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5d CIDR-Heatsynch improves the estrus expression and conception rate in anestrus buffalo of Palwal district of Haryana

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Abstract
The present study aimed to investigate whether increasing estradiol (E_2) concentrations during preovulatory period would increase the estrus expression and conception rate in 5d CIDR based timed AI (TAI) protocol. A total of 80 anestrus buffalo (3rd to 5th parity) were allocated (40 for each group) to either 5d CIDR-Cosynch or 5d CIDR-Heatsynch group. Pregnancy was confirmed on day 35. The buffalo in 5d CIDR-Heatsynch had greater estrus induction rate and increasing trend for conception rate (55% vs. 40%) than 5d CIDR-Cosynch. The pregnant buffalo that exhibited estrus signs was greater in 5d CIDR-Heatsynch than 5d CIDR-Cosynch. In conclusion, 5d CIDR-Heatsynch improved the conception rate in anestrus buffaloes of Palwal district of Haryana.

Keywords: Buffalo, conception, estrus, pregnancy

Introduction
Buffalo is the premier animal of the Indian dairy industry and is considered a better option than cattle for rearing farmers because of its better adaptability in harsh climatic conditions (Gasparrini, 2013) [21]. To continue to take advantage of available resources and increase producer profit margins, we are looking to increase productivity by improving the reproductive efficiency of the buffalo. A buffalo with good reproductive efficiency is expected to begin ovarian activity after 30 to 45 days of calving, conceive within 90 days of calving and maintain 12 to 13 months calving interval (Abdalla, 2003) [1]. Field surveys on reproductive disorders found anestrus as the most common single cause of infertility in buffaloes (El-Wishy, 2007a; Kumar *et al*., 2014) [18, 25]. Meager endocrine support from the hypothalamic-pituitary axis, resulting in inactive ovaries (acyclic conditions) and thus, the buffalo suffers from anestrus for a longer period (Tanwar *et al*., 2003; El-Wishy, 2007b) [40, 19].

In order to improve the reproductive potential of buffaloes, using progesterone, estradiol, prostaglandin, GnRH, and eCG hormones, only in recent years have many protocols been developed to control the cyclicity, time of ovulation and thus, avoid the need for estrus detection (Baruselli *et al*., 2013; Karuppanasamy *et al*., 2017; Bisen *et al*., 2018; Kavita *et al*., 2018) [2, 22, 5, 24]. The synchronization of estrus and timed-artificial insemination (TAI) regimen enabled dairy farmers to increase the use of frozen semen in buffalo. Although, the fertility following synchronized ovulation is sub-optimal (Santos *et al*., 2004) [34] due to premature ovulation of the follicle consequently, preovulatory follicle size is reduced (Colazo *et al*., 2003; Mussard *et al*., 2007) [16, 29] that results into development of small-sized CL (Pandey *et al*., 2011) [30]. Despite the improved conception rate, there is always a scope to increase the fertility rate by modifying the TAI protocol to achieve the maximum reproductive potential of buffalo.

Progesterone based estrus synchronization protocols have been found better in anestrus buffalo. Inclusion of Controlled internal drug release (CIDR) into 7d Co-synch protocol, improved the pregnancy rates in anestrus cows (Schafer *et al*., 2007; Wilson *et al*., 2010) [36, 42]. Various studies have reported improved pregnancy rate is associated with the higher circulatory progesterone concentrations at the time of $PGF_{2\alpha}$ administration (Bello *et al*., 2006; Bisinotto *et al*., 2010) [3, 6] and increased circulatory concentrations of estradiol at preovulatory period (Cerri *et al*., 2004; Bridges *et al*., 2014) [14, 12]. Recent studies (Bridges *et al*., 2008; Santos *et al*., 2010; Bridges and Lake, 2011; Pereira *et al*., 2013; Bridges *et al*., 2014) [11, 33, 10, 32, 12] have reported increased pregnancy rates by decreasing the progesterone exposure from 7 to 5 days in 5d CIDR-Cosynch protocol in cows.

