

Research Article:**COMPARING THE EFFECTIVENESS OF 7-DAY VS 9-DAY CIDR PLACEMENT FOR THE TREATMENT OF POSTPARTUM ANESTRUS IN CROSSBRED DAIRY COWS****Niraj Shrestha^{a,b}, Gokarna Gautam^{a*} and Bhuminand Devkota^a**^aFaculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal^bShree Aadharbhoot Secondary Technical School, Rampur, Palpa, Nepal

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DOI: <https://doi.org/10.3126/jafu.v6i1.78164>**ABSTRACT**

This study was conducted to compare the effectiveness of duration of Controlled Internal Drug Release (CIDR) placement in CIDR-PGF_{2α}-GnRH protocol for the treatment of postpartum anestrus in dairy cows. Forty cows that were not detected in estrus at least until 60 days postpartum were selected and divided randomly into two treatment groups. The CIDR device containing 1.9 g progesterone was placed into the vagina of cows for seven days (T1, 7-day group, n=20) and 9 days (T2, 9-day group, n=20). One day before CIDR removal, all the cows were injected with PGF_{2α} analogue (Cloprostenol 500 µg). In both groups, GnRH analogue Buserelin 20 mcg was injected intramuscularly 48 hours after CIDR removal. Then cows were artificially inseminated at fixed time (FTAI) 16-20 hours after GnRH injection. Both the estrus expression and the pregnancy rates were similar between 9-day and 7-day groups. Overall estrus expression and pregnancy rates were 97.5%, and 45%, respectively. None of the factors influenced the estrus expression and pregnancy outcomes except that the ovarian cyclicity at the start of protocol tended to affect the pregnancy rate (P=0.06). In conclusion, while applying CIDR-PGF_{2α}-GnRH protocol, the application of CIDR for 7 days and 9 days were equally effective on inducing estrus and pregnancy outcome in postpartum anestrus cows, but the pregnancy outcome depended on the status of ovarian cyclicity at the start of the protocol.

Key words: CIDR-PGF_{2α}-GnRH protocol, duration comparison, estrus expression, pregnancy rate

INTRODUCTION

With continued genetic selection for high milk production, the fertility in dairy cattle has been decreasing worldwide (Lucy, 2001). Infertility refers to the temporary disorder in reproductive function whereby animal cannot get pregnant. Anestrus, sub estrous, delayed ovulation, cystic ovaries, repeat breeding, endometritis etc. are the forms of infertility (Roine & Saloniemi, 1978). Among these, postpartum anestrus is the major cause of infertility in dairy cattle (Hadush et al., 2013). Anestrus is the state of ovarian acyclicity, reflected by complete sexual inactivity without manifestation of estrus (Wright & Malmo, 1992), and anovulation accompanied by serum progesterone concentrations less than 0.5 ng/ml. In cattle, anestrus in the postpartum period were recognized as a problem for the first time more than 60 years ago (Short et al., 1990). Postpartum anestrus is the period after parturition during which cows do not show any behavioral signs of estrus. The anestrus condition is associated with the presence of static ovaries, and even though there is follicular development, none of the ovarian follicles becomes mature enough for ovulation. The main factors affecting the duration of postpartum anestrus in cattle are the nutritional status (measured by BCS) and suckling (Randel, 1990). Some other

factors such as breed, age, parity, milk yield, calving season, presence or absence of the bull, delay uterine involution, dystocia and general health status influence duration of postpartum anestrus.

Since, the cause of postpartum anestrus are multifactorial, its treatment involves nutritional supplementation, management practices and hormonal intervention. In cows on good nutritional plane, estrus synchronization is one of the effective methods for the treatment of postpartum anestrus in dairy cows (Sprott & Carpenter, 2005). Estrus synchronization is the induction of the estrus in a group of animals approximately at a same time. It is an advanced management tool through which the human errors and the managerial costs can be reduced (Islam, 2011).

