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Effect of Ovsynch and Ovsynch with CIDR protocols on synchronization of ovulation and conception in postpartum buffaloes during peak breeding season

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Abstract
Ovulation synchronization is an irresistible tool to augment fertility, but, efficacy of various protocols has not been reported in buffaloes. Twenty numbers of graded Murrah buffaloes were selected for the study and divided into two equal groups (I and II). The group I buffaloes were treated with Ovsynch protocol and group II buffaloes were treated with Ovsynch treatment plus CIDR protocol (Additional to Ovsynch protocol, CIDR was inserted intra-vaginally for 7 days from day 0 of Ovsynch and removed on the day of $PGF_{2}\alpha$ on day 7. The buffaloes returned to estrus following TAI were inseminated artificially in the subsequent estrus. The interval to onset of induced estrus following ovulation synchronization in group I and group II buffaloes were 46.80 ±1.19 and 42.10±2.39 hours, respectively. The duration of induced estrus in groups I buffaloes differed significantly from group II buffaloes. The percentage of buffaloes with intense, intermediate and weak estrus 0 (0), 5 (50.00) and 5 (50.00) in group I and 2 (20.00), 7 (70.00) and 1 (10.00) in group II. The ovulatory response in group I and II buffaloes were 90.00 and 100.00 per cent, respectively. The conception rate after first and second insemination and overall conception rate in this study were 30.00, 20.00 and 50.00 per cent in group I buffaloes and 30.00, 40.00 and 70.00 per cent in group II buffaloes. The progesterone concentration in group I and group II buffaloes did not differ significantly, however, the progesterone concentration differed significantly within the groups. The serum estrogen concentration in group I and group II buffaloes did not differ significantly, however, the estrogen concentration differed significantly on the day of AI from other days of the treatment within the groups. The study concluded that the Ovsynch with CIDR protocol following mineral supplementation resulted in better conception than the Ovsynch group.

Keywords: Ovsynch, Ovsynch plus CIDR, postpartum buffaloes, peak breeding season

Introduction
Genetic improvement for augmenting production could be achieved using artificial insemination (AI). Establishment of pregnancy depends on estrus detection; however, buffaloes exhibit subtle estrus expression (Baruselli *et al.*, 2003) [3] and highly aberrant estrus duration (Ohashi, 1994) [12] hindering the timing of ovulation and hence, fixed time insemination could be the only solution. Therefore effective estrus detection would ideally shorten the calving intervals and better reproductive performance.

Synchronization of estrus and ovulation could help in resolve these problems and removes the necessity of estrus detection. Various programmes for synchronization of estrus and ovulations have been practiced using Fluorogesterone acetate (Selvaraju and Kathiresan, 1997) [25], medroxyprogesterone acetate (Selvaraju *et al.*, 1997) [28], norgestomet and CIDR (Selvaraju *et al.*, 2004) [23] in goats and norgestomet – eCG (Narayanan *et al.*, 2006) [11] in sheep. Similar protocols were also framed using Norgestomet and $PGF_{2}\alpha$ (Selvaraju *et al.*, 1997[28] and Selvaraju and Veerapandian, 2010) [21], norgestomet (Selvaraju *et al.*, 2009) [22], $PGF_{2}\alpha$ (Selvaraju *et al.*, 2010a) [24], norgestomet and hCG (Selvaraju *et al.*, 2010b) [26], hCG plus $PGF_{2}\alpha$ (Selvaraju *et al.*, 2010c) [27] in repeat breeding cows. Velladurai *et al.*, (2015) [33] 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. However, the exact time of ovulation is essential for obtaining major reproductive advancement in buffaloes and hence ovulation synchronization is mandate to treat infertility in buffaloes (Selvaraju *et al.*, 2021) [20].

The Ovsynch protocol (Pursley *et al.*, 1995) [13] is the most commonly used TAI protocol, however, response to the Ovsynch protocol vary according to the stage of the estrous cycle during initiation. The Ovsynch protocol initiated between day 5 and 12 of the cycle resulted in better conception rate (Vasconcelos *et al.*, 1999) [32].

Similar studies were done by Ravikumar *et al.* (2021) [16] during peak and low breeding seasons and suggested that Ovsynch protocol resulted in increased pregnancy rate in both seasons.

