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M Selvaraju
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

P Kumaresan
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

R Ezakial Napolean
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

D Gopikrishnan
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

Corresponding Author:
M Selvaraju
Department of Veterinary Gynaecology and Obstetrics, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Tamil Nadu, India

Effect of GnRH and hCG on the fertility rate in river buffaloes following estrus synchronization with progesterone impregnated intra vaginal device (PIVD)

M Selvaraju, P Kumaresan, R Ezakial Napolean and D Gopikrishnan

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Abstract
Estrus synchronization program results in effective synchronization of stage of the estrous cycle which is associated with a relatively higher conception if combined with fixed time insemination in buffaloes. 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, II, and III. Group I buffalo were inserted with PIVD intravaginally for 9 days and PIVD was removed on 9th day and 10 µg of GnRH was administered during artificial insemination (AI) was performed 48 hours after withdrawal of PIVD. Group II buffaloes were inserted with PIVD intravaginally for 9 days and PIVD was removed on 9th day and 2000 IU of hCG 25 mg of PGF2α was administered intramuscularly one day prior to the removal of PIVD. Group III buffaloes were kept as control and artificially inseminated during natural estrus. The conception rate after first and second service and overall observed in this study were 33.33, 16.67 and 50.00; 16.67, 33.33 and 50.00 and 16.67, 16.66 and 33.33 in groups I, II and III, respectively. PIVD treatment resulted in 100 per cent oestrus response in buffaloes. Administration of hCG during FTAI increased the conception rate when compared to PIVD treatment alone. But injection GnRH during FTAI resulted in higher conception rate than hCG group in postpartum buffaloes.

Keywords: PIVD, Progesterone, GnRH, hCG, fertility, buffaloes

Introduction
Poor heat detection and prolonged postpartum period is some of the main contributing factors to the prolonged intercalving period and decreased reproductive efficiency in buffaloes (Selvaraju *et al.*, 2005 and El-Wishy, 2007) [17, 4]. Sah and Nakao (2010) [12] from their studies opined that 34-49 per cent buffaloes showed estrus signs within 90 days postpartum, while 31-42 per cent remained acyclic for more than 150 days postpartum. The reproductive efficiency of the buffaloes can be improved by adoption of assisted reproductive techniques such as estrus synchronization protocols. Various synchronization programmes for estrus and ovulations have been framed in domestic animals using Fluorogesterone acetate (Selvaraju and Kathiresan, 1997) [22], medroxyprogesterone acetate (Selvaraju *et al*., 1997) [22], norgestomet (Senthilkumar *et al*., 1998) [27] and CIDR (Selvaraju *et al*., 2003 and Selvaraju *et al.*, 2004) [26, 19] in goats and Norgestomet – eCG (Narayanan *et al*., 2006) [9] in sheep. Similar protocols were also framed using Norgestomet and $PGF_2\alpha$ (Selvaraju *et al*., 1997 and Selvaraju and Veerapandian, 2010) [22], Norgestomet (Selvaraju *et al*., 2009a) [8], $PGF_2\alpha$ (Selvaraju *et al*., 2010a) [21], Norgestomet and hCG (Selvaraju *et al*., 2010b) [23], hCG plus $PGF_2\alpha$ (Selvaraju *et al*., 2010c) [24] in repeat breeding cows. Velladurai *et al.*, (2015) [30] reported that the use of GnRH to synchronize the follicle growth and ovulation is the most common protocol used for the synchronization in cows and buffaloes. Most of the estrus synchronization protocols in buffaloes are based on protocols developed for cattle, either shortening the luteal phase or prolonging the luteal phase resulting in variable fertility. In buffaloes, synchronization has been done by conventional use of $PGF_2\alpha$, and/ or use of progesterone devices which ensued acceptable fertility. However synchronization of ovulation will be more helpful for ensuring fertilization and pregnancy.

The ovulation can be synchronized by addition of gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG). The present study explains the influence of GnRH and hCG along with PIVD on the fertility rate in postpartum buffaloes.

