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Synchronization and Resynchronization as a Novel Approach for Improving Reproductive Performance of Postpartum Dairy Cows

A.H. Shahzad[1], A. Sattar[1,*], A. Husnain[1], I. Ahmad[2], N. Ahmad[1], D. Nak[3] and Y. Nak[3]

[1]*Theriogenology Department, University of Veterinary and Animal Sciences, Lahore*
[2]*Physiology Department, University of Veterinary and Animal Sciences, Lahore*
[3]*Department of Obstetrics & Gynecology, Uudag University, Bursa, Turkey*

ABSTRACT

Estrus synchronization and suitable resynchronization intervention is crucial part of improved reproductive management in dairy industry. Our objectives were to appraise CIDR-EB based estrus synchronization and used CIDR-Ovsynch based resynch protocol in lactating Holstein cows at two different geographical locations. On location *A*, 160 postpartum cows were enrolled in standard CIDR-EB protocol. Cows were subjected to timed AI and randomly assigned into two groups: 1) control (n=70), subjected to AI on detected estrus (AIDE) from d18-d30 post TAI. Pregnancy rate was diagnosed on d30, d60 and d90 post TAI, 2) resynch (n=90) received CIDR from d14-d21 post TAI. On day 23, all cows received GnRH shot and open cows received remaining Ovsynch protocol. Blood was collected on d14,d16 and d30 for P4 profile. Cross-sectional area of luteal tissue was measured on d30 in pregnant animals in both groups. In experiment II, both control (n=64) and resynch (n=54) groups were similarly treated as in experiment I, without P4 profile and luteal cross-sectional area measurement. Pregnancy rate(PR) was compared by PROC FREQ of SAS. Effect of treatment on circulatory P4 profile and luteal tissue cross-sectional area were analyzed using GLM procedures ($P<0.05$). The PR on d30 in control (43%) and resynch (48%) groups was non-significant. On d60 and d90 overall PR was 44 and 73% (P=0.002) and 43 and 72% ($P=0.002$) while overall pregnancy loss was 11 and 4% in control and resynch groups, respectively. In experiment II, PR on d30, d60 and d90 was 44 versus 54% ($P=0.28$), 53 versus 72% (P=0.03) and 50 versus 70% (P=0.025) in control and resynch groups with overall pregnancy loss of 14 and 3%. Mean P4 profile (ng/mL) was non-significant on d14 while significant on d16 and d30 post TAI in control and resynch groups. Mean luteal cross-sectional area on d30 post TAI has significant difference between both groups. In conclusion, a significant increase in overall PR in resynch group makes it an efficient reproductive management tool in comparison with control group.

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Authors' Contribution
AHS, AS and YN designed and conceived the experiments. AHS, DN, YN, AH and AS performed the experiment. IA and NA anayesd the data. AHS and AS wrote the manuscript. AS reviewed and revised the manuscript.

Key words
Postpartum cows, CIDR-EB based synchronization, Progesterone profile, Reproductive management, Pregnancy rate.

INTRODUCTION

Modern dairy industry is facing multiple issues like estrus expression and decreased PR around the globe. The success of commercial dairy farming is based on the optimal inter calving interval (ICI). Hence, cows must return to ovarian cyclicity, express estrus and be bred within 85 days postpartum to achieve ICI of 365 days. Calving is a traumatic event and the ability to control ovarian and uterine events in the postpartum cow could play an important role in achieving subsequent fertility (Mobashar et al., 2018). Two major physiological factors which influence the reproductive success in postpartum dairy cow are: ovarian cyclicity and uterine health. For this purpose, estrus synchronization is a useful reproductive management tool. In recent years; resynchronization in a suitable manner is also a good management tool for improved conception rate (CR).

Early pregnancy diagnosis (EPD) and rebreeding of open cows are crucial to improve reproductive performance and enhanced CR in dairy herds (Fricke *et al*., 2003). Without early pregnancy diagnosis and resynchronization protocols, there may be extended inter estrus interval, prolonged days in milk (DIM), and decreased milk production (Dewey *et al*., 2010). In high producing cows, CR is low (< 40%) leading to majority of non-pregnant cows (Fricke *et al*., 1998). Despite the better understanding of reproductive physiology and lower number of services per conception following by first AI, early postpartum successful breeding is still a goal to be achieved (Galvao *et al*., 2007; Chebel *et al*., 2006). This situation becomes worse if the pregnancy diagnosis and resynchronization strategy is not executed in a timely manner especially for the remaining 60% of non-pregnant animals. After early

* Corresponding author: masattar@uvas.edu.pk
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pregnancy diagnosis, open cows require to be enrolled promptly for the subsequent breeding. To minimize the inter-insemination intervals, an ideal resynchronization protocol needs to be standardized.