Other studies have reported the beneficial effect of estrogen administration during the final stages of TAI protocols (Cerri *et al*., 2004; Mirmahmoudi *et al*., 2014; Dysart *et al*., 2021) [14, 28, 17], may be a rational approach to improve fertility in TAI protocols for lactating animals. To date, only one investigation found by Singh *et al*. (2022) [37] for reproductive response with 5d CIDR-Cosynch and 5d CIDR-Heatsynch protocols in anestrus buffaloes in winter season. Therefore, the study was designed with the objective to compare the synchronized AI conception rate between 5d CIDR-Cosynch and 5d CIDR-Heatsynch protocols.

Materials and Methods

The present study was conducted on pluriparous (between 3rd and 5th parity) anestrus buffaloes (n = 80) during the winter season (from December to January), when the environmental temperature ranges between 5 and 25 °C. The healthy pluriparous buffaloes (Bodyweight: between 380 and 550 Kg and BCS: between 3 & 4 out of 5 scale), having more than 60 days post-partum with a history of normal parturition and having no pathological conditions of the reproductive organs were selected. Buffaloes housed under a semi-loose housing system.

Animals were divided into two groups, comprising 40 animals in each group. All animals in respective groups were subjected to the two estrus synchronization protocols.

Group-1 (5d CIDR-Cosynch)

The buffalo received GnRH analogue (Buserelin acetate (BA), 10µg, i.m.; Inj. Receptal, MSD animal health, India) and Controlled internal drug release (CIDR; 1.38 gm progesterone, Zoetis, India) inserts into the vagina on day -5. At CIDR removal (day 0), $PGF_{2\alpha}$ (Cloprostenol, 500µg, i.m.; Inj. Vetmate, Vetcare Pharma, Bengaluru) was administered, followed later 72 h by a 2nd dose of GnRH analogue (Buserelin acetate 10µg, i.m.). All buffaloes were subjected to timed artificial insemination (TAI) with frozen-thawed semen.

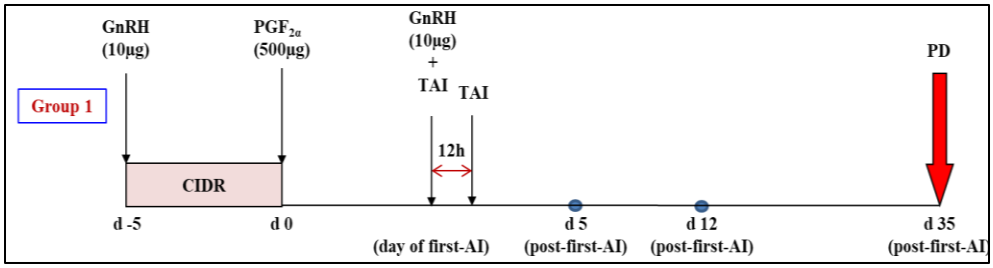

Diagram 1: Schematic diagram of schedule of 5d CIDR-cosynch protocol in buffaloes; (d: Day, PD: pregnancy diagnosis, TAI: timed artificial Insemination

Group-2 (5d CIDR-Heatsynch)

The Heatsynch protocol is modified in the present experiment compared to the conventional heat synch (Mirmahmoudi *et al*., 2014) [28] protocol in buffalo. All buffalo received GnRH analogue (Buserelin acetate, 10µg, i.m.; Inj. Receptal, MSD animal health, India) and Controlled internal drug release (CIDR; 1.38gm progesterone, Zoetis, India) inserts into the vagina on day -5. On the day of CIDR removal (d 0), $PGF_{2\alpha}$ (Cloprostenol, 500µg, i.m.; Inj. Vetmate, Vetcare Pharma, Bengaluru) was administered, followed by estradiol benzoate (Inj. Pregheat; Virbac Animal Health India Pvt. Ltd.) 1mg i.m. 24 h after CIDR removal. All buffaloes were subjected to timed artificial insemination (TAI) with frozen-thawed semen.

