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Effect of progesterone releasing intra vaginal device and Prostaglandin F_2 alpha on the fertility rate in postpartum buffaloes

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Abstract
Synchronization of estrus is a reliable technique which results in higher pregnancy in buffaloes when combined with timed artificial insemination. A total number of 18 healthy pluriparous buffaloes at 60 days postpartum were selected for the study and equally divided into three groups' *viz*., Group I, Group II, and Group III. Group I buffaloes were inserted with PIVD intravaginally for 9 days and was removed on 9th day and inseminated artificially 48 hours after PIVD removal. Group II buffaloes were inserted with PIVD for 9 days and 25mg of $PGF_2\alpha$ was administered one day prior to PIVD removal. Artificial Insemination was done 48 hours after the PIVD removal. Group III buffaloes were kept as control and inseminated artificially during natural estrus. The conception rate after first and second service and overall conception rates observed in this study were 33.33, 16.67 and 50.00 in group I; 16.67, 33.33 and 50.00 in group II and 16.67, 16.66 and 33.33 in group III, respectively. It could be concluded that the PIVD can be effectively used for synchronization of estrus in postpartum buffaloes.

Keywords: PVID, Progesterone, PG, Buffaloes

Introduction
Buffalo has been a traditionally poor breeder characterized by delayed puberty and sexual maturity, seasonality, poor estrus expresser possessing lower conception rate and long calving intervals (Selvaraju, *et al*., 2005 and Ganesh *et al*., 2022) [15, 5]. Synchronization of estrus in goats was successfully performed using FGA (Selvaraju and Kathiresan, 1997) [23], MAP (Selvaraju *et al*., 1997) [23], and norgestomet (Senthilkumar *et al*., 1998 [25] and CIDR (Selvaraju *et al*., 2003) [24] However, Narayanan *et al*. (2006) [8] utilized norgestomet - eCG to induce multuiple births in ewes. Similarly, in repeat breeding cows synchronization was performed by utilizing norgestomet (Selvaraju *et al*., 2009a) [16], $PGF_2\alpha$ (Selvaraju *et al*., 2010a) [18], norgestomate plus hCG (Selvaraju *et al*., 2010b) [21], $PGF_2\alpha$ plus hCG (Selvaraju *et al*., 2010c) [22]. As a consequence, majority of buffaloes (18-40 per cent) are culled and slaughtered due to infertility. To overcome these discrepancies and to enhance fertility, estrus synchronization is the major and applicable assisted reproductive technique. Fixed Time Artificial Insemination (FTAI) is one among the modifications that overcome the difficulties in estrus detection and to improve fertility in buffaloes (Baruselli *et al.*, 2003) [3]. In spite of multiple protocols in cows, buffaloes were not synchronized for improving the fertility using different combinations of hormones. Synchronization of estrus using progesterone devices such as controlled internal drug release (CIDR), progesterone releasing intra vaginal device (PRID) and progesterone releasing intra vaginal sponges had been discussed earlier with varying conception rates.

Materials and Methods
A total number of 18 healthy pluriparous buffaloes at 60 days postpartum with regular estrous cycle length, no palpable genital tract abnormalities based on gynaeco clinical examination and negative for white side test were selected for the study. The selected buffaloes were equally divided into three groups' *viz*., Group I, Group II, and Group III. The experimental buffaloes were dewormed and supplemented with 30g of TANUVAS mineral mixture for 20 days.
A progesterone impregnated intra vaginal device (PIVD), TRIU-B® (Virbac animal health Ltd., Argentina) containing 3 medicated rings (green color) and one non medicated ring (white color) was selected as progesterone device. Each medicated ring (green color) contains 186 mg

of progesterone. One additional ring (pink colour) contains 400 mg of progesterone has also been provided. The barrel and the plunger provided were used after proper washing with antiseptic solution and with lubricant.

Group I buffaloes were inserted with PIVD intravaginally for 9 days. The PIVD was withdrawn on 9th day and artificial insemination was done 48 hours after withdrawal of PIVD. Group II buffaloes were inserted with PIVD intravaginally for 9 days and 25mg of $PGF_2\alpha$ (Lutalyse ®, Pfizer India Ltd.,) was administered intramuscularly one day prior to the removal of PIVD (on 8th day) and timed artificial Insemination was done 48 hours after the PIVD withdrawal. Group III buffaloes were kept as control and artificially inseminated during natural estrus.

The estrus response (per cent), onset of estrus (hours), duration of estrus (hours), intensity of estrus, ultrasonography of the ovaries and serum progesterone profile were studied in the experimental group.

