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## Evaluation of estrus synchronization protocols (Ovsynch vs CIDR) in recipient cows in embryo transfer programme

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### Abstract

The present work was conducted to evaluate estrus character in cows subjected to different estrus synchronization protocol. Estrus synchronization can play important role in faster multiplication of superior germplasm to ensure conservation of cattle breeds and increase in milk production. About 52 cows were distributed into 3 groups randomly. Group 1 (Ovsynch, n=15) 10µg GnRH on 0<sup>th</sup> day, PGF<sub>2</sub>α (500 µg) on 7<sup>th</sup> day and second GnRH dose on 9<sup>th</sup> day. Group 2 (CIDR group, n=10) 0<sup>th</sup> day CIDR, removal of CIDR and 500 µg of PGF<sub>2</sub>α at 7<sup>th</sup> day. Group 3 (control, n=27) cows in natural estrus were included in this group. The results showed significant difference ( $p<0.05$ ) in estrus response rate (%) between treated groups (Ovsynch (86.66), CIDR (100.00) and control group (55.55)). The duration of onset of estrus (hrs.) has no significant ( $p>0.05$ ) difference between Ovsynch (53.69±1.90) and CIDR (49.1±0.48) group, but both CIDR and Ovsynch group differed significantly ( $p<0.05$ ) with control group (109.73±18.74). There was significant difference observed ( $p<0.05$ ) in mean estrus intensity score between CIDR (16.30±1.59) and control group (11.07±1.05), but CIDR and Ovsynch group (13.38±0.94) has no difference significantly ( $p>0.05$ ). From the present study it was concluded that among all treated group, CIDR protocol was most effective and efficient synchronization protocol for improving the efficacy of synchronization and conception rates in embryo transfer programme.

**Keywords:** Estrus synchronization, Ovsynch, CIDR, Estrus response rate

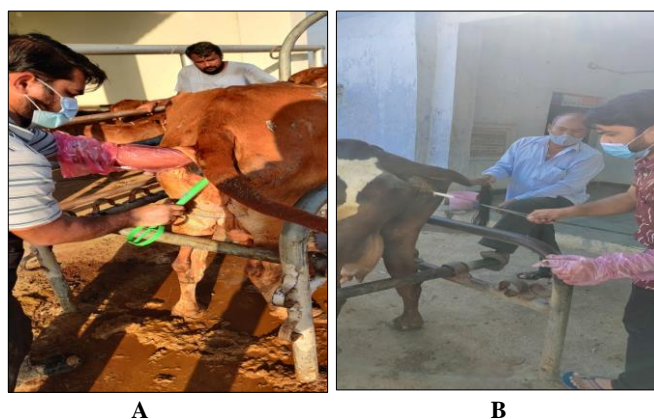
### Introduction

Estrus synchronization implies estrous cycle manipulation or estrus induction in animals so that females would come into estrus in a short time which is predetermined (Odde, 1990) [23]. Estrus synchronization is an advanced process through which minimization of human errors and cost of management could be done. This synchronization of estrus during favourable season helps in fixation of breeding time in a predefined short period so that parturition time can be scheduled to rear the new-borns in a suitable environment to enhance their survival (Islam, 2011) [13]. Increase in the fertility of farm animals due to the timely breeding can be made possible with this technique. The progesterone in the form of a controlled internal drug release (CIDR) device and its combination with PGF<sub>2</sub>α is a successful estrus synchronization protocol, effective in inducing estrus at a younger age in suckling beef cows, heifers (Kajaysri *et al.*, 2017) [16]. The exogenous intravaginal impregnation of CIDR for 7-12 days induces negative feedback in hypothalamus and pituitary and inhibition of gonadotropin hormone release (Macmillan and Burke, 1996) [20]. When the CIDR removed, large quantities of gonadotropin is releases which causes stimulation of follicle development, estradiol release, resuming of estrus and synchronization of ovulation or luteinization of large dominant follicles (Cerri *et al.*, 2009) [7]. The present work was taken up to the synchronise estrus in cows by using Ovsynch and CIDR plus PGF<sub>2</sub>α protocols to monitor the estrus characters and CL quality in both natural estrus and synchronized estrus cows.