A routinely employed resynchronization protocol includes a shot of GnRH to the all cows irrespective of pregnancy status seven days prior to pregnancy evaluation. On pregnancy diagnosis, open cows are subjected to $PGF_{2\alpha}$ and GnRH at 2 days interval of PGF2α followed by TAI (Fricke *et al*., 2003). It is a cost effective method and potential reproductive managemental tool to decrease the interbreeding intervals (Galvao *et al*., 2013; Giordano *et al*., 2013; Tenhagen *et al*., 2004). Reduced pregnancy rates have been reported following resynchronization protocols in comparison with synchronized based TAI. One major reason for reduced PR is improper intervention in estrous cycle with synchronization and resynchronization protocols (Bilby *et al*., 2013; Bruno *et al*., 2013). Research data have shown that in resynchronization group, large proportions of cows were not on anticipated stage of the estrous cycle at the time of initial GnRH of Ovsynch (Bisinotto *et al*., 2010; Bartolome *et al*., 2009). Other important explanation for decreased PR following resynchronization protocol is that 15-26% animals don't have functional luteal tissue leading to suboptimal P4 in circulation (Silva *et al*., 2009; Sterry *et al*., 2006; Fricke *et al*., 2003). To overcome lower conception rate, many researchers used CIDR inserts in resynchronization protocol and obtained beneficial impact on PR in lactating dairy cows (Bilby *et al*., 2013; Bisinotto *et al*., 2010; Chebel *et al*., 2010; Dewey *et al*., 2010).

There is no literature available on the implementation of a CIDR based GnRH resynchronization treatment followed by CIDR-EB synchronization protocol in lactating dairy cows. Therefore, it was hypothesized that a similar treatment used in conjunction with this protocol (CIDR-GnRH-PG-GnRH) followed by TAI will result in a highly synchronized follicular wave emergence and subsequent ovulation in open cows for resynch and increased TAI pregnancy rates in lactating dairy cows with beneficial effects of P4 supplementation on existing pregnancy. Primary objectives of the present study were to examine the effect of CIDR-EB protocol on PR in lactating dairy cows, 2) to determine the effect of CIDR on PR and P4 supplementation on pregnancy loss and to investigate the CIDR insertion on resynch protocol for insemination of open lactating cows on d33 after US without waiting period for resynchronization, and 3) to compare P4 concentration before and after CIDR insertion and luteal tissue cross-sectional area in control and resynch group.

