

**Research Article****EFFECTIVENESS OF DURATION OF CIDR APPLICATION ON REPRODUCTIVE PERFORMANCE OF POSTPARTUM ANESTROUS DAIRY COWS****G. Gautam<sup>1\*</sup>, B. B. Ratna<sup>1</sup>, A. K. Sah<sup>2</sup>, and B. N. Devkota<sup>1</sup>**<sup>1</sup>Agriculture and Forestry University, Rampur, Chitwan, Nepal<sup>2</sup>Nepal Agricultural Research Council, National Buffalo Research Program, Tarahara, Nepal

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**ABSTRACT**

This study was done to compare the effectiveness of duration of Controlled Internal Drug Release (CIDR) application on reproductive performance of postpartum anestrous dairy cows while applying CIDR-PG protocols. Cows (n=19) in a large commercial dairy farm in Chitwan, Nepal 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 (7-day group, n=10) and for 11 days (11-day group, n=9). One day before the removal of CIDR, all the cows were treated with PGF2 alpha analogue (Cloprostenol 500 µg). Vaginal electrical resistance (VER) was measured at 48 hours after CIDR removal. Cows were observed for estrus signs at least three times a day starting from the day of CIDR removal until five days after that, and those detected in estrus were artificially inseminated (AI) according to am/pm rule. Pregnancy was diagnosed 60 days after AI using trans-rectal palpation and ultra-sonography. Overall estrus expression and pregnancy rates were 73.7% and 52.6%, respectively. 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. There was no effect of Body Conditioning Score (BCS), breed, ovarian status before treatment on estrus expression rate and pregnancy outcome. There was a tendency ( $p < 0.1$ ) that estrus expression rate was higher in primiparous than in pluriparous cows. Moreover, the pregnancy rate tended to be higher in cows with high VER than in cows with low VER. In conclusion, while applying CIDR-PG protocol, application of CIDR for 7 days was more effective than for 11 days on inducing estrus in postpartum anestrous cows while pregnancy rate was not different between two CIDR application durations.

**Key words:** Duration comparison, estrus expression, pregnancy, vaginal electrical resistance**INTRODUCTION**

Livestock plays a significant role in the Nepalese economy that contributes around 13% to the National Gross Domestic Product. The livestock sector has a high potential for growth in Nepal. Cow is the second most important livestock species in Nepal. Cow milk contribution to total milk production is around 34.7%. Cattle population and cow milk production is in increasing trend (Livestock Statistics of Nepal, 2017). With the commercialization of dairy industry, exotic crossbred dairy cows have been introduced and their number is increasing for last few decades. However, crossbred cows have been suffering from various health problems including infertility. Infertility problem in crossbred and exotic cattle has been reported to be the most prioritized problem in dairy pocket areas in Nepal (Jha, 1999). Anestrus was the major form of infertility in dairy cattle (Moellers & Riese, 1988; Khanal, 1996; Sankhi, 1999; Chakurkar et al., 2008).

Postpartum anestrous in dairy cows can be treated using various hormonal protocols. Since, the most of the postpartum anestrous cases are due to inactive ovaries, progesterone based protocols are useful to treat such cases (Johnson et al., 2011; Cheison, 2006; Imwalle et al., 1998). Progesterone plays an important role in controlling the estrous cycle of the bovine female. Progestins were shown to induce estrus cycle by increasing LH pulse frequency and down-regulating E2 receptors in the hypothalamus, eliciting the greatest effect after progestin removal (Nash et al., 2011). CIDR (Controlled Internal Drug Release) device has been used alone or in combination with prostaglandin to treat anestrous condition (Macmillan & Peterson, 1993). CIDR device is inserted intravaginally in cattle. CIDR contains progesterone which is released from it into blood circulation and blood concentration of progesterone is elevated. After removal of CIDR there is a rapid fall in plasma progesterone which results in synchronization of estrus in animal (Macmillan & Peterson, 1993).

There are variable results regarding the effectiveness of CIDR application in terms of duration of its application. It was reported that longer the insertion period, better was the estrous expression rate, however, the pregnancy rates were reduced (MacMillian & Peterson, 1993). Islam (2011) reported that the longer the progestin is administered to cattle, the higher rate of estrus synchronization was found. However, Thomas et al. (2016) reported that the proportion of cows expressing estrus following CIDR removal tended to be higher among cows assigned to the 9-d CIDR-PG treatment (93%) than 14-d CIDR-PG protocol (81%). Thus, the duration of CIDR insertion in CIDR-PG protocol affects the effectiveness on its outcomes. Therefore, this study was done to compare

the effectiveness of CIDR-PG protocols in terms of duration of CIDR application on estrus synchronization and pregnancy outcomes in long-standing postpartum anestrous crossbred dairy cows, and to determine the factors affecting estrus expression and pregnancy outcomes in CIDR-PG treated anestrous cows.