### Materials and Methods

The selected cows were randomly distributed into a group of 3. Group 1 (Ovsynch) cows of this group were estrus synchronized by using Ovsynch protocol of GnRH-Buserelin acetate (Receptal) (10µg) on 0<sup>th</sup> day, cloprostenol (Estrumate) (500 µg) on 7<sup>th</sup> day and GnRH second dose on 9<sup>th</sup> day given I/M). Group 2 (CIDR) (day 0 insert CIDR, day 7 CIDR remove and PGF<sub>2</sub>α 500 µg of cloprostenol (Estrumate) given I/M).

Group 3 (control) cows in natural estrus were included in control group. Estrus detection was done by visual observation. The animals were checked twice a day for estrus for 30 min each time. The estrus signs such as bellowing, standing to be mounted, vulval mucus membrane redness, vulval swelling and clear mucous discharge were recorded. In this study, treatment efficacy was measured in terms of estrus response rate, onset of estrus, intensity of estrus and duration of estrus.



**Fig 1:** A) Introducing CIDR with applicator gun into vagina. B) Removal of CIDR by holding tail of CIDR on 7<sup>th</sup> day.

### Estrus response rate

The percentage treated cows that exhibited estrus was used to calculate the estrus response rate. Based on behavioural, physical, and gynaecological evaluation, estrus was determined. The PGF<sub>2</sub> (Estrumate) injection was followed by a physical examination, 24 hours (hrs) later. At every 12 hrs to confirm the estrus, indication signs such as mucus discharges, edema and vulval congestion, behaviour of mounting and stand to be mounted in Ovsynch, CIDR and Control groups of cows were observed. Estrus response rate was expressed in percentage.

### Onset of estrus

The estrus onset was measured in hours (hrs) from the time of PGF<sub>2</sub> treatment to the first appearance of estrus signs in treated cows and for control group animals it was calculated from the day of selection to exhibition of estrus up to 10-day period.

### Duration of estrus

The estrus duration was calculated as the number of hours (hrs) between onset and termination of estrus. The estrus duration in cows was determined by a history obtained from the dairy farms' technical staff based on visual examination of external genital organs and rectal examination of internal genital organs for the appearance (or disappearance) of estrus symptoms and structures on the ovaries.

### Intensity of estrus

The degree of estrus in cows were determined using the Rao and Rao (1981)<sup>[24]</sup> estrus intensity score card, which included a visual and rectal inspection of the external and internal genitalia for demonstrated estrus symptoms and certain physiological changes in the reproductive organs. According to the modified and score card, the estrus intensity was evaluated as weak, normal or intermediate and intense based on scoring of <10, 10 to 15 and >15 points, respectively.

### Statistical analysis

The statistical analysis was carried out using SPSS software, version 20.0. Using the chi-square test, the estrus response rate in distinct groups was compared. Data on the onset of estrus, its intensity, and its duration were utilised in a one-way analysis of variance (ANOVA) to compare variation between groups.

### Results and Discussions

The estrus synchronization efficacy was recorded based on estrus response (Fig 2), onset of estrus, duration of estrus and intensity of estrus.

### Estrus response rate

The Estrus response rates were (13/15) 86.66, (10/10) 100.00 percent in Ovsynch and CIDR groups respectively, and (15/27) 55.55 percent in control group. In the current investigation, statistical analysis showed that there was a significant difference ( $P < 0.05$ ) between the treatment groups (Ovsynch, CIDR) and control group (Table 1). The highest estrus induction rate was observed in CIDR group (100.00 percent) followed by Ovsynch group (86.66 percent) and lowest in control group (55.55 percent). Similar result of estrus response rate with Ovsynch group was reported by Hirole *et al.* (2018)<sup>[12]</sup>. Contrary to this, the greater estrus response rate was reported by Velludurai *et al.* (2014)<sup>[29]</sup>, Buchecha *et al.* (2015)<sup>[6]</sup>, Ahmed *et al.* (2016)<sup>[11]</sup> and Linga Swamy (2017)<sup>[18]</sup> and Doodram (2018)<sup>[10]</sup>. But the lesser estrus response was observed by Al Al-Katanani *et al.* (2002)<sup>[2]</sup> and Ambrose *et al.* (1999)<sup>[3]</sup>. The similar results of estrus response rates were reported with CIDR synchronization protocol by Dhimi *et al.* (2015)<sup>[6]</sup>. While Murugavel *et al.* (2010)<sup>[22]</sup>, Jyothi *et al.* (2012)<sup>[14]</sup>, Haider *et al.* (2017)<sup>[11]</sup> reported lower estrus response rate than the CIDR group.