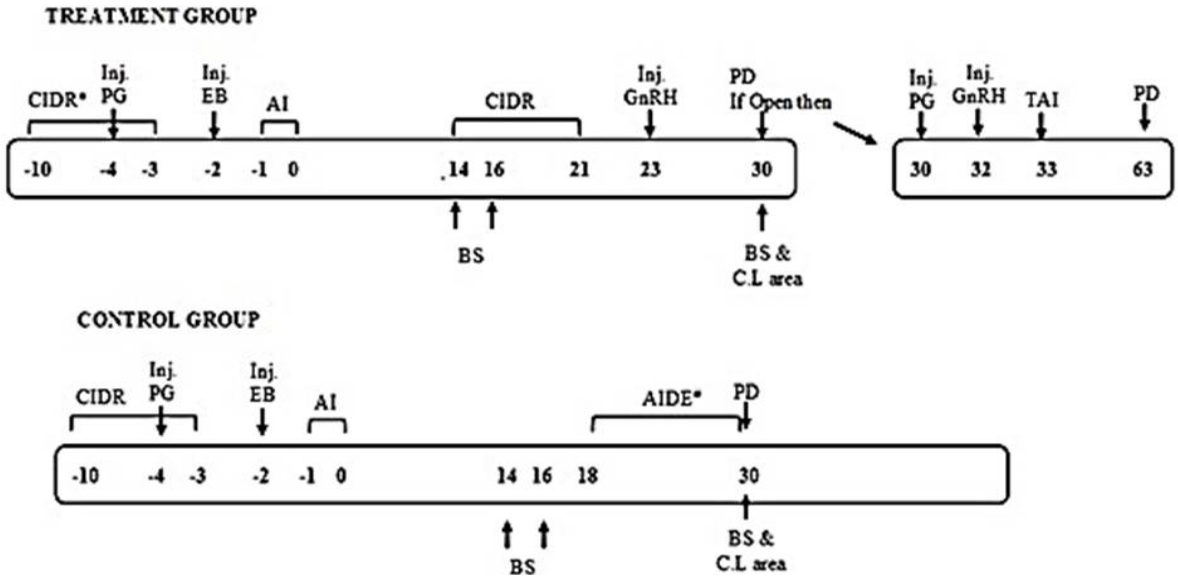

Fig. 1. Scheme of the estrus synchronization protocols in experiment 1. Cows in resynch group were synchronized by CIDR-EB (CIDR on day-10, PG on day-6, Estradiol Benzoate (EB) on day-2. CIDR withdrawal on day-7, TAI 48 h later was marked on day-0). On day 14 post TAI previously used autoclaved CIDR was inserted for 7 days. On 23rd day post TAI GnRH was injected intramuscularly. Pregnancy diagnosis was done on day-30. In non-pregnant PG (Dalmazin, d-cloprostenol 0.150 mg; Fatro, Italy) was injected. GnRH (Dalmareline, lecirelin, Fatro Italy) after 48 h and TAI 16-20 h post GnRH. BS=blood sampling on day 14, 16 and 30 post TAI. CL area on day 30 post TAI. In control group (CIDR-EB) lactating dairy cows (n = 70) were enrolled without further treatment except AIDE (artificial insemination at detected estrus) between 18 to 30 days post TAI and PD was performed 30 days after AI, respectively.

MATERIALS AND METHODS

Experiment I, Location A

Animals, nutrition, and location

Normally calved multiparous lactating Holstein cows (n = 160) with 45-110 DIM, housed in cross-ventilated free stall barns on a commercial dairy farm in Yenişehir Bursa, Turkey (40°15′52″N 29°39′11″E) were used in the present study. Before the start of the study, all the cows were subjected to BCS as described by Ferguson *et al*. (1994) and cyclicity evaluation through ultrasonography. Cows with BCS ranging 2.50-3.25 were included in the study. Estrus response was evaluated for CIDR-EB protocol. Animals were kept open barns with shade and a yard with a concrete floor under stall barn housing system, fed TMR (total mixed ration consisting of alfalfa hay, straw, maize silage and concentrates) twice daily *ad libitum* and water. Mineral mixture and salt blocks were made available in the barns. Feed formulation was in accordance with NRC requirements for lactating dairy cattle. Feed increment was offered to high producing cows accordingly. During the whole course of experiment, cows were milked thrice daily. The experiment was conducted during the months of April to October.

Experimental design

All animals (n = 160) were synchronized using standard CIDR-EB protocol as described previously (Naseer *et al*., 2011). Cows were inseminated using frozen thawed semen at 48-60 h after CIDR removal. On day 14 post AI, all the cows were randomly allocated to two groups: 1) resynch (n = 90) received previously used autoclaved (Zuluaga *et al*., 2008) CIDR for 7 days on day 14th post TAI. GnRH was injected to all cows on d23. On d30, all cows were subjected to PD through US. Cows found non-pregnant received 2 ml (im) $PGF_{2\alpha}$ on the same day and 100 µg (im) GnRH two days later. TAI was done 64-68 h post PG injection 2) control (n = 70) group was only inseminated at detected estrus and no further treatment was carried out (Fig. 1).

Blood sampling and RIA for P4 profile

Blood sampling from all the cows was done on d14, d16 and d30 post TAI. Five ml blood was collected from the coccygeal vein in Vacutainer coated with EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ). Blood samples were spun in a centrifuge @ 2800X g for 20 min to separate plasma. After harvesting the plasma, it was transferred to eppendorf tubes and stored at –20 °C till P4 analysis. A subset of plasma samples (resynch; n = 30 and control; n = 27) of the cows found pregnant on day 30 post insemination were further processed for P4 analysis through radio immune assay (RIA) while the remaining stored plasma samples were discarded. Progesterone values were measured using a solid-phase, RIA kit (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA) described by Patterson et al. (1995).