### MATERIAL AND METHODS

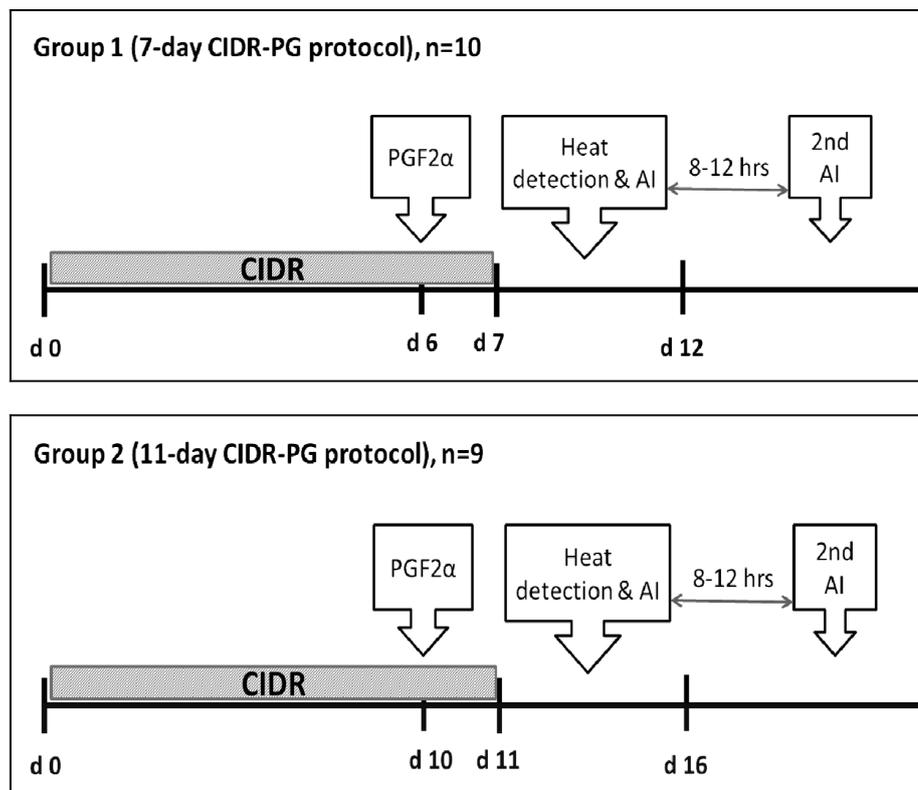
This study was conducted in a commercial dairy farm operated by a cooperative located at Khairahani municipality in Chitwan district of Nepal, from June 2017 to March 2018. The environmental fluctuation is typical with cold and semi-dry to dry winter (December-February), rapidly increasing hot and dry spring (March-May), very hot and rainy monsoon summer (June-August) and moderate autumn (September-November) (Devkota & Bohora, 2009).

#### Data collection and clinical examination

Data regarding breed, parity, date of calving, date of AI, breeding history since last calving were collected from herd records. Reproductive organs of all the cows in experiment were clinically examined using transrectal ultrasonography at the start of clinical trial, and the body condition score (BCS, 1-5 range) and ovarian status at the start of treatment protocols were recorded.

#### Treatment protocols

Cows that were not detected in estrus at least until 60 days postpartum (n=19) were divided into two treatment groups treated as shown in Figure 1. Briefly, the controlled internal drug release (CIDR) device containing 1.9 gm progesterone (Zoeitus, Australia) was inserted into the vagina of cows for seven days (7-day group, n=10) and for 11 days (11-day group, n=9). In both groups of cows, the PGF2 alpha analogue (Cloprostenol 500 µg) was injected intramuscular one day before the removal of CIDR. Cows detected in estrus after CIDR removal was artificially inseminated (AI).



**Figure 1. Estrous synchronization protocols varying on the duration of CIDR insertion CIDR: Controlled Internal Drug Release containing 1.9 g progesterone PGF2α: prostaglandin analogue (Cloprostenol 500 µg).**

#### Heat detection and AI

Starting from the day after CIDR removal for 5 days, all the treated cows were observed for estrus signs at least three times a day. Cows showing any of the following signs were considered to be in estrus: standing to be

mounted, clear mucus discharge, bellowing, and restlessness, mounting on other cows, swelling and congestion of vulva. AI was performed as per am/pm rule by a trained technician using frozen thawed semen produced at National Livestock Breeding Center, Pokhara; and then second AI was done 8-12 hours after the first AI.