Various intra-vaginal devices impregnated with different amounts of progesterone (0.5-1.9 g) are commercially available. CIDR® (Controlled Internal Drug Release) came to the fore front in various countries throughout the world cattle industry (Macmillan & Peterson, 1993). On the basis of duration of CIDR placement, CIDR can be applied in two ways as- longer duration (i.e., 9-14 days) or shorter duration (i.e., 5-8 days). Studies in dairy cows and heifers showed that the longer duration CIDR placement resulted on more estrus expression rate but lower conception. Unlikely, the estrus expression rate was found to be lower with moderate conception rate from CIDR-treated animals for shorter duration (Macmillan & Peterson, 1993).

Previous study showed that the estrus expression rate within five days after CIDR removal was higher ($p < 0.05$) in 7-day group than in 11-day group, whereas pregnancy rate did not differ significantly between the groups (Gautam et al., 2019). However, it is still unclear whether the placement of CIDR for 9-days would be more effective than for 7-days for estrus expression as well as pregnancy outcome. So, this study was designed to determine the effectiveness of CIDR insertion in terms of duration of its placement (7-days vs 9-days) for the treatment of postpartum anestrus in crossbred dairy cows.

MATERIALS AND METHODS

Study area and animals

This research was conducted in a commercial dairy farm in Chitwan district, Bagmati Province of Nepal. This farm had altogether 550 crossbred cattle including parous cows, heifers and calves. A total of forty postpartum anestrus crossbred Jersey and Holstein cows, at least two months postpartum, with no estrus activity for the past two months, free from any anatomical defects or reproductive anomalies, and with a recorded body condition score (BCS) ≥ 2.25 were selected for the present study.

Clinical examination

Cows in the experiment were examined to assess their BCS (1-5 scale with 0.25 increment, Ferguson et al., 1994). Transrectal-ultrasonography (Farm Scan® L60, BMV Technology, China) was performed to determine the ovarian cyclicity status and to rule out any possible anatomical defects or anomalies.

Treatment protocols

Before the commencement of hormonal protocols, all the cows in experiment were dewormed with broad-spectrum anthelmintics: Oxytoclozanide+Levamisole (Nepal Pharmaceutical Ltd., Birgunj, Nepal) at the recommended doses followed by supplementation of liver tonics for five days and mineral mixtures for three weeks. Then the cows were randomly divided into two treatment groups in terms of duration of CIDR® (Controlled Internal Drug Release, Zoites, Australia, containing 1.9 gm of progesterone) placement viz. 7 days (T1, n=20) and 9 days (T2, n=20) (Fig. 1). In both groups, CIDR was inserted on day 0 (d0). The CIDR was inserted into the vagina of each cattle using CIDR applicator after disinfecting vulva. In both groups, a

PGF_{2α} analogue, cloprostenol 500 µg (Interchem, Netherlands) was injected intramuscularly 24 hours before CIDR removal. In both groups, GnRH analogue Buserelin acetate 20 mcg (Intas Pharmaceuticals, India) was injected intramuscularly 48 hrs after CIDR removal. Irrespective of estrus, the fixed time artificial insemination (FTAI) was done 16-20 hours after GnRH analogue injection with good quality frozen-thawed semen of Jersey and Holstein cattle produced from National Livestock Breeding Office, Pokhara, Nepal. Cows were observed for estrus signs at least three times a day after removal of CIDR until the time of FTAI. Standing to be mounted was taken as a primary sign of estrus. Secondary estrous signs (mucus discharge, bellowing, mounting, vulva congestion and uterine contraction at FTAI) were also recorded. Pregnancy diagnosis was done after two months of AI by using trans-rectal palpation.

