Original Article
Buffalo Bulletin (April-June 2019) Vol.38 No.2

EFFECT OF PER-VAGINAL INSERTION OF ONCE-USED TRIU-B ON POST-OVULATION PLASMA PROGESTERONE AND CONCEPTION RATE IN BUFFALO

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ABSTRACT

The present study evaluated the impact of once-used intravaginal progesterone inserts (TRIU-B) from day 4 to day 10 post-ovulation on luteal profile (corpus luteum diameter, CL; plasma progesterone) and conception rate in buffalo (n=80) during summer season. The buffaloes were estrus synchronized by Cloprostenol (500 µg, i.m.) administered 11 days apart, followed by AI during standing estrus. All the buffaloes were equally categorized into control (without TRIU-B) or treatment (pervaginally inserted with once-used TRIU-B) group. From each group, 15 buffaloes were subjected to ovarian ultrasonography for CL diameter measurements on day 4 and day 10 post-ovulation followed by jugular vein blood collection for plasma progesterone analysis. On day 10 post-ovulation, the control group buffalo had larger ($P<0.05$) CL diameter (15.2±0.5 vs. 14.0±0.3 mm), whereas, plasma progesterone was higher ($P<0.05$) in treatment group (3.4±0.2 *vs.* 1.7±0.1 ng/ml). Conception rates were 55.0 and 37.5% in treatment and control groups, respectively ($\chi2=2.46$, $P>0.05$). Moreover, pregnant and non-pregnant buffalo of treatment group exhibited higher ($P<0.05$) plasma progesterone on day 10 post-ovulation compared to their control counterparts. In conclusion, per-vaginal insertion of once-used TRIU-B enhanced post-ovulation plasma progesterone but failed to improve conception rate in buffalo.

Keywords: *Bubalus bubalis*, buffaloes, conception, progesterone, TRIU-B

INTRODUCTION

In the recent decades, dairy farmers have developed interest in buffalo rearing mainly due

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to the quality milk production (Singh, 2009). However, reproductive efficiency of buffaloes is considerably affected by summer anestrus, silent estrus, long postpartum anestrus and lesser pregnancy retention, which are major impediments in the popularization of buffalo rearing (El-Wishy, 2007). The plasma progesterone during initial days of post-conception is positively correlated with subsequent conception rate in buffalo (Pandey *et al*., 2016; 2018). Sub-optimal plasma progesterone on day 6 post-AI is a main cause of conception failure (Green *et al*., 2010). Progesterone is essential for orchestrating the histotrophic environment for the nourishment of the conceptus (Santos *et al*., 2004). It is well established that not only optimal plasma progesterone is important for pregnancy but delay in post-ovulatory rise in progesterone compromises production of interferon tau (IFN-τ) on day 16 post-AI by embryo, which results in reduced conception rates (Wathes *et al*., 2003; Green *et al*., 2010).

The circulating progesterone could either be increased by external sources or by stimulating ovary to develop additional CL following GnRH or hCG administration on d5 or day 12 post-ovulation (Campanile *et al*., 2007; Pandey *et al*., 2013a; 2013b; 2015). The accessory CL starts to secrete progesterone from day 8, which attains maximum release on day 16 to 17 of estrus cycle (Kerbler *et al*., 1997). However, this delay in progesterone increase may not be beneficial for subsequent embryo survival (Howard *et al*., 2006). Consequently, the exogenous progesterone insertion could be beneficial for embryo development and increase in conception rate. Earlier studies have suggested that progesterone supplementation at an early post-conception stage leads to increase in pregnancy rate; however, supplementation following d6, had no beneficial impact on pregnancy rate (Mann and Lamming, 1999).

The attempts made to economize the cost of hormonal treatments in dairy industry by investigating the reuse of intravaginal progesterone inserts during estrus synchronization protocols, suggested that insert reuse reduces the cost of synchronization with effects like new insert (Long *et al*., 2009; Abdallah and Rahim, 2014; Monteiro *et al*., 2014; Spurlock *et al*., 2016). Nevertheless, the efforts were not made to determine the impact of reuse of progesterone insert following AI on conception rate in buffalo. Therefore, the objective of the present study was to investigate the efficacy of once-used (during five-day estrus synchronization protocol) intravaginal progesterone inserts (TRIU-B) supplementation from day 4 to day 10 post-ovulation on CL diameter, plasma progesterone and conception rate in buffalo during summer season.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana (India).