Ovulatory response

Ovulatory response was assessed by the presence of CL in any one of the ovary detected by rectal examination in group I, II and III.

Conception rate

Conception rate was calculated as percentage of animals that conceived to insemination at induced estrus and subsequent estrus and pregnancy verified 45 days after insemination.

Serum endocrine profile

In all the experimental buffaloes (Group I and II), blood collection was done during (i) animal selection, (ii) TRIU-B insertion (PIVD), (iii) PIVD removal, (iv) artificial insemination, (v) 7 days after AI and (vi) 45 days after AI for estimating the progesterone profile by radioimmunoassay (RIA). Additionally in group II buffaloes serum samples were collected at during $PGF_2\alpha$ administration. In control animals the blood collection was done at selection, at AI and 7 days after AI.

Results and Discussion

The PIVD retention rate and estrus expression in the present study were 100 per cent group I and group II buffaloes as reported by Ganesh *et al*., 2022 [5]. No complications were observed during the treatment period with PIVD and both the protocols applied for estrus synchronization effective inducing estrum in all the experimental buffaloes.

The time of onset, duration and intensity of induced estrus and conception rate are presented in table 1. The onset of standing estrum is the best indicator for insemination timing (Selvaraju *et al*., 2009) [17]. The mean (±SE) values for the onset of induced estrus were 48.17±1.05 and 47.33±1.99 in groups I and II, respectively. Among the experimental groups, group I buffaloes had the higher mean (±SE) onset of estrus (48.17±1.05 h) followed by groups II. However, the difference ($p \geq 0.5$) observed in mean onset of induced estrus among experimental groups was not statistically significant ($p \geq 0.5$). The results of this study indicated that the PIVD did not affect the onset of estrus following synchronization of estrus. However, onset of estrum following CIDR removal was 36-72 (Lakra *et al*., 2003) [7], 46-47 (Azawi *et al.*, 2012), 42.92 (Ravikumar *et al.*, 2014) [12] and 72-96 h (Singh, 2003) [26] in buffaloes. The earlier onset of *Oestrus* might be due to the early acceleration of oestradiol production after withdrawal of CIDR (Lakra *et al*., 2003) [7].

The mean (±SE) values of duration of induced estrus in groups I and II were 22.83±1.19 and 24.00±0.45, respectively. Among all the experimental groups, group II had the higher mean (±SE) duration of induced estrus (23.10±0.37 h). It was clear that the synchronization of estrus with PIVD did not influence the duration of induced estrus. The result of the study is in accordance with the findings of Ganesh (2013) [4] 27.31± 0.67 and Ravi Kumar *et al.* (2014) 27.60±0.31 h in postpartum buffaloes. The mean (±SE) values of duration of natural estrus in group I was 22.67±080 h. This was in accordance with the reports of Ravikumar *et al.* (2014) [12].

In the present study the intensity of estrus following synchronization in buffaloes was classified as intense, intermediate and weak. During synchronization of estrus with PIVD, the percentages of buffaloes with intense, intermediate and weak estrus intensities were 33.33, 33.33 and 33.34 in group I; 50.00, 16.67 and 33.33 in group II and 50.00 per cent in group III, respectively. In this study, the intensity of induced *Oestrus* was classified as intense, intermediate and weak based on the manifestations of *Oestrus* symptoms, the intensity of the *Oestrus* was classified as weak, normal and intense (Velladurai *et al.*, 2015) [28] in estrus synchronized cows. The PIVD treatment in the present study resulted in good *Oestrus* expression in buffaloes which might be due to the reason that progesterone used might have increased the follicular sensitivity to elevated serum LH concentration and increased estradiol secretion (Sanchetz *et al*., 1993) [13]. Minerals are the co-enzymes for the production of steroid hormones (Pandey *et al*., 2007) [9] and calcium plays a vital role in utilization of cholesterol by mitochondria for hormone synthesis. The ovulatory response following estrus synchronization in buffaloes were 83.33, 83.33 and 66.66 per cent in groups I, II and III, respectively. Similar finding was reported in buffaloes by Ravi kumar *et al.,* (2014) [12] and (Ganesh, 2013) [4].

Pregnancy diagnosis by rectal examination and ultrasonography was done at 45 days post AI in all the groups. The conception rates following first and second service and overall conception rates observed in this study were 33.33, 16.67 and 50.00 in group I; 16.67, 33.33 and 50.00 in group II and 16.67,16.66 and 33.33 in group III, respectively. Similar conception rates were obtained by Ravikumar *et al.,* (2014) [12] with CIDR in postpartum buffaloes. However, Andurkar and Kadu, (1995) [1] recorded 100 per cent conception rate in CIDR plus $PGF_2\alpha$ treated buffaloes. The result of this study indicated that inclusion $PGF_2\alpha$ in PIVD protocol did not improve the conception rate.