**Fig 2:** The cow is in standing estrus (heat) after 48 hours following the removal of CIDR and PGF<sub>2</sub> injection.

### Onset of estrus

The mean duration of onset of estrus was recorded as  $53.69 \pm 1.90$ ,  $49.1 \pm 0.48$  and  $109.73 \pm 18.74$  hrs in Ovsynch, CIDR and control groups, respectively. The estrus onset has no difference significantly ( $p > 0.05$ ) between Ovsynch and CIDR treated group, but both CIDR and Ovsynch group were different significantly ( $p < 0.05$ ) with control group.

The mean duration of onset of estrus in Ovsynch study group has similarity to results of Linga Swamy (2017)<sup>[18]</sup>, Hirole *et al.* (2018)<sup>[12]</sup>. Contrary to this, longer onset of estrus was observed by Buhecha *et al.* (2015)<sup>[6]</sup> and Senthilkumar and Chandrahasan (2015)<sup>[27]</sup>.

The mean duration of onset of estrus in CIDR group was in accordance with observation of Jyothi *et al.* (2012)<sup>[14]</sup>, Haider

*et al.* (2017) [11] and Amin *et al.* (2019) [4]. Contrary to this longer time for onset of estrus as observed by Larson *et al.* (2006) [17], Jyothi *et al.* (2011) [15] and Murugavel *et al.* (2010) [22]. Shorter time for onset of estrus was observed by Lucy *et al.* (2001) [19], Degefa *et al.* (2016) [8]. The CIDR group differ significantly ( $p<0.05$ ) with control group, as CIDR group

required shorter time for estrus induction. The shorter induction time of estrus in CIDR group was due to the application of short-term progesterone treatment for 7 days and PGF<sub>2</sub>α administration on the day of CIDR removal which results in close synchrony between estrus and ovulation.

**Table 1:** Estrus response rate in cows treated with therapeutics

S. No	Group	No. of cows treated	No. of cows exhibited estrus	Percentage of cows responded	Chi- Square value
1	Ovsynch	15	13	86.66	9.31
2	Cidr	10	10	100.00	
3	Control	27	15	55.55	

There was significant difference between Ovsynch, CIDR and Control group ( $p<0.05$ )

**Table 2:** Onset of estrus(hrs) in therapeutic groups of cows after injection of PGF<sub>2</sub>α and in control group from beginning of selection

S. NO	Name of the group	No. of cows treated	No. of cows exhibited estrus	Mean onset of estrus(hrs)
1	Ovsynch	15	13	53.69±1.90 <sup>a</sup>
2	Cidr	10	10	49.1±0.48 <sup>a</sup>
3	Control	27	15	109.73±18.74 <sup>b</sup>

<sup>a, b</sup> superscript means with different superscripts in a column differ significantly: ( $p<0.05$ )

**Intensity of estrus**

The overall intensity of estrus was 15.79, 44.74 and 39.47 percent (Table 3) as weak, normal or intermediate and intense, respectively. The experimental cows exhibited the weak estrus intensity was 7.69, 0, and 33.33 percent in Ovsynch, CIDR, and control groups, respectively. Normal or intermediate estrus intensity was 61.53, 30, and 40 percent in Ovsynch, CIDR, and control groups, respectively. Similarly, the intense estrus was observed 30.66, 70.00, and 26.66 percent cows of Ovsynch, CIDR and control groups, respectively. The mean score for estrus intensity were observed as 13.38±0.94, 16.30±1.59 and 11.07±1.05 in Ovsynch, CIDR and control groups, respectively (Table 4). The statistical analysis showed significant difference ( $p<0.05$ ) in mean estrus score between CIDR and Control group, but CIDR and Ovsynch group has no significant difference ( $p>0.05$ ). Similarly, there was no significant difference ( $p>0.05$ ) between Ovsynch and Control group (Table 4). The Ovsynch study group cows exhibited 7.69, 61.53 and 30.76 percent as weak, normal and intense estrus respectively (Table 3, Fig.2). Similar results were reported by Ahmed *et al.* (2016) [1] and Senthilkumar and Chandrahasan (2015) [27] where incidence of normal or intermediate and intense estrus was higher in Ovsynch treated animals.