Materials and Methods

Eighteen healthy pluriparous buffaloes at 60 days postpartum with no palpable genital tract abnormalities and negative for white side test with regular estrous cycle were form the large animal Gynaecology ward, Department of Clinics, Veterinary College and Research Institute, Namakkal were utilized for the study. The buffaloes were randomly divided into three groups *viz*., Group I, II, and III each with six buffaloes. All the selected buffaloes were dewormed before the initiation of the study and supplemented with 30-50 g of TANUVAS mineral mixture per day.

A progesterone impregnated intra vaginal device (PIVD), TRIU-B® (Virbac animal health Ltd., Argentina) were used as progesterone source. Group I buffaloes were inserted with PIVD intravaginally for 9 days. The PIVD was withdrawn on day 9 day and artificial insemination (AI) was performed 48 hours after withdrawal of PIVD. Ten µg of GnRH (2.5 ml, Buserelin acetate, Ovulanta®, Vet Mankind, New Delhi) was administered intramuscularly at the time of artificial insemination.

Group II buffaloes were inserted with PIVD intravaginally for 9 days. The PIVD was removed on day 9 and artificial inseminated 48 hours after withdrawal of PIVD. But instead of GnRH, 2000 IU of hCG (Folyson®, Indian immunologicals Ltd.,) was administered intramuscularly at the time of FTAI. 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. The serum progesterone concentration was studied during selection, PIVD insertion, PIVD removal, FTAI, 7 days after AI and 45 days after AI and conception rate were also studied. 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 was confirmed by ultrasonography.

Pregnancy was confirmed by ultrasonography at 45 days post insemination. In Group I and II buffaloes, blood collection was done at the time of (i) animal selection, (ii) TRIU-B insertion, (iii) PIVD removal, (iv) artificial insemination, (v) 7 days after AI and (vi) 45 days after AI for estimating the progesterone profile. In control animals the blood collection was done at selection, at AI and 7 days after AI. Serum samples were analysed for progesterone by radio-immunoassay technique (RIA).

Table 1: Onset, duration, intensity of induced estrus and conception rate (Mean ± SE) following synchronization of estrus with PIVD, GnRH and hCG 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	43.33±1.78	22.33±0.99	3(50.00)	2(33.33)	1(16.67)	100.00(6/6)	3/6(50.00)	2/6(33.33)	5/6(83.33)
Group II	46.33±0.62	23.67±0.62	4(66.67)	1(16.66)	1(16.67)	100.00(6/6)	2/6(33.33)	2/6(33.33)	4/6(66.66)
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)

Values in the parenthesis denotes the percentage

Table 2: Serum progesterone concentration in buffaloes treated with progesterone intravaginal device**,** GnRH and hCG

Group	Selection	PIVD insertion	PIVD removal	FTAI	7 days after AI	45 days after AI
I	1.73^{apq}±0.07	2.58^{aqr}±0.11	3.42^{ar}±0.12	0.82^{ap}±0.07	1.99^{apq}±0.54	3.72^{ar}±0.70
II	1.97^{aq}±0.08	2.39^{aq}±0.18	3.36^{ar}±0.17	0.42^{ap}±0.16	1.23^{apq}±0.48	3.72^{ar}±0.65
III	-	-	-	1.98^{bp}±0.35	2.47^{ap}±0.12	2.43^{ap}±0.20

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).

Results and Discussion

The retention rate of the PIVD and estrus expression was 100 per cent in both group I and group II buffaloes in the present study which was reported by Ganesh *et al*. (2022) [7]. Both the protocol applied for estrus synchronization was highly effective to induce estrus in all the postpartum experimental buffaloes.

The onset of induced estrus, duration of induced estrus, intensity of induced estrus and conception rate are presented in table 1. Onset of standing estrus is considered to be ma\jor determinant of insemination timing in synchronization programme (Selvaraju *et al*., 2009b) [20]. The mean (±SE) interval for the onset of induced estrus was 43.33±1.78 hrs and 46.33±0.62 hrs in groups I and II, respectively. Among the experimental groups, group I buffaloes had the higher mean onset of estrus followed by group II, however, no statistically significant difference was observed (p≥0.5).

The mean duration of induced estrus in groups I and II were 22.33±0.99 and 23.67±0.62, respectively and this is in accordance with Selvaraju *et al.* (2008) [14] in repeat breeding cows. Even though the group I buffaloes had short duration of estrus the difference was not statistically different suggesting that the synchronization of estrus with PIVD did not influence the duration of induced estrus in postpartum buffaloes.