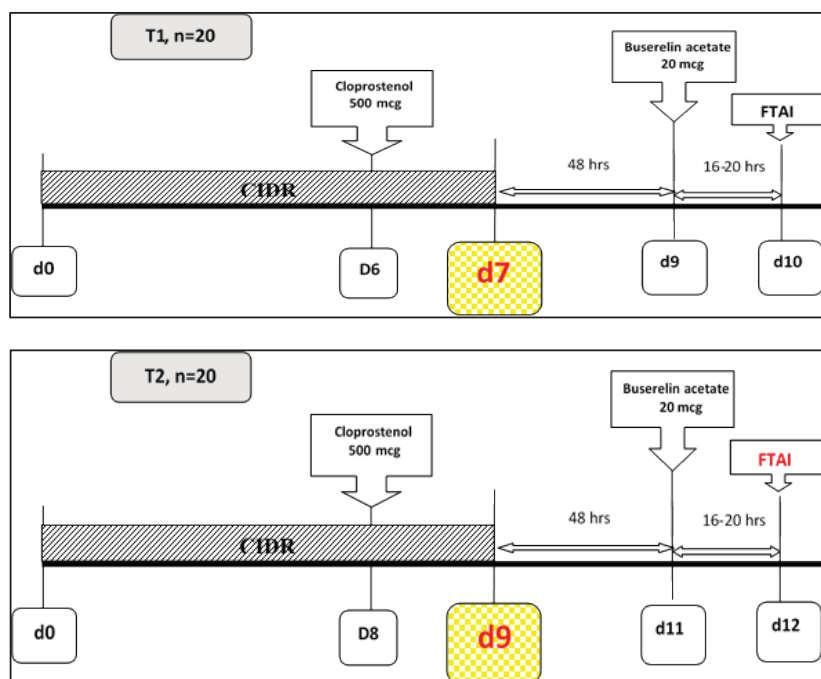


Fig.1. Estrous synchronization protocols varying in the duration of CIDR insertion

Statistical analyses

The data analysis was done by using Excel spreadsheet (Microsoft Corporation, 2007) and SPSS (Version 25). For analysis of various factors on estrus expression and pregnancy outcomes, the cows were categorized into primiparous and pluriparous, BCS low (<2.75) and good (≥ 2.75), milk production more than average (>12.4lit/day) and less than or equal to average (≤ 12.4 lit/day), calving season (summer and winter), presence or absence of uterine contraction at FTAI and ovarian cyclicity at CIDR insertion (cyclic and noncyclic).

Data entry and simple mathematical calculations were done by using Ms-Excel 2007. Estrus expression rate and pregnancy rate between two groups were compared using Chi-square test. Similarly, the effect of various factors like parity, BCS, season of calving, milk yield and ovarian condition on estrus induction and that on pregnancy outcome were also analyzed by using Chi-square-test. If the expected frequency was <5 in more than 20% of the cells, Fisher's exact probability test was used. Probability (P) value ≤ 0.05 was considered as significant whereas $0.05 < P \leq 0.1$ was considered to have tendency effect.

RESULTS

Estrus expression

The proportion of cows that showed primary and various secondary signs of estrus are shown in Table 1. There was significant difference in the proportion of cows showing primary estrus

sign i.e. standing to be mounted between the two treatment groups. However, there was no significant difference in other estrus signs between 7-day and 9-day groups i.e. mucus discharge rate, bellowing rate, mounting rate and vulva congestion. Similarly, the overall estrus expression rate was also not different between 7-d group (95%) and 9-d group (100%).

Table 1. Estrus expression rate in cows treated with CIDR-PGF_{2α}-GnRH protocols in terms of duration of CIDR placement

Estrus signs	Treatment group		P-value
	T1 (7-day, n=20)	T2 (9-day, n=20)	
Standing to be mounted (%)	30	65	0.03
Mucus discharge (%)	65	17	0.14
Bellowing (%)	75	55	0.18
Mounting (%)	55	75	0.18
Vulva congestion (%)	45	45	1.00
Uterine contraction at FTAI (%)	70	80	0.46
Overall estrus expression rate (%)	95	100	1.00

Pregnancy rates

There was no significant difference ($P=0.16$) in pregnancy rate (35% vs 55%, respectively) between 7-day and 9-day groups. Overall pregnancy rate among all cows was 45% (18/40).