Pregnancy diagnosis and measurement of luteal tissue cross-sectional area

Pregnancy was diagnosed by ultrasonography at d30, d60 and d90 post TAI. It was confirmed by presence of amniotic vesicle (AV), heartbeat of embryo and intraluminal uterine fluid as pregnancy markers as described by Fricke (2003). Pregnancy losses (%) were recorded on d60 and 90 post TAI. Luteal tissue cross-sectional area was measured in cows which were positive for pregnancy on d30 post TAI. Cross-sectional area of luteal tissue was measured with following formulae (Kastelic *et al*., 1990):

$$\text{Area without a cavity} = wCl \times hCl \times \pi/4$$

$$\text{Area with cavity} = (wCl \times hCl \times \pi/4) - (wC \times hC \times \pi/4)$$

Where, w is width of luteal tissue, h is height of luteal tissue and C is cavity inside luteal tissue.

Statistical analyses

Effect of treatments in resynch and control groups on first-service conception rates and overall pregnancy rates were determined by chi square analysis in PROC FREQ of SAS (Statistical Analysis System, Version 9.1 for Windows; SAS Institute, Cary, NC, USA). Unadjusted odd ratios were calculated using the logistic procedure of SAS. Interactions of BCS, parity, cyclicity and estrus response were calculated on pregnancy odds. Effect of treatments on circulatory P4 profile and luteal tissue cross sectional area were analyzed by using GLM procedures of SAS. Differences were declared significant if $P < 0.05$ and a trend towards significant was supposed when $0.05 \leq P \leq 0.10$.

Experiment II, Location B

Animals, nutrition, and location

Normally calved multiparous lactating Holstein cows (n = 118) with 45-110 DIM, housed in cross-ventilated free stall barns on a commercial dairy farm in Chunian Distt. Kasur, Pakistan (30° 58’ 0N 73° 58’ 60 E) were used in the present study. Multiparous cows with 45-110 DIM were enrolled in this study. Before the start of study, all the cows were subjected to BCS as described by Ferguson *et al*. (1994) and cyclicity evaluation through ultrasonography. Cows with BCS ranging 2.50-3.25 were included in the study. Animals were kept open barns with shade and a yard with a concrete floor under stall barn housing system, fed TMR (total mixed ration consisting of alfalfa, straw, maize silage and concentrates) twice daily *ad libitum* and water.

