present study, the overall conception rate in POVS group buffaloes under field condition during the breeding season was in tune with the findings of Konrad *et al*. (2013) who found the conception rate as 55.80 per cent. Whereas, Ravikumar *et al*. (2014) observed a higher conception rates during breeding (70.00%) and low breeding seasons (50.00%) under field conditions.

The present findings evidently explained that higher conception rates in postpartum subestrus buffaloes might be due to pre-synchronization with prostaglandin analogue that caused lysis of the CL before the initiation of Ovsynch protocol. This resulted in most the buffaloes being in mid luteal phase at the time of initiation of Ovsynch protocol, so that the first GnRH helped in luteinization and/or ovulation of follicle administration of $PGF_{2\alpha}$ alpha on 7th day lead to lysis of CL and resulting in initiation of new follicular wave. The second GnRH injection allowed maturation and ovulation of Graffian follicle in turn improved the conception rate as opined by Mendonca *et al*. (2019).

CONCLUSION

The combination Ovsynch protocol with Pre-synch (Presynch-ovsynch) might be considered as suitable protocol for postpartum subestrus buffaloes. The disadvantage with the adoption of Presynch-Ovsynch protocol was its cost effectiveness and long duration that could be outweighed in terms of result *i.e.* initiation of new follicular wave, maturation of follicle, ovulation of Graffian follicle and less embryonic loss with improved pregnancy rate.

Conflict of interest

All authors declare that they have no conflict of interest.

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