Effect of various factors on estrus expression

All cows were pooled together to analyze the effect of various factors on estrus expression in cattle treated with CIDR-PGF_{2α}-GnRH protocols (Table 2). It was found that parity, BCS, season of calving, milk yield and ovarian cyclicity status at CIDR insertion had no significant association with the estrus expression rate.

Table 2. Effect of various factors on estrus expression in cows treated with CIDR-PGF_{2α}-GnRH protocols

Factors	Level	No. of cows	Estrus expression rate (%)	P-value
Parity	Primiparous	1	100 (1/1)	0.67
	Pluriparous	39	66.7 (26/39)	
BCS	Low (<2.75)	22	63.6 (14/22)	0.56
	Good (≥2.75)	18	72.2 (13/18)	
Season of calving	Summer	15	66.7 (10/15)	0.47
	Winter	19	73.7 (14/19)	
Milk yield	≤average (12.4 L/day)	24	87.5 (21/24)	0.47
	>average (12.4 L/day)	16	93.75(15/16)	
Ovarian cyclicity status at CIDR Insertion	Non-cyclic	22	86.4 (19/22)	0.38
	Cyclic	18	94.4 (17/18)	

Effect of various factors on pregnancy outcomes in cows treated with CIDR-PGF_{2α}-GnRH protocols

There was no difference in pregnancy rate between 7-d and 9-d groups. Therefore, in order to analyze the factors affecting pregnancy outcomes, cattle from both groups were pooled

together and analyzed (Table 3). There was no effect of parity, BCS, season of calving and milk yield on pregnancy outcome. However, ovarian cyclicity at CIDR insertion tended to affect the pregnancy outcome. Pregnancy rate was higher ($P=0.06$) in cyclic cows (61.1%) than in non-cyclic cows (31.8%).

Table 3. Effect of other factors on pregnancy rate in cows treated with CIDR-PGF_{2α}-GnRH protocols

Factor	Level	No. of cows	Pregnancy rate (%)	P-value
Parity	Primiparous	1	0 (0/1)	0.55
	Pluriparous	39	46.2 (18/39)	
BCS	Low (<2.75)	22	54.5 (12/22)	0.18
	Good (≥ 2.75)	18	33.3 (6/18)	
Season of calving	Summer	15	46.7 (7/15)	0.73
	Winter	19	52.6 (10/19)	
Milk yield	\leq average (12.4 L/day)	19	42.1 (8/19)	0.73
	$>$ average (12.4 L/day)	21	46.6 (10/21)	
Ovarian cyclicity status at CIDR insertion	Non-cyclic	22	31.8 (7/22)	0.06
	Cyclic	18	61.1 (11/18)	

DISCUSSION

Present study demonstrated that the ovarian cyclicity status at the initial of the protocol, rather than the duration of CIDR placement, influenced the success of CIDR-PGF_{2α}-GnRH protocol for the treatment of anestrus in crossbred dairy cattle.

It was found that the overall estrus expression rate was not affected by the duration of CIDR placement in CIDR-PGF_{2α}-GnRH protocol. However, the length of duration of CIDR insertion (either 7 days or 9 days) significantly affected the ‘standing to be mounted’ as a primary sign of estrus (30% vs 65%). Previous studies also indicated that as compared to 7-d insertion period, the longer duration of CIDR insertion resulted on clearer estrus expression both in heifers and cows (Macmillan & Peterson, 1993).