### Measurement of vaginal electrical resistance (VER)

Vaginal electrical resistance (VER) was measured at 48 hour after CIDR removal by using an Estrous Detector (Draminski, Poland). The probe was inserted into the vagina, and the electrical resistance from three different locations of cranial vagina was recorded. Mean value of three different readings was used for the analysis. As per the manufacturer's manual, the VER value  $\geq 300$  units was considered as high and  $< 300$  units as low.

### Pregnancy diagnosis

Pregnancy was diagnosed 60 days after AI using transrectal palpation and/or ultrasonography.

### Statistical analyses

Statistical analyses were carried out using SPSS (Version 20) and Ms-Excel 2007. The estrus expression rate and pregnancy rates between two treatment groups were compared using Chi-square test. Similarly, effects of various factors on estrus expression and pregnancy outcome were also analyzed using chi-square test. If the expected frequency was less than 5 in any cell, Fisher Exact Probability test was used. VER values between different groups of cows were compared using students' *t*-test assuming equal variances. P-value  $\leq 0.05$  was considered to have significant effect, and  $0.05 > P \leq 0.1$  was considered to have tendency.

## RESULTS

Overall, 73.7% (14/19) cows showed estrus signs within five days after CIDR removal and 52.6% (10/19) cows became pregnant. Estrus expression rate was significantly ( $p=0.02$ ) higher in 7-day group (100%; 10/10) than in 11-d group (44.4%; 4/9). However, pregnancy rate was similar between two groups (60% and 44.4% in 7-day and 9-day groups, respectively,  $p=0.66$ ).

### Factors affecting estrus expression rate in cows treated with CIDR-PG protocols

Table (1) shows the factors affecting estrus expression rate in cows treated with CIDR-PG protocols. Estrus expression rate was significantly higher in 7-day group than in 11-day group. Likewise, there was a tendency that the estrus expression rate was higher in primiparous ( $p=0.1$ ) than in pluriparous cows. However, there was no effect of BCS, breed, ovarian status before treatment and vaginal electrical resistance (VER) at 48 hours after CIDR removal on estrus expression rate.

**Table 1. Factors affecting estrus expression rate in cows treated with CIDR- PG protocols**

Factors	No. of cows	Estrus expression Rate (%)	p- value
Protocol	7-day protocol	10	100
	11-day protocol	9	44.4
BCS	Low ( $< 2.75$ )	11	63.6
	Good ( $\geq 2.75$ )	8	87
Parity	Primiparous	7	100
	Pluriparous	12	58.3
Breed	Jersey cross	7	71.4
	Holstein cross	12	75
Ovarian status before treatment*	Inactive ovaries	3	66.7
	DF	5	100
	CL	11	63.6
VER at 48 hours after CIDR removal	Low ( $< 300$ units)	13	61.5
	High ( $\geq 300$ units)	6	100

\* DF= Dominant follicle; CL= Corpus luteum

### Factors affecting pregnancy outcomes in cows treated with CIDR- PG protocols

Table (2) shows the factors affecting pregnancy rate in cows treated with CIDR-PG protocols. There was no effect of protocols, BCS, parity, breed and ovarian status before treatment on pregnancy outcomes. However, there was a tendency that pregnancy rate was higher in cows with high VER readings than in cows with low VER at 48 hours after CIDR removal.

**Table 2. Factors affecting pregnancy rate in cows treated with CIDR- PG protocols**

	Factors	No. of cows	Pregnancy rate (%)	p- value
Protocol	7-day protocol	10	60	0.66
	11-day protocol	9	44.4	
BCS	Low (< 2.75)	11	45.4	0.65
	Good ( $\geq 2.75$ )	8	62.5	
Parity	Primiparous	7	71.4	0.40
	Pluriparous	12	41.7	
Breed	Jersey cross	7	57.1	1.00
	Holstein cross	12	50	
Ovarian status before treatment*	Inactive ovaries	3	33.3	0.30
	DF present	5	80	
	CL	11	45.4	
VER at 48 hours after CIDR removal	Low (<300 units)	13	46.1	<b>0.10</b>
	High ( $\geq 300$ units)	6	66.7	

\* DF= Dominant follicle; CL= Corpus luteum

### Vaginal electrical resistance (VER) at 48 hours after CIDR removal

There was no significant difference in VER values at 48 hours after CIDR removal between 7-day ( $305 \pm 17.7$  units) and 11-day ( $319.7 \pm 43.0$  units) groups. When cows were grouped according to whether they expressed estrus signs (n=14) or not (n=5), the VER value at 48 hours after CIDR removal was higher (p=0.001) in cows that expressed estrus than in cows that did not express estrus within five days after CIDR removal (Table 3).