Animals and management

The present experiment was conducted on 80 healthy Murrah buffalo (Body weight range: 400 to 500 kg; parity: 2 to 5; body condition score: 3 to 4 on 5 point scale) during summer month (April to June, temperature ranged between 40 and 45^{o}C). The animals were offered chaffed green fodder, wheat straw, concentrates (barley 12%, maize 30%, wheat bran 25%, groundnut cake 20%, linseed cake 10%,), 2% mineral mixture, 1% common salt and *ad* *libitum* drinking water.

Experimental design

Before the start of double PG (prostaglandin) protocol, all the buffaloes were examined for the presence of CL through ultrasonography at 10 day interval, and further confirmed by plasma progesterone levels (>1.0 ng/ml on any one day of two examinations). The buffaloes were synchronized for estrus with double $PGF_{2\alpha}$ protocol. In this protocol, two injections of a synthetic $PGF_{2\alpha}$ analogue (500 µg, Cloprostenol Sodium, Vetmate, Vetcare, Banglore, India) were administered (i.m.) at 11 day apart. All the buffaloes expressed estrus between 48 and 72 h of second PG injection. Estrus was confirmed by teaser bull parading twice daily at morning and evening for an hour. Artificial insemination was carried out during mid to late estrus and thereafter at 24 h interval once again (Figure 1). Artificial insemination was done using frozen-thawed semen of a same bull. All the inseminations were carried out by the same person.

The buffaloes were examined through ultrasonography to observe preovulatory follicle (POF) diameter from the day of estrus until ovulation. Thereafter, based upon the POF diameter (mean POF diameter equal in both groups), the buffaloes were categorised into two groups (Group 1, control (n=40, no treatment) and Group 2) treatment group (n=40, 5 day used TRIU-B for estrus synchronization: Progesterone, 958 mg; Virbac animal health India Private limited, Mumbai). Once-used TRIU-B was washed in running tap water to remove the mucus and debris, followed by autoclaving at 121°C under 724 mm Hg for 20 minutes (Zuluaga and Williams, 2008) to sterilize the device. The buffalo of treatment group was inserted with autoclaved used TRIU-B from day 4 to day 10 post-ovulation. Per-rectal ovarian ultrasonography was carried out using a battery-operated B-mode ultrasound scanner (Agroscan AL, ECM, Angouleme, France) equipped with inbuilt interchangeable 5/7.5 MHz linear-array rectal transducer (ALR 575 probe, ECM, Angouleme, France). The scanning was carried out starting from the day of onset of estrus at 24 h interval until ovulation, and thereafter on day 4, 10 and 35 post-ovulation (Figure 1). Ovaries were systematically scanned, and images were recorded in the recorder. The average diameter of the follicle/corpus luteum (CL) was determined by mean of the largest and smallest diameter of the follicle/CL. All measurements were made using the built-in, on-screen calipers. The follicle which disappeared after the end of estrus was considered as the ovulatory follicle. Day of ovulation (day 0) was the last day, when the follicle was found intact before disappearing at subsequent examination about 24 h later (Pandey *et al*., 2016). Pregnancy was confirmed on day 35 post-ovulation by ultrasound.

Blood sampling and hormone analysis

Jugular vein blood was collected (10 ml) from 15 buffalo of both the groups in a heparinized vaccutainer vials after each ultrasonographic examination (Figure 1). Plasma was separated immediately after blood collection by centrifugation at 1500 *X* g for 15 minutes, and plasma aliquots were stored at -20°C until analysis.

Plasma progesterone was assayed with a solid-phase radioimmunoassay (Ghuman *et al*., 2009). The mean intra- and inter-assay coefficients of variance were 6.2 and 9.5%, respectively. The sensitivity of progesterone assay was 0.1 ng/ml.