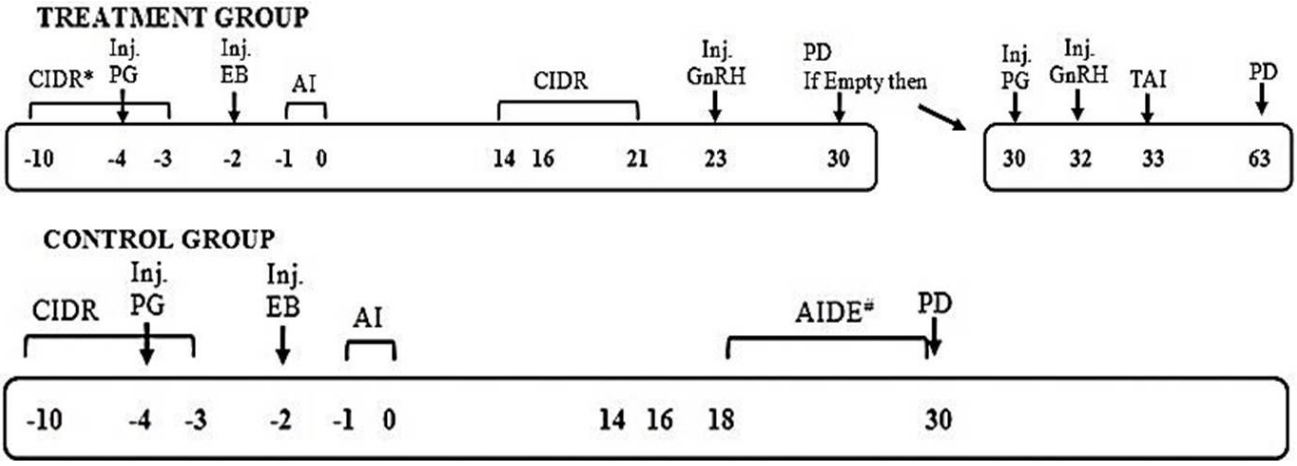

Fig. 2. Scheme of the estrus synchronization protocols in experiment 2. Cows in resynch group (n = 54) were synchronized by CIDR-EB (CIDR on day-10, PG on day-6, Estradiol Benzoate (EB) on day-2. CIDR withdrawal on day-7, TAI 48 h later was marked on day-0). On day 14 post TAI when previously used autoclaved CIDR was inserted for 7 days. On 23rd day post TAI injection of GnRH was injected intramuscularly. Pregnancy diagnosis was done on day-30. In case of non-pregnant PG (Dalmazin, d-cloprostenol 0.150 mg; Fatro, Italy) was injected. GnRH (Dalmareline, lecirelin, Fatro Italy) after 48 h and TAI 16-20 h post GnRH. In control group (CIDR-EB) lactating dairy cows (n = 64) were enrolled without further treatment except #AIDE (artificial insemination at detected estrus) between 18 to 30 days post TAI and PD was performed 30 days after AI, respectively.

Mineral mixture and salt blocks were made available in barns. Feed formulation was in accordance with NRC requirements for dairy cattle. Food increment was offered to high producers accordingly. The cows were milked twice daily during the experiment which continued from October to July.

Experimental design

Treatments of all the animals (n = 118) in this experiment were same as in study 1 but without blood sampling and luteal tissue measurement. Parameters under study in resynch group (n = 54) were compared with those of control (n = 64) group (standard CIDR-EB protocol; Fig. 2).

Pregnancy diagnosis

Pregnancy was diagnosed by ultrasonography (Aloka 900 V equipped with a 5.0 MHz linear array transducer, Aloka, Wallingford, CT, USA) at d30, d60 and d90 post TAI. It was confirmed by presence of amniotic vesicle (AV), heartbeat of embryo and intraluminal uterine fluid as pregnancy markers as described by Fricke *et al*. (2003). Pregnancy losses (%) were recorded on d60 and d90 post TAI.

Statistical analyses

Effect of CIDR based synchronization and resynch treatment on first-service conception rates and overall pregnancy rates were determined by chi square analysis in PROC FREQ of SAS (Statistical Analysis System, Version 9.1 for Windows; SAS Institute, Cary, NC, USA). Odd ratios, unadjusted, were calculated using the logistic procedure of SAS. Interactions of BCS, parity, cyclicity and estrus response were calculated on pregnancy odds. Differences were declared significant if $P< 0.05$.

RESULTS

Experiment I

Pregnancy rate and pregnancy loss

The PR on d30 post insemination in resynch group was higher (48%) than that of control group (43%) but this difference was statistically non-significant ($P = 0.50$; Table I). On d60 and d90 post insemination, overall pregnancy rates were significantly ($P = 0.002$) higher (73 and 72%) in cows of resynch group as compared to those of control (44 and 43%) group. Nevertheless, BCS, cyclicity and parity did not show any impact on PR either independently or in interaction with treatment.

Pregnancy loss in experiment I was recorded between d30 and d60 post insemination which was non-significantly ($P = 0.18$) lower for cows in resynch group (5%) in comparison to control group (13%) with no CIDR insertion post insemination. Overall pregnancy loss between d30 and d90 was also reduced ($P = 0.20$) for cows in resynch group (4%) in comparison to control group (11%) with no CIDR insertion post AI (Table I).

Table I.- Pregnancy rate on days 30, 60 and 90 and pregnancy loss for lactating dairy cows in experiment I, II and cumulative pregnancy rate of both experiments.

Variables	Treatment[1]	
	Resynch	Control
Experiment I		
Pregnancy rate on d30 post AI[2]	48.0% (43/90)	43.0% (30/70)
Overall pregnancy rate on d60 post AI[3]	73.0%* (66/90)	44.0% (31/70)
Overall pregnancy rate on d90 post AI[3]	72.0%* (65/90)	43.0% (30/70)
Pregnancy loss between d30 and d60 post AI	5.0% (2/43)	130% (4/30)
Pregnancy loss between d30 and d90 post AI	4.0% (3/68)	110% (4/36)
Experiment II		
Pregnancy rate on d30 post AI[2]	54.0% (29/54)	44.0% (28/64)
Overall pregnancy rate on d60 post AI[3]	72.0%* (39/54)	53.0% (34/64)
Overall pregnancy rate on d90 post AI[3]	70%* (38/54)	50% (32/64)
Pregnancy loss between d30 and d60 post AI	0%	7.0% (2/28)
Pregnancy loss between day 30 and 90 post AI	3.0% (1/39)	14.0% (5/36)
Both experiments		
Cumulative pregnancy rate on d30 post AI[2]	50.0% (72/144)	43.0% (58/134)
Cumulative pregnancy rate on day 60 post AI[3]	72.9%* (105/144)	48.50% (65/134)
Cumulative pregnancy rate on day 90 post AI[3]	71.50%* (103/144)	46.0% (62/134)
Cumulative pregnancy loss between day 30 and 60 post AI	3.0% (2/62)	10.0% (6/58)
Cumulative pregnancy loss between day 30 and 90 post AI	4.0%* (4/107)	13.0% (9/72)