The CIDR study group cows exhibited 0, 30, and 70 percent as weak, normal and intense estrus, respectively (Table 3). The result was in accordance with observation of Jyothi *et al.* (2011) [15], Reshma *et al.* (2020) [25] where incidence of intense estrus is higher followed by normal and weak estrus.

The higher mean score of intensity of estrus in CIDR group

was because of higher incidence of intense estrus among all study group. The variation in estrus intensity was attributed to climatic conditions, nutritional status and managerial condition of cows.

**Duration of estrus**

The mean of estrus duration recorded was 21.61±0.77, 20.30±0.72, and 20.47±0.70 hrs in Ovsynch, CIDR and control groups, respectively in the current experiment (Table 5). Statistical analysis showed no significance difference ( $p>0.05$ ) observed in mean duration of estrus in Ovsynch, CIDR and control group. The present study results for estrus duration in Ovsynch group was in accordance with the studies of Ahmed *et al.* (2016) [1] and Hirole *et al.* (2018) [12]. Contrary to this result, the longer duration of estrus in Ovsynch group was reported by Velladurai *et al.* (2014) [29] and shorter estrus duration was reported by Shahid *et al.* (2019) [28] and Anjum *et al.* (2009) [5].

The estrus duration in CIDR group were is in accordance with the observation of Degefa *et al.* (2016) [8]. Contrary to these results, the shorter duration of estrus in CIDR protocol was observed by Sauls *et al.* (2016) [26] and Mengchao *et al.* (2010) [22] and longer duration of estrus was reported by Reshma *et al.* (2020) [25] and Devi *et al.* (2021) [9]. The shortening in the duration of estrus might be because use of progesterone device might have increased the degree of the hypothalamus-pituitary gonadal axis for estrus generation and the surge in LH due to the increase of endogenous estradiol response to ovulation.

**Table 3:** Showing the intensity of estrus in synchronized group of cows

S. No	Group	No. of cows exhibited estrus / No. of cows treated	Scale of intensity of estrus					
			<10 points		10-15 points		>15 points	
			(weak)		(Normal)		(Intense)	
			No.	%	No.	%	No.	%
1	Ovsynch	13/15	1	7.69	8	61.53	4	30.76
2	Cidr	10/10	-	-	3	30.00	7	70.00
3	Control	15/27	5	33.33	6	40.00	4	26.66
Total		38/52	6		17		15	
Overall Percent				15.79		44.74		39.47

**Table 4:** Mean estrus intensity score in synchronized groups of cows

S. No	Group	No. of cows treated	No. of cows exhibited estrus	Mean estrus intensity score
1	Ovsynch	15	13	13.38±0.94 <sup>ab</sup>
2	Cidr	10	10	16.30±1.59 <sup>b</sup>
3	Control	27	15	11.07±1.05 <sup>a</sup>

\*a,b Means with different superscripts in a column differ significantly ( $p < 0.05$ )

**Table 5:** Duration of estrus (hrs) in synchronized groups of cows

S. NO	Group	No of cows exhibited estrus	Mean duration of Estrus (hrs)
1	Ovsynch	13/15	21.61±0.77 <sup>a</sup>
2	Cidr	10/10	20.30±0.72 <sup>a</sup>
3	Control	15/27	20.47±0.70 <sup>a</sup>

There was no significant difference in duration of estrus among 3 groups ( $p > 0.05$ )

## Conclusion

Estrous synchronisation involves influencing the bovine estrous cycle so that most animals display standing estrus in a brief amount of time. It is a very efficient way to raise the percentage of animals that reproduce at the start of the breeding season. From present study it was concluded that among all treated group, CIDR protocol was most effective and efficient synchronization protocol for improving the efficacy of synchronization and conception rates in embryo transfer programme.

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