Statistical analysis

Numerical data are represented as mean ± SE. Conception rate was defined as the number of buffaloes diagnosed pregnant on day 35 divided by the number of inseminated buffaloes in a group. Three-way ANOVA with repeated measures were used to determine the effects of fixed factors (group and pregnancy), and repeated factor (days) on CL diameter and plasma progesterone. Logistic regression analysis was employed to assess association between groups and pregnancy. Probabilities of < 5% ($P<0.05$) were considered statistically significant. All statistical analysis was performed in SPSS (16.0) system for windows (SPSS 16.0).

RESULTS

Overall CL diameter of control and treatment groups was similar ($P>0.05$) on day 4 post-ovulation, however, the former group had larger ($P<0.05$) CL diameter than the latter on day 10 post-ovulation (Table 1). However, the pregnant and non-pregnant buffalo of control group had similar ($P>0.05$) CL diameter as compared to their treatment group counterparts (Table 1). Following the insertion of TRIU-B on day 4 post-ovulation, significantly higher ($P<0.05$) plasma progesterone was recorded in treatment group on day 10 compared to controls (Table 1). Furthermore, pregnant and non-pregnant buffalo of treatment group exhibited higher ($P<0.05$) plasma progesterone on day 10 post-ovulation compared to their control counterparts (Table 1). The conception rate was observed 37.5 and 55% for control and treatment group, respectively ($\chi^2=2.46$, $P>0.05$).

DISCUSSION

We hypothesized that the supplementation of progesterone through once-used TRIU-B during early luteal phase would mimic the diestrus phase of estrous cycle and will result to increased conception rate during summer in buffalo. Insufficient plasma progesterone during early stages of embryo development is one of the major causes for reduced conception rate in hot summer months (Wiltbank *et al*., 2011). In the current study, higher concentrations of plasma progesterone were recorded in treatment compared to control group buffaloes. To the best of our knowledge, no study evaluated the impact of intravaginal supplementation of used progesterone device during the post-ovulation period, however, the use of new CIDR (controlled internal drug release) intravaginally from day 3.5 to 10 post-AI increased plasma progesterone in cows on day 4 post-AI (Larson *et al*., 2007). Other studies have also reported similar plasma progesterone concentrations following reuse of an intravaginal progesterone insert as compared to a new insert (Long *et al*., 2009; Spurlock *et al*., 2016). Thus, the present study suggests that 5 day used TRIU-B following autoclaving could be reused as progesterone supplement in buffalo during summer season.

In the present study, following the insertion of progesterone insert, a decrease in diameter of CL was recorded in treatment than controls. Unlike present study, Pandey *et al.* (2013a) observed similar CL diameter between CRESTAR treated and control group buffalo. The discrepancy in the results between current and previous study might be due to differences in the type of progesterone supplement and mode of administration. Similar to present study, use of progesterone releasing intravaginal device (PRID) have led to reduction in CL diameter (Campanile *et al*., 2007; Stevenson *et al*., 2007). The decrease in CL diameter on day 10 post-AI in treatment group might be ascribed lower Luteinizing hormone in blood circulation due to elevated plasma progesterone following supplementation of used TRIU-B (Van Cleeff *et al*., 1996).

In the present study, conception rate was not significantly different between treatment and control group. Like present study, use of PRID between day 5 and 15 post-AI, had no influence on pregnancy rate in buffalo (Campanile *et al*., 2007). The results from similar studies in dairy cattle are also not conclusive and have shown either no (Arndt *et al*., 2009; Marques *et al*., 2014), negative (Van Cleeff *et al*., 1996) or positive (Larson *et al*., 2007; Mehni *et al*., 2012; Souza *et al*., 2015) effect of progesterone supplementation during early luteal phase. Luteal insufficiency is a known cause of embryonic mortality that contributes to repeat breeding in dairy cows (Villarroel *et al*., 2004). Progesterone administration will only improve the fertility when there is lack of progesterone in the blood (Bó *et al*., 2007). Repeat breeder cows treated with intravaginal progesterone implant post-AI have tendency to reduce the embryonic mortality (Villarroel *et al*., 2004; Ergene, 2012). Reuse of intravaginal progesterone device may transmit the pathogens and spread the diseases, which would contribute to embryonic mortality in early stage of pregnancy. We have neither recorded any contamination in vagina in term of dirty mucus discharge nor any adverse systemic clinical signs following reuse of TRIU-B. Autoclaving may be the best option when re-using CIDR inserts, as it reduces maximally the risk of disease transmission (Zuluaga and Williams, 2008). On the basis of an apparent benefit of increase in plasma progesterone following reuse of TIRU-B, study is warranted in

Table 1. Luteal profile (corpus luteum diameter and plasma progesterone concentrations) and conception rate in buffalo inserted with used progesterone intravaginal insert (TRIU-B) between d4 and 10 post-ovulation (P: Pregnant; NP: Non-pregnant; POF: Preovulatory follicle).