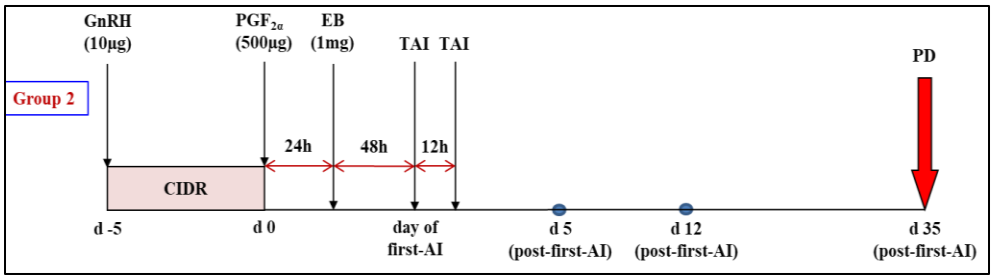

Diagram 2: Schematic diagram of schedule of 5d CIDR-heatsynch protocol in buffaloes; (d: Day, PD: pregnancy diagnosis, TAI: timed artificial Insemination

Statistical analyses

The two-way ANOVA was used to determine any significant difference between two treatment groups and pregnancy status (pregnant/non-pregnant) for various parameters. All statistical analyses were performed in SPSS 20 version.

Results

Estrus synchronization with 5d CIDR-Heatsynch increased estrus response (1.20 times) compared with 5d CIDR-Cosynch at timed-AI (85 vs. 70%, 5d CIDR-Heatsynch vs. 5d CIDR-Cosynch. The synchronized AI conception rate day 35 in the buffaloes with 5d CIDR-Heatsynch (55%) tended to be higher (1.32-folds) than 5d CIDR-Cosynch (40%) treated group. The proportion (%) of pregnant buffalo that did exhibit estrus signs was found to be greater (p<0.01) in 5d CIDR-Heatsynch than 5d CIDR-Cosynch treated buffaloes (60 and 38%, 5d CIDR-Heatsynch and 5d CIDR-Cosynch, respectively). In addition, the proportion of pregnant buffalo that did not exhibit estrus signs was recorded to be higher (p<0.01) in the 5d CIDR-Cosynch compared with 5d CIDR-Heatsynch treated buffaloes (55 vs 30%, 5d CIDR-Cosynch vs. 5d CIDR-Heatsynch, respectively.

Table 1: Estrus synchronization with 5d CIDR-Heatsynch increased estrus response (1.20 times) compared with 5d CIDR-Cosynch at timed-AI (85 vs. 70%, 5d CIDR-Heatsynch vs. 5d CIDR-Cosynch

Parameters	Group	
	5d CIDR-Cosynch	5d CIDR-Heatsynch
Estrus rate (%)	70[a]	85[b]
Synchronized AI Conception rate (%)	40	55[#]
Proportion of pregnant buffalo that did exhibit estrus signs (%)	38[c]	60[d]
Proportion of pregnant buffalo that did not exhibit estrus signs (%)	55[c]	30[d]

[a vs b] differ significantly ($p<0.05$); [c vs d] differ significantly ($p<0.01$); [#]differ significantly ($p<0.08$) in a row

Discussion

Anestrus is a major cause of poor reproductive efficiency in buffalo. The estrus induction by hormonal approaches is a novel method to overcome this problem. To formulate an effective reproductive strategy for increasing productivity, it becomes imperative to evaluate estrus synchronization protocols to treat anestrus females for harvesting the maximum calf in the whole reproductive life of the buffalo. Bridges *et al*. (2008) reported that increasing time duration from CIDR removal to TAI in 5 day CIDR protocol, improved fertility because of increased circulation of estrogen during proestrus that enhances the developmental competence of ovulatory follicle. In order to improve fertility, we hypothesized that extra support of estrogen subsequent to 5d CIDR-Cosynch protocol would enhance the fertility in buffalo. Moreover, combination protocols of estrogen and progesterone are commonly used in South America, reporting pregnancy rates between 41 and 60% (Bo and Baruselli, 2014). The present study was the first to compare two of the estrus synchronization protocols 5d CIDR-Cosynch and 5d CIDR-Heatsynch in palwal district of Haryana focused on the acyclic buffaloes, undertaken to investigate the conception rate between these protocols. Earlier these protocols in anestrus buffaloes in winter season were studied by Singh *et al*. (2022) [37].