An approach to improve synchronization is the use of short-term progesterone supplementation using intra vaginal devices for sustained release from the first GnRH to the $PGF_2\alpha$ injections maintaining blood progesterone concentration. This might prevent premature estrus signs, LH surge, and ovulation (El-Zarkouny *et al.*, 2004 and McDougall, 2010) [6, 10]. However, the success rates of these treatments are unfolded in buffaloes.

Materials and Methods

Twenty healthy postpartum (more than 90 days) graded Murrah buffaloes aged between 2nd and 5th parity were selected and divided equally into group I to group II.

The selected buffaloes were supplemented with TANUVAS mineral mixture for 15 days before the initiation of the protocol. The buffaloes of group I were treated with Ovsynch protocol as described by Pursley *et al.* (1995) [12]. The buffaloes were administered with 10 µg of GnRH (2.5 ml, Buserelin acetate, Ovulanta®, Vet Mankind) on the day 0 (Initiation of the protocol), 500 µg of $PGF_2\alpha$ (2 ml, Cloprostenol, Pragma®, INTAS Pharmaceuticals, Ahmedabad, India) on day 7 after first GnRH administration and second GnRH injection (10 µg) was administered on day 9 (48 hours after the $PGF_2\alpha$ administration). Timed artificial insemination (TAI) was done on day 10 (24 hrs after the second GnRH injection). In group II, in addition to the Ovsynch treatment, controlled internal drug release device (CIDR, EAZI-BREED, Pfizer Animal Health, India) was inserted intra-vaginally for 7 days starting from day 0 (1st GnRH injection) and removed on day 7 ($PGF_2\alpha$ injection) of Ovsynch protocol.

The estrus response, pattern of induced estrus, ultrasonographic evaluation ovaries, conception rate and progesterone concentration were studied. The experimental buffaloes were closely observed for estrus signs after $PGF_2\alpha$ injection in groups I and II buffaloes.

The onset of estrus (hrs) was calculated from the time of $PGF_2\alpha$ administration to first appearance of estrus signs and duration of estrus (hrs) was calculated from the time of first appearance of estrus signs to the disappearance of estrus signs.

The ovulatory response was assessed by ultrasonography on day 10 after induced estrus. Ovarian structures of group I and group II buffaloes subjected to ultrasonography during(i) selection (ii) initiation of Ovsynch protocol (iii) $PGF_2\alpha$ injection (iv) first AI and (v) 10 days following TAI. Ultrasound scanning of ovaries was done transrectally with a real time ultrasound scanner (SONOVET 600) equipped with liner array, 5–7.5 MHz frequency transrectal transducer.

Pregnancy was confirmed ultrasonography at 45 days post insemination and pregnancy rate was assessed in per cent. The serum samples were collected for progesterone and estradiol-17β estimation. The progesterone and estradiol-17β concentration in serum samples was measured by RIA (Immunotech, Beckman Coulter, France).

Table 1: Pattern of induced estrus following Ovsynch and Ovsynch plus CIDR protocols in buffaloes

S. No.	Treatment groups	No of buffaloes treated	Onset of induced estrus (Mean ± SE) (hours)	Duration of estrus (Mean ± SE) (hours)	Intensity of the estrus		
					Intense	Intermediate	Weak
1.	Group I	10	$46.80^{b}\pm1.19$	$26.30^{a}\pm0.47$	-	5 (50.00)	5 (50.00)
2.	Group II	10	$42.10^{a}\pm2.39$	$28.00^{b}\pm0.79$	2 (20.00)	7 (70.00)	1 (10.00)

Figures in the parentheses are in percentage, mean values bearing different superscripts (a, b, c, d) among groups within a same column differ significantly ($p\leq0.05$).

Table 2: Follicular activity and ovulatory response by ultrasonography following ovsynch and ovsynch plus cidr protocols in buffaloes

S. No.	Treatment Groups	No of buffaloes treated	Time of selection		d 0 (GnRH injection)		d 7 ($PGF_2\alpha$ injection)		d 10 (First AI)		10 days Post AI	Conception rate		
			Follicles > 8mm	CL	Follicles> 8mm	CL	Follicles> 8mm	CL	Follicles> 8mm	CL	CL	First service	Second service	Overall
1.	Group I	10	3/10 (30.00)	7/10 (70.00)	4/10 (40.00)	8/10 (80.00)	3/10 (30.00)	9/10 (90.00)	9/10 (90.00)	2/10 (20.00)	9/10 (90.00)	3/10 (30.00)	2/10 (20.00)	5/10 (50.00)
2.	Group II	10	3/10 (30.00)	8/10 (80.00)	4/10 (40.00)	7/10 (70.00)	3/10 (30.00)	8/10 (80.00)	10/10 (100.00)	1/10 (10.00)	10/10 (100.00)	3/10 (30.00)	4/10 (40.00)	7/10 (70.00)