[1]According to treatment scheme of Figures 1 and 2. [2]Pregnancy rate to initial AI determined by ultrasound on day 30 post AI. [3]Pregnancy rate to all AIs (resynch: CIDR+EB synchronization + TAI to open cows on d30 PD; Control: initial TAI+AIDE between day 18-30 post TAI).

Plasma progesterone

Plasma progesterone (P4) profile on d14, d16 and d30 post TAI was measured in a subset of study 1 (resynch, n = 30; control, n = 27). At day 14, cows in both groups have non-significant ($P > 0.05$) difference of progesterone concentration. However, after 48 h, cows of resynch group had significantly ($P< 0.05$) elevated P4 values as compared to those of control. Similarly, on d30 post AI, P4 values of both groups differed significantly ($P< 0.05$, Table II).

Table II.- Plasma progesterone concentrations (mean ± SD) on days 14, 16, and 30 for pregnant dairy cows in experiment I.

Days post AI	Treatment[1]	
	Resynch (n = 30)	Control (n = 27)
14	5.50 ± 1.12	5.38 ± 0.96
16	6.47* ± 0.99	5.60 ± 1.15
30	7.57* ± 1.14	6.34 ± 1.32

[1]According to treatment scheme of Figure 1. *In the same row indicates significant differences ($P < 0.05$).

Luteal tissue cross-sectional area

Based on the mean luteal tissue cross-sectional area, the corpora lutea of the cows in resynch (n = 30) and control (n = 27) group, on d30 post TAI was 422 ± 98 mm^2 and 490 ± 127 mm^2, respectively ($P = 0.03$) as shown in Table III.

Table III.- Luteal tissue cross-sectional area (mean ± SD, mm^2) for pregnant cows on day 30 post insemination in experiment I.

Resynch (n = 30)	Control (n = 27)
422* ± 98	490 ± 127

*In the same row indicates significant differences ($P < 0.05$).

Experiment II

Pregnancy rate and pregnancy loss

The PR, in second study, was observed to be 54% on day 30 post AI in the cows of resynch group which was significantly ($P = 0.028$) higher than the control group (44%). Similarly, overall PR at d60 and d90 post AI in resynch group of cows was higher (72 and 70%) as compared to those of control group (53 and 50%) and the differences were significantly higher ($P = 0.033$ and 0.025) in both groups, respectively (Table I). No pregnancy loss was observed between d30 and d60 in resynch group while it was 7% in control group ($P = 0.090$) of cows. Overall pregnancy loss between d30 and d90 was also reported to

be lesser (3%) for cows in resynch group in comparison to control group (14%) with no CIDR insertion post TAI. These pregnancy losses were statistically significant *(P*= 0.071; Table I) in resynch versus control groups.

Combined pregnancy rate and loss in both studies with different geographical locations

Combined PR in both experiments (I and II) was observed to be 50% on day 30 post AI in the cows of resynch group which was higher ($P = 0.26$) than the cows of control group (43%). Similarly, overall PR on d60 and d90 post TAI in resynch group of cows (73 and 72%) was higher as compared to those of control group (48.5 and 46%) and the differences were significantly higher ($P = 0.00003$ and 0.00002) in both groups, respectively (Table I). Between day 30 and 60, pregnancy loss in resynch group was lower as compared to pregnancy loss of 10% in control group ($P = 0.12$). Overall pregnancy losses between d30 and d90 was reported to be significantly less ($P = 0.027$; 4%) for cows in resynch group in comparison to control group (13%) with no CIDR insertion post AI (Table I).