The cattle in this experiment were kept in loose housing system, so the estrus detection after treatment was based on observation of both primary and secondary estrous signs such as mucus discharge from vulva, bellowing, mounting, vulva congestion and uterine contraction at FTAI. Among these, the most frequently observed signs were uterine contraction at FTAI (80%) in 9-day group and bellowing (75%) in 7-day group. No other estrous signs were found to be significantly different between two groups except ‘standing to be mounted’ (primary sign). CIDR contains progesterone which is released from it into blood circulation and thus, progesterone in peripheral blood circulation is elevated. High level of progesterone in peripheral blood circulation results in negative feedback effect on hypothalamus to secrete GnRH. After removal of CIDR there is a rapid fall in plasma progesterone that removes the negative feedback effect of progesterone on hypothalamus which results in synchronization of estrus in animal (Macmillan & Peterson, 1993). It was found that progesterone treatment increased the concentrations of estradiol receptors in the ventromedial hypothalamus of the ewe (Caraty & Skinner, 1999),

which suggests the possible priming effect of progesterone in the brain for various estradiol-mediated functions (Sauls et al., 2017).

In this study the overall estrus expression rate was found to be 97.5% (39/40), which was almost similar to the findings of previous studies that used CIDR-PG_{2α} protocols of various days (Gautam et al., 2019; Macmillan & Peterson, 1993; Romano et al., 2013).

This study found no significant difference in pregnancy rate (35% vs 55%) between 7-day and 9-day groups. Romano et al. (2013) also obtained similar findings i.e, pregnancy rate (40.9% vs 39.8%) in day-7 group and day-9 group. Gautam et al (2019) also found no significant difference in pregnancy rate (60% vs 44.4%) between 7-day group and 11-day group. Thus, it can be recommended that the CIDR can be placed into the vagina of treated cows for either 7 or 9 days in CIDR-PGF_{2α}-GnRH protocol while treating anestrus in crossbred dairy cattle.

The overall pregnancy rate after FTAI in the present study was 45%. This was in agreement with most of the studies where at least 26% pregnancy rate was achieved in dairy cows by using CIDR-PG_{2α} protocol (Dhami et. al., 2015; Gautam et al., 2019; Lucy et.al., 2001). However, the pregnancy rate obtained in this study was higher than that reported by previous studies that used CIDR-PG_{2α} protocols [9.5% reported by Crane et al. (2006); 12.5% reported by Sah et al. (2019)].

It was found that none of the factors like parity, BCS, season of calving, milk yield and ovarian cyclicity at CIDR insertion were associated with the estrus expression rate. Previous study also found no significant association of BCS, breed and ovarian status with estrus expression rate (Gautam et al., 2019). Although a previous study indicated that the cows having low BCS were 0.48 times less likely to be expressed estrus signs than cows with a high BCS (Crane et al., 2006), no difference in estrus expression rate between low and good BCS in this study might be due to the fact that all cows in this study had BCS ≥ 2.25 i.e. cows with very poor BCS were not included in the study. Ovarian cyclicity at the time of CIDR insertion tended to influence pregnancy outcome, with cyclic cows showing a higher pregnancy rate (61.1%) compared to non-cyclic cows (31.8%). However, other factors like parity, BCS, season of calving, milk yield and ovarian cyclicity were not associated with pregnancy outcomes. These results were consistent with the findings of a previous study that compared the efficacy of 7-day vs 11-day CIDR-PG_{2α} protocols (Gautam et al., 2019).

Although a good pregnancy outcome was achieved with CIDR-PG_{2α}-GnRH protocol in the present study, this protocol required frequent (four times) handling of cows. Thus, further study is recommended to examine whether injecting GnRH simultaneously at the time of FTAI would have similar efficacy as that of the present protocol so that there will be less (three times) handling of the cows.

CONCLUSION

The duration of CIDR placement (7-d vs 9-d) had no effect on the overall efficiency of CIDR-PG_{2α}-GnRH protocols for the treatment of postpartum anestrus in crossbred dairy cows except that the proportion of cows showing primary estrus signs was higher in 9-d group than in 7-d group. A good pregnancy outcome (45%) was achieved with CIDR-PG_{2α}-GnRH protocol, however the pregnancy outcome depended on the status of ovarian cyclicity at the start of the protocol.

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