Figures in the parentheses are in percentage

Table 3: Mean (±se) serum progesterone (ng/ml) and estradiol (pg/ml) levels in ovsynch and ovsynch plus cidr protocols in buffaloes

S. No.	Treatment groups	Time of selection	d 0 (GnRH injection)	d 7 ($PGF_2\alpha$ injection)	d 10 (First AI)	10 days Post AI
	Progesterone (ng/ml)					
1.	Group I	$1.11^{bp}\pm0.10$	$1.49^{bp}\pm0.11$	$2.03^{cp}\pm0.13$	$0.43^{ap}\pm0.05$	$3.32^{dp}\pm0.11$
2.	Group II	$1.26^{bq}\pm0.11$	$1.55^{bp}\pm0.12$	$2.67^{cp}\pm0.13$	$0.48^{ap}\pm0.05$	$3.88^{dp}\pm0.11$
	Estradiol(pg/ml)					
1.	Group I	$15.11^{ap}\pm1.19$	$15.75^{ap}\pm1.19$	$16.38^{ap}\pm1.15$	$34.33^{bp}\pm0.85$	$18.72^{ap}\pm2.27$
2.	Group II	$15.41^{ap}\pm1.50$	$16.75^{ap}\pm1.63$	$17.28^{ap}\pm1.08$	$36.22^{bq}\pm0.78$	$18.76^{ap}\pm0.93$

Mean values bearing different superscripts (a, b, c, d) between different days of blood collection within a same row differ significantly ($p\leq0.05$).
Mean values bearing different superscripts (p, q, r, s) between groups within a same column differ significantly ($p\leq0.05$)

Results and Discussion

The pattern of induced estrus (onset, duration and intensity of induced estrus) was studied in group I and group II buffaloes and are presented table 1. The onset of induced estrus following ovulation synchronization in the present study were 46.80 ±1.19 and 42.10±2.39 in group I and group II buffaloes, respectively and the difference was statistically significant. The onset of estrus was less than that reported by Stevenson *et al.* (2006) [31] who reported 54.00 ± 13.00 and 55.00 ± 4.40 hrs in Ovsynch 33 and Ovsynch 48 protocol, respectively in treated dairy cows. Ravikumar (2003) [15] reported that the time of onset of estrus in Ovsynch treated sub-estrus and anestrus buffaloes as 48.80±7.74 and 52.67±4.18 hrs and in Ovsynch plus CIDR treated sub-estrus and anestrus buffaloes as 36.71±2.65 and 41.40±8.33 hrs, respectively. It might be due to the fact that following the $PGF_2\alpha$ injection of Ovsynch protocol might have lysed the corpus luteum completely (Schmitt *et al.,* 1996) [19] as evinced by low serum progesterone concentration during first AI in all the experimental buffaloes.

The duration of induced estrus following ovsynch and ovsynch plus CIDR protocols was 26.30±0.47 in group I buffaloes and 28.00±0.79 hours in group II buffaloes and the findings were in accordance with Ravikumar (2003) [14], Zaabel *et al*. (2009) [34] and Ganesh (2013) [9] in buffaloes, however, Flores *et al*. (2006) [8] reported 6.50 hrs in anestrus cows. The duration of induced estrus differed significantly between group I and group II buffaloes in the present study which is in accordance with Richardson *et al*. (2002) [17] who recorded 12 hours as duration of estrus in heifers following CIDR treatment.

The intensity of estrus following ovsynch and ovsynch plus CIDR protocols was classified as intense, intermediate and weak. The number and percentages of buffaloes with intense, intermediate and weak estrus intensities were 0 (0), 5 (50.00) and 5 (50.00) in group I and 2 (20.00), 7 (70.00) and 1 (10.00) in group II buffaloes, respectively. In the present study, the percentage of intense estrus in group II was higher (20.00) when compared to groups I buffaloes (0.00). The percentages of intermediate estrus were higher in group II (70.00) compared to group I buffaloes (50.00) and the percentages of weak estrus intensity was higher in group I (50.00) than in group II (10.00) buffaloes. In the present investigation, CIDR treatment resulted in good estrus expression in buffaloes. Sanchetz*et al*. (1993) [18] opined that progesterone from CIDR resulted in more pronounced estrus intensity after CIDR withdrawal. Further Fabre-Nys and Martin, (1991) [7] suggested that progesterone increased sensitivity of estrogen in hypothalamus.