The serum progesterone concentrations (ng/ml) in all the experimental buffaloes are presented in Table 2. The serum progesterone concentrations (ng/ml) during selection, PIVD insertion, PIVD removal, FTAI, 7 days after AI and 45 days after AI were 1.85±0.13,2.50±0.16, 3.36±0.14, 0.86±0.66, 2.13±0.50 and 2.55±0.62 in group I buffaloes and the progesterone levels (ng/ml) during selection, PIVD insertion, Inj.$PGF_2\alpha$, PIVD removal, FTAI, 7 days after AI and 45 days after AI were 1.59±0.05, 2.47±0.07, 2.82±0.28, 3.56±0.12, 0.79±0.10, 3.07±0.47 and 3.49±0.53 hours in group II buffaloes.

Progesterone levels in peripheral blood directly reflected the functionality of the corpus luteum. From this study, it was observed that the serum progesterone levels increased from selection of buffaloes to the PIVD removal but reached below 1 ng/ml at the time of estrus and elevated at 7 days post AI as

suggested by Ganesh *et al*. (2022) [5].

The serum progesterone concentration in the current study ranged from 1.73 to 3.42 from selection to PIVD removal in an increasing manner followed by basal concentration on day of AI and increases on day 7 and day 45. Similar finding was found by Kabir *et al*. (2001) [6] following CIDR and Rajamahendran and Thamotharam (2003) [10] PRID treatment in postpartum buffaloes and in Malabari goats (Selvaraju *et al.,* 2007). The serum progesterone concentration during PIVD removal ranged between 3.36 and 3.56 ng/ml and this was in agreement with the findings of Singh and Singh (2006) [27] and Rajamahendran and Thamotharam (2003) [10]. Supplementation of mineral mixture in the present study might have altered the progesterone and estrogen ratio and increased the number of large size follicles (Ravikumar *et al*., 2018 and Selvaraju *et al*., 2020) [11, 14]. Hence concluded that, PIVD treatment resulted in 100 per cent *Oestrus* response in buffaloes. The administration of $PGF_2\alpha$ at the time of withdrawal of PIVD did not improve the conception rate in buffaloes. Hence, it is recommended PIVD may be used as a progesterone source to augment fertility in buffaloes under field conditions.

Table 1: Onset, duration, intensity of induced estrus and coneption rate (mean ± se) following synchronization of estrus with pivd in buffaloes

Treatment groups	Onset of induced estrus (Mean ±SE hours)	Duration of estrus (Mean ±SE hours)	Intensity of the estrus			Ovulatory response (per cent)	Conception rate		
			Intense	Intermediate	Weak		First service conception rate	Second service conception rate	Overall conception rate
Group I	48.17±1.05	22.83±1.89	2 (33.33)	2 (33.33)	2 (33.34)	83.33 (5/6)	2/6 (33.33)	1/6 (16.67)	50.00 (3/6)
Group II	47.33±1.99	24.00±0.45	3 (50.00)	1 (16.67)	2 (33.33)	83.33 (5/6)	1/6 (16.67)	2/6 (33.33)	50.00 (3/6)
Group III	-	22.67±0.80	1 (16.67)	2 (33.33)	3 (50.00)	66.66 (4/6)	1/6 (16.67)	1/6 (16.66)	33.33 (2/6)

Table 2: Serum progesterone concentration in buffaloes treated with pivd and $pgf_2\alpha$

Group	Selection	PIVD insertion	$PGF_2\alpha$	PIVD removal	FTAI	7 days after AI	45 days after AI
I	1.85^{apq}±0.13	2.50^{aqr}±0.16	-	3.36^{ar}±0.14	0.86^{ap}±0.66	2.13^{aq}±0.50	2.55^{aqr}±0.62
II	1.59^{ap}±0.05	2.47^{aq}±0.07	2.82^{qr}±0.28	3.56^{ar}±0.12	0.79^{ap}±0.10	3.07^{aqr}±0.47	3.49^{aqr}±0.53
III	-	-	-	-	1.98^{bp}±0.35	2.47^{ap}±0.12	2.43^{ap}±0.20

Mean values bearing different superscripts between columns (a,b,c,d) among different groups and between rows (p, q, r) on different days of blood collection in the group differ significantly ($p<0.05$)

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