Day of AI	**Control (*n*=15)**	**TRIU-B (*n*=15)**	**Control (*n*=15)**		**TRIU-B (*n*=15)**	
			P (*n*=5)	**NP (*n*=10)**	**P (*n*=9)**	**NP (*n*=6)**
POF diameter, mm	11.7 ± 0.4	12.0 ± 0.4	11.1 ± 0.4	11.9 ± 0.6	11.7 ± 0.5	12.4 ± 0.6
Corpus luteum diameter, mm						
Day 4 Post-Ovulation	12.9 ± 0.4	13.2 ± 0.3	12.2 ± 0.4	13.3 ± 0.5	12.9 ± 0.4	13.6 ± 0.6
Day 10	15.2 ± 0.5[a]	14.0 ± 0.3[b]	15.4 ± 0.8	15.1 ± 0.6	13.7 ± 0.4	14.5 ± 0.6
Plasma progesterone, ng/ml						
Day 0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Day 4	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
Day 10	1.7 ± 0.1[a]	3.4 ± 0.2[b]	1.8 ± 0.2[e]	1.6 ± 0.1[c]	3.3 ± 0.2[f]	3.6 ± 0.3[d]
Conception Rate (%)	37.5% (15/40)	55% (22/40)				

[a *vs* b, c *vs* d, e *vs* f] Values in a row differ significantly at P< 0.05

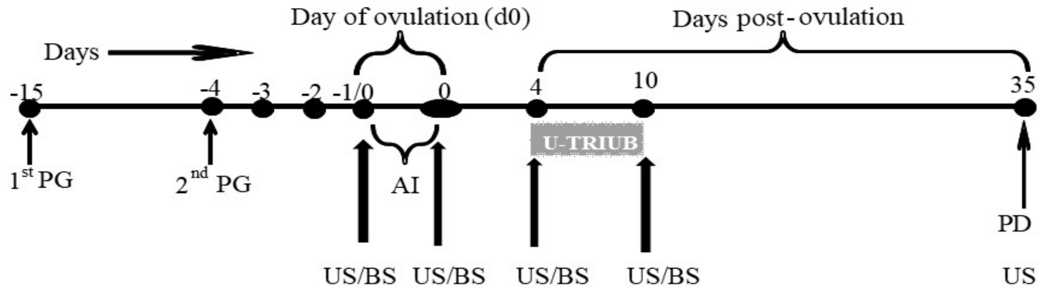

Figure 1. Experimental design of control and treatment group (onec-ues TRU-B) buffalo during summer.
AI : artificial inseminaton;
BS : blood sampling;
d : day;
PD : pregnancy diagnosis;
PG : prostaglandin;
US : ultrasonography

repeat breeding buffaloes to investigate its impact on conception rate.

In conclusion, post-ovulation supplementation with once 5 day used TRIU-B could be used between day 4 and day 10 as intravaginal progesterone supplement to increase the circulating progesterone, but it couldn't increase conception rate in buffalo during summer.

Conflict of interest statement

We hereby declare that there were no conflicts of interest among authors, institutions and/or organizations involved in the study.

ACKNOWLEDGEMENTS

This work was carried out under the research project entitled "Diagnosis of infertility owing to hormonal imbalance and optimizing fertility through hormonal approaches in dairy animals" (RKVY Scheme 4030-C (g)-TVCC-3-O.A.). The authors are highly thankful to Dr. Sunil Bishnoi, Dr. Pankaj Gunwant, Dr. Prem Singh, Dr. R. K. Chandolia and my colleagues of Department of Veterinary Clinical Complex for their unconditional help at every step of experimentation.

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