DISCUSSION

In this experiment, two studies were conducted to evaluate whether quick resynchronization protocol would result in an early scheduled synchronized estrus to facilitate the FTAI followed by synchronization in lactating dairy cows deemed open on PD. It was aimed to reduce the optimal time period in resynch groups in both studies. The potential benefit from the anticipated increase in overall PR following resynch protocol response would be an advantage if resulted in increased PR resulting from FTAI. There is no information available about CIDR-EB based and resynch protocols in lactating dairy cows. Major hindrance in using synchronization protocols containing EB (estradiol benzoate) are availability and restrictions on this product in dairy industry. Availability and usage issues make GnRH and gonadotropins (LH, hCG) as products of choice for ovulation induction in synchronization protocols. But in many areas where it is being used has resulted in promising outcomes. In present study, standard CIDR-EB protocol has been investigated as postpartum reproductive management tool in lactating dairy cows followed by resynch manipulation for open animals on d30 post TAI. In a previous study, Souza et al. (2009) has shown CIDR-EB based synchronization protocol has resulted in emergence of synchronized follicular wave in 84.4% animals within 1-5 days post treatment. Another advantage of using EB in CIDR based protocol, it does not require to check/monitor follicular wave status (Bó *et al*., 1994) which makes it a protocol of choice in tropical and subtropical regions where heat stress has dominant impact on follicular development resulting in decreased probability of ovulation followed by GnRH based synchronization protocol (Vasconcelos *et al*., 1999). Using CIDR in Ovsynch protocol has resulted in elevated P4 concentration leading to lowered LH pulsatility which in turn improves competency of ovulated oocyte (Revah and Butler, 2001) and subsequent improvement in PR (Vasconcelos *et al*., 1999).

Main objective of the present study on both geographical locations was to evaluate the effect of CIDR insert on post TAI in CIDR-EB based standard protocol and early resynch protocol after the diagnosis of non-pregnant cows in resynch group. In present study, selection of animals was on random basis as the start protocol consisted of CIDR insert. In previous studies, improved pregnancy per AI has been reported (Bilby *et al*., 2013) in 7-day resynch protocol when either absence of CL (27.0 *versus* 19.2%) or low (<1 ng/mL) P4 (29.4 *versus* 15.0%) profile. In present study, pregnancy outcome on location A was 43% and 48% in control and resynch group ($P = 0.50$), respectively. In our study, resynch protocol was initiated on day 14 post AI by CIDR insert while in another study, Pulley and Stevenson (2015) has shown negative impact of P4 supplementation, when introduced at day 35 post AI, on P/AI. In another 5-d resynch protocol, Bisinotto *et al*. (2010) observed tendencies ($P = 0.07$ to 0.10) for increased P/AI on day 32 and 60 post AI, when resynch protocol was introduced at day 34 post AI, in open cows with CL+CIDR device insert and CL bearing cows without CIDR insert. In another study conducted by Mehni *et al*. (2012), it was found that insertion of P4 implant in Holstein cows from d5-d19 post AI resulted in increased PR as compared to control group (56 *versus* 25%). This significant increase in PR was the possible effect of reduced embryonic mortality by elevated P4 profile in treated cows. Similar observations were recorded by Villarroel *et al*. (2004) who demonstrated a trend towards lower pregnancy loss ($P = 0.077$) in repeat breeder cows supplemented with P4 implants from d5-d19 post AI in treatment group *versus* control. In our study, no comparison was possible as we started it on 14 days post TAI when virtually all cows were supposed to have CL. In present study PR followed by first service at both locations in control groups was less than P4 supplementation groups. A meta-analysis study by Mann and Lamming (1999) has shown that P4 supplementation within one week of AI yielded higher fertility but beyond this in 2-3rd week post AI, P4 failed to have any impact on PR.

Treatment of cows with CIDR inserts during resynchronization protocols improves P/AI to re-insemination by approximately 5 percentage units

when the resynchronization protocols were initiated at approximately 38 ± 3 d after AI (Bilby *et al*., 2013; Dewey *et al*., 2010). It is not clear, however, whether the use of a CIDR insert during a resynchronization protocol would increase P/AI to re-insemination of cows that had their estrous cycle presynchronized with GnRH or $PGF_{2\alpha}$. In present study, overall PR was higher in resynch group on both locations. This is first study of its kind in which CIDR-EB was used for synchronization and CIDR-Ovsynch was used for resynch protocol. Resynchronization with P4 supplementation improves and synchronizes induction rate in non-pregnant cows (Colazo *et al*., 2006; Stevenson *et al*., 2003). This P4 supplementation after 5d post AI improved fertility in Holstein cows (Villarroel *et al*., 2004), but resulted in decreased fertility when introduced within 48 h of AI (van Cleeff *et al*., 1996). It is obvious that P4 supplementation could be used to resynchronize cow but introduction timings may influence the survival of earlier established pregnancy and subsequent fertility of the following ovulation of the succeeding estrous.