In the present study, estrus response was recorded higher in 5d CIDR Heatsynch than in the 5d CIDR-Cosynch group was nearly same as investigated by Singh *et al*. (2022) [37] in anestrus buffaloes in winter season. It is well placed on record that the progesterone priming increases the sensitivity of the hypothalamus to estrogen to improve the estrus expression (McDougall, 1992; El-Wishy, 2007a) [27, 18]. In the present study, as the use of CIDR for 5 days in the current study improved the estrus expression in either group (85% and 70% in 5d CIDR-Heatsynch and 5d CIDR-Cosynch, respectively). Nevertheless, the expression of estrus was higher in estradiol treated buffalo. Estrus expression is significantly dependant on the circulating concentrations of estrogen during proestrus (Kyle *et al*., 1992) [26]. It seems logical that a higher estrus induction rate in the CIDR-Heatsynch group could be associated with the administration of estradiol benzoate 24 h after removal of the CIDR. The treatment with estradiol benzoate 24 h after a luteolytic dose of $PGF_{2\alpha}$ exposes higher estradiol concentrations during proestrus and estrus, and behavioural signs of estrus in anestrus cows, which likely creates a better uterine environment for embryonic development (Stevenson *et al*., 2002; Bridges *et al*., 2012) [39, 13]. Estradiol administration induces a more extended LH surge than GnRH (Thatcher *et al*., 2002) [41]. However, in beef heifers, treatment with various forms of estradiol for synchronizing the estrus led to suppression of gonadotropins and regression of growing follicles (Bo *et al*., 1993, 1994) [7-8].

In the present study, a tendency of higher synchronized AI conception rate was observed for the buffalo treated with 5d CIDR-Heatsynch (55%) than 5d CIDR-Cosynch (40%). These findings strongly support our main hypothesis that the administration of estradiol rather than GnRH would improve fertility. Although, not measured in the present study, however, otherwise the estradiol administration induces a more extended LH surge than GnRH (Thatcher *et al*., 2002) [41] could be responsible for a higher conception rate. Similar to the present study, Singh *et al*. (2022) [37] and Scandolo *et al*. (2020) [35] reported an increased number of pregnant Buffaloes and cows respectively that were treated with estradiol benzoate before AI. Additionally, the majority of buffalo expressed estrus (85%) subsequent to the 5d CIDR-Heatsynch protocol might have also contributed to the increased conception rate. Similar to the previous studies (Galvao *et al*., 2007; Kasimanickam *et al*., 2005; Souza *et al*., 2007; Pereira *et al*., 2013; Pereira *et al*., 2014) [20, 23, 38, 32-31], the estrus expression was associated with increased fertility in the present study. Among buffalo that showed estrus signs, the higher synchronized AI conception rate (60%) subsequent to 5d CIDR-Heatsynch protocol than 38% for 5d CIDR-Cosynch is also an explanation of improved pregnancy rate in 5d CIDR-Heatsynch protocol. Unexpected but interesting, in the present study, the 5d CIDR-Cosynch group showed a higher synchronized AI conception rate than the 5d CIDR-Heatsynch group among the buffalo that did not express estrus signs. The reason for the lower proportion of pregnant buffalo in this group might be ascribed to the lower number of buffalo present in this category for better statistical analysis and/or it may be speculated that the group of buffalo with the absence of estrus expression might have low fertility. In another experiment, Souza *et al*. (2007) [38] reported lower PP/AI in cows with a lack of expression of estrus in the Heatsynch protocol than in Ovsynch. They postulated that this group of cows would be of lower fertility and had a lower body condition score, which might have contributed to the lower PP/AI even after E_2 treatment.

Conclusion

In conclusion, estradiol administration 24 h of CIDR removal and $PGF_{2\alpha}$ administration in 5d CIDR-Heatsynch protocol improved the estrus expression and increased the synchronized AI conception rate in buffalo. In addition, a higher synchronized AI conception rate was observed for the buffalo that exhibited estrus expression in 5d CIDR-Heatsynch. Nevertheless, a higher estrus expression rate in 5d CIDR-Heatsynch may have contributed to the increased conception rate in this group. Overall, the findings lead to the conclusion that estradiol is the treatment of choice for synchronizing estrus in 5d CIDR-Heatsynch protocol in pluriparous buffaloes.

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Conflict of interest statement
All authors declare that there was no any conflict of interest.

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