The intensity of estrus following synchronization of estrus with PIVD in buffaloes was classified as intense, intermediate and weak. In the present study the percentages of buffaloes with intense, intermediate and weak estrus intensities were 50.00, 33.33 and 16.67 in group I; 66.67, 16.67 and 16.67 in group II and 16.67, 33.33 and 50.00 per cent in group III, respectively. 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) [10] and calcium plays a vital role in utilization of cholesterol by mitochondria for hormone synthesis.

In oestrus induction treatments, progesterone increased hypothalamus sensitivity of estrogens with subsequent increase in the intensity of oestrus (Fabre-Nys and Martin, 1991) [5]. GnRH stimulation to increase LH secretion was a calcium dependent mechanism and even meagre deficiency of phosphorus could cause pituitary-ovarian axis disturbances (Arosh *et al*., 1998) [1]. The study suggests that the supplementation of minerals improved the estrus expression in buffaloes.

The ovulatory response following estrus synchronization on day 7 after insemination in buffaloes were 100.00, 100.00 and 66.66 per cent in group I, II and III, respectively. The ovulatory response in group II was 83.33 per cent. The ovulatory response in group I and II in the present study might be due to the administration of GnRH or hCG at the time of AI as explained by Carvalho *et al.* (2007) [3]. Pregnancy diagnosis by rectal examination and ultrasonography was done at 45 days post AI in all the groups. The pregnancy rates in this study following estrus synchronization estrus with PIVD in buffaloes are documented in table 1. The conception rates following first and second service and overall conception rates observed in this study were 50.00, 33.33 and 83.33 in group I; 33.33, 33.33 and 66.66 in group II and 16.67,16.66 and 33.33 in group III, respectively. Group I buffaloes had 83.33 per cent of overall conception. Similar findings were observed by Carvalho *et al.* (2007) [3] and Azawi *et al*. (2012) in CIDR+GnRH treated buffaloes.

Group II buffaloes had the overall conception rate of 66.66 per cent. A similar conception rate was obtained by Carvalho *et al.* (2007) [3] and Murugavel *et al.* (2009) [8] in CIDR+hCG treated buffaloes. The serum progesterone concentrations (ng/ml) during selection, PIVD insertion, PIVD removal, FTAI, 7 days after AI and 45 days after AI were 1.73±0.07, 2.58±0.11, 3.42±0.12, 0.82±0.07, 1.99±0.54 and 3.72±0.70 in group I;1.97±0.08, 2.39±0.18, 3.36±0.17, 0.42±0.16, 1.23±0.48 and 3.72±0.65 in group II buffaloes and are presented in table 2.

In the present investigation, all the treatment groups had higher conception rate than control groups. This result clearly indicated that supplementation of mineral mixture along with PIVD treatment had the greater impact on conception rate in buffaloes. Administration of GnRH at FTAI further increased conception rate in postpartum buffaloes. Progesterone levels in peripheral blood directly reflected the function of corpus luteum and were considered as an indicator of ovarian function. The results of the study shows that the serum progesterone levels in all experimental groups increased from selection of buffaloes to the removal of PIVD but at the time of estrus, the serum progesterone levels reached below 1 ng/ml and at 7 days post AI, there was an elevated progesterone levels in all the groups as suggested by Ganesh *et al*. (2022) [7]. The serum progesterone concentration in the current study ranged from 1.73 to 3.72 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. In this current investigation, the serum progesterone recorded during PIVD removal was ranged between 3.36-3.42 ng/ml. According to Selvaraju *et al*. (2020) [16] mineral mixture supplemented in the present study might have been due to altered oestrogen and progesterone ratio and decreased atretic follicle number.

Hence, it was concluded that PIVD treatment resulted in 100 per cent oestrus response in buffaloes. Administration of hCG during FTAI increased the conception rate when compared to PIVD treatment alone. But injection GnRH during FTAI resulted in higher conception rate than hCG group in postpartum buffaloes. Hence, it is recommended PIVD in combination with GnRH at AI may be used to augment fertility in buffaloes under field conditions.

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