The follicular study and ovulatory response in the present study are presented in table 2. The percentage of buffaloes with large size (LF) follicles (>8 mm) and corpus luteum during first GnRH (D 0) were 40.00 and 80.00 in group I buffaloes and 40.00 and 70.00 in group II buffaloes, respectively. The percentage of buffaloes with large size (LF) follicles (>8 mm) and corpus luteum during $PGF_2\alpha$ (D 7) were 30.00 and 90.00 in group I buffaloes and 30.00 and 80.00 in group II buffaloes, respectively. The percentage of buffaloes with large size (LF) follicles (>8 mm) and corpus luteum during first GnRH (D 10) were 90.00 and 20.00 in group I buffaloes and 100.00 and 10.00 in group II buffaloes, respectively. The percentage ovulatory response following ovulation synchronization on day 10 post insemination were 90.00 and 100.00 in groups I and II buffaloes, respectively as reported by Ali and Fahmy (2007) [1] in buffaloes and Pursley *et al.* (1995) [12] in cows. Reduced ovulatory responses (60-80 per cent and 54.54 per cent) were observed by Berber *et al.* (2002) [4] and Ravikumar (2003) [14] following Ovsynch programs in buffaloes.

Pregnancy diagnosis in the present study was performed by ultrasonography at 45 days after insemination in the groups I and II and documented in table 2. The conception rate following first and second insemination and overall conception rate observed were 30.00, 20.00 and 50.00 per cent, respectively in group I and 30.00, 40.00 and 70.00 per cent, respectively in group II buffaloes. The overall conception rate in the group II buffaloes were higher than group I buffaloes. According to Drost (2007) [5] the conception rate of 57.50 was reported in dairy cows following Ovsynch plus CIDR treatment. Senger (2005) [29] suggested that exogenous progesterone (CIDR) limited the follicular development and maturation delaying ovulation until the CIDR removal. These observations might explain the increased conception rates of Ovsynch plus CIDR (group II) treatment than Ovsynch group (group I) as reported by Ravikumar *et al.* (2009) [14]. Similar observations were given by Shahzad *et al.* (2019) [30] suggested that Ovsynch plus CIDR (5 days) protocol can be used as a postpartum reproductive management tool to increase pregnancy rate in cows.

The mean serum concentration of progesterone and estradiol in the present study is presented in table 3. The mean progesterone concentration in group I buffaloes were 1.11±0.10. 1.49±0.11, 2.03±0.13, 0.43±0.05 and 3.32±0.11 during selection, first GnRH (D0), $PGF_2\alpha$ injection (D7), First AI (D10) and Day 10 after AI, respectively. The mean progesterone concentration in group II buffaloes was 1.26±0.10. 1.55±0.12, 2.67±0.13, 0.48±0.05 and 3.88±0.11 during selection, first GnRH (D0), $PGF_2\alpha$ injection (D7), First AI (D10) and Day 10 after AI, respectively.

The mean estradiol concentration in group I buffaloes were 15.11±1.19. 15.75±1.19, 16.38±1.15, 34.33±0.85 and 18.72±2.27 pg/ml during selection, first GnRH (D0), $PGF_2\alpha$ injection (D7), First AI (D10) and Day 10 after AI, respectively. The mean estradiol concentration in group II buffaloes was 15.41±1.50. 16.75±1.63, 17.28±1.08, 36.22±0.78 and 18.76±0.93 pg/ml during selection, first GnRH (D0), $PGF_2\alpha$ injection (D7), First AI (D10) and Day 10 after AI, respectively. Peak estradiol-17β level was observed by Bansal *et al.* (2004) [2] on the day of estrus. The serum estradiol concentration in group I buffaloes differed significantly from group II buffaloes in the present study. The increased estrogen level at d 10 (induced estrus) indicated the presence of preovulatory follicles in the ovaries and were further confirmed by ultrasonography in this study.

Conclusion

The study concluded that the Ovsynch plus CIDR is superior over ovsynch in postpartum buffaloes and suggested that, Ovsynch with CIDR protocol might improve the fertility in postpartum buffaloes during peak breeding season.

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