In present study pregnancy loss at location *A* was found to be low, between first and second pregnancy check, in resynch group (5.0%; 2/43) compared to control group (13.0%; 4/30). Similarly, overall pregnancy loss between day 30 and 90 was also reduced ($P< 0.05$) for cows in resynch group (4.0%; 3/68) in comparison to control group (11%; 4/36). On location *B* pregnancy loss was 0.0% between d30 and d60 in resynch group as compared to control group (7.0%; 2/28) with no CIDR insertion post TAI. Overall pregnancy loss between day 30 and 90 was also reduced ($p<0.05$) for cows in resynch group (3.0%; 1/39) in comparison to control group (14.0%; 5/36) with no CIDR insertion post TAI. On both locations overall pregnancy loss was recorded as 4%. This, reduced, loss is may be attributed to higher mean P4 profile in resynch group as reduced P4 profile during days 28-37 post-insemination has also been associated with late embryonic mortality (Inskeep and Dailey, 2005). Most critical period for embryonic losses is 28-45 days of pregnancy ranging from 3.2%-11.4% of all detected pregnancies (Giordano *et al*., 2013; Santos *et al*., 2004; Silke *et al*., 2002). In other studies, embryonic loss was reported to be 10.5-17.7% between first and second pregnancy check (Giordano *et al*., 2013; Gumen *et al*., 2003; Lopez-Gatius et al., 2002; Silke *et al*., 2002). In many studies, supplementation of P4 in synchronization has resulted in improved PR and reduced pregnancy loss. Yilmazbas-Mecitoglu *et al*. (2014) found 48.6 % versus 52.6% in control and P4 supplemented group respectively on d32 and 42.9 and 48.7% on day 62 post AI. Pregnancy loss in control and treatment groups was 11.8% and 7.3%. Herlihy *et al*. (2011) has reported improved PR of 58 versus 55% in P4 treated versus control group on day 32 post AI. Pregnancy losses were 6.7 versus 6.5% on day 62 post AI, respectively. Stevenson *et al*. (2006) found 43% versus 48% in control and P4 supplemented group respectively on d32 and 34.6 versus 42.4% on day 62 PTAI. Pregnancy loss in control and treatment group was 19.2% and 11.8%, respectively.

In the present study, P4 profile was measured at one location and before CIDR insertion on d14 post TAI and was non-significant ($P> 0.05$). On d16 and d30 P4 profile was significantly elevated in resynch group. Reason on d16 is obvious but on d30 is fortuitously. At the same time luteal tissue area was significantly smaller in resynch group in comparison with control group. This, unexpected, outcome may be due to some synergistic effect of supplemented P4 on luteal progesterone production or GnRH based LH surge dependent supplementary P4 production by small luteal cells (Niswender and Nett, 1988). In literature no data are available for the comparison.

At both locations there was huge difference between two herds on milk production basis. Despite this difference, reproductive performance did not differ in control *versus* control and resynch protocol at locations *A* and *B*. In literature erratic findings have been reported on account of production impact on reproduction. In one study decreased, although slight, reproductive efficiency was documented in high producing group (Lucy, 2001). In current similar weather conditions prevailed at both location. In another review Santos *et al*. (2004) observed that early and late fetal mortality has no link with high production in dairy cows. Lucy (2001) has shown negative correlation between high yielding and fertility but this might be the result of other contributory factors like increased incidence of postpartum issues and heat stress. In our experiment production factor did not altered fertility which was in accordance to the previous results (Lopez-Gatius, 2003).

CONCLUSION

In conclusion, standard CIDR-EB based synchronization protocol is practicable synchronization protocol for postpartum dairy cows which has resulted in similar PR at different geographical locations. Resynch intervention is, at the same time, also a viable protocol for early breeding in open cows after pregnancy diagnosis to reduce the days open in lactating dairy cows. Further investigations are warranted at large scale and use of autoclaved CIDR in resynch protocol to make it more economical one. Results of present study also support the hypothesis that P4 supplementation when used, between days 14 and 21 after TAI program, synchronized the subsequent estrus in the majority of non-pregnant lactating

cows and reduced embryonic losses for resynch group containing CIDR insert compared to control group.

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Statement of conflict of interest

Authors declare that they have no conflict of interest.

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