**Table 3. Vaginal electrical resistance at 48 hours after CIDR removal in cows showing estrus or not showing estrus**

VER at 48 hours after CIDR removal (Mean $\pm$ SE)	Groups		p-value
	Estrus expressed (n=14)	Estrus not expressed (n= 5)	
	340.7 $\pm$ 25.1	231.4 $\pm$ 12.8	0.001

### DISCUSSION

The study evaluated the effectiveness of CIDR-PG protocols in terms of duration of CIDR insertion on estrus expression and pregnancy outcomes in cross-bred dairy cows in a large commercial farm in subtropical area of Nepal. It also determined the effects of various factors on estrus expression and pregnancy outcome in CIDR-PG treated anestrus cows. The average estrus response (74%) in this study is considered good compared with the average from 50% to 74% of estrus detection for non-synchronized estrus (Stevenson & Britt, 1977; Williams et al., 1981).

In the present study, the estrus expression rate was higher in 7-day protocol than in 11-day protocol. However, the pregnancy rate was similar between two treatment groups. It has been reported that in CIDR synchronization protocols, the estrous synchrony was more precise with insertion periods of 14 days or more, but pregnancy rates were reduced (Macmillan & Peterson, 1993). However, present study revealed that with seven days insertion period, all cows expressed estrus within five days after CIDR removal although expression of estrus was spread over five days. Such a good estrus response might be due to inclusion of luteolytic agent in the protocol so that

after removal of CIDR there would have been abrupt decline of progesterone resulting in withdrawal of negative feedback effect of progesterone on hypothalamus. On the other hand, lower estrus expression rate in 11-day protocol might be due to the fact that 11 days might be quite long duration to maintain high progesterone level in blood of lactating cows so that there might have gradual or slow decline of progesterone starting from before CIDR removal rather than abrupt decline in progesterone after CIDR removal. Similarly, though not significantly different, the pregnancy rate was numerically higher in 7-day protocol than in 11-day protocol. These findings suggest that insertion of CIDR for seven days as compared to 11 days would be more effective for reproductive performance in cross-bred dairy cows. This is supported by the findings of previous studies that reported the proportion of cows expressing estrus following CIDR removal tended to be higher among cows assigned to 9-day CIDR-PG treatment (93%) than 14-day CIDR-PG treatment protocol (81%) (Thomas et al., 2016). However, it is not known whether nine days insertion period is better than seven days insertion. Thus, further study is recommended to compare the effectiveness of 7-days vs 9-day CIDR-PG protocols in lactating cross-bred dairy cows in Nepalese context.

In this study, the estrus expression rate was not affected by BCS, breed, ovarian status before the start of treatment and VER at 48 hours after CIDR removal. However, parity affected the estrus expression- there was a tendency that the estrus expression rate within five days after CIDR removal was higher in primiparous cows than in pluriparous cows. This result is in agreement with Aziz & Waheb (2017) who reported that primiparous Holstein cows responded better than multiparous cows to the 12-day presynch-ovsynch protocol as evidenced by better estrus response and higher conception rate. For ovarian status before treatment, estrus expression rate was numerically higher in cows with dominant follicle (100%) than in cows with inactive ovaries (67.7%) and corpus luteum (63.6%). Seven out of 10 cows in 7-day protocol had presence of dominant follicle on the day of CIDR insertion. This might be the reason why estrus expression was good in 7-day protocol. Two of three cows with inactive ovary also showed estrus. This finding is similar to the findings of Islam (2011) who reported that short term exposure to progesterone caused some postpartum anestrus cattle to begin cycling.

Pregnancy rate, although not significantly different between two protocols, was numerically higher in 7-d protocol than in 11-d protocol. This is ultimately due to better estrus expression rate since there was no fixed time artificial insemination; only the cows showing estrus were inseminated. Thus, further study with fixed time artificial insemination using GnRH injection after CIDR removal is recommended to determine the effect of these two protocols on pregnancy outcome.

The vaginal electrical resistance at 48 hours after CIDR removal was similar in both protocols. It has been stated that VER falls to minimum (below 300 units) during estrus and again rises to high level (above 300 units) after estrus (Draminski Estrus Detector Manual, 2015). However, in our study it was found that the value of VER at 48 hours after CIDR removal was higher in cows that showed estrus than that in cows that did not show estrus. From this finding, it can be speculated that the decline of VER at 48 hours after CIDR removal might not be associated with behavioral estrus signs. This was supported by the finding that pregnancy rate tended to be lower in cows with low VER than in cows with high VER. It can be assumed that in cows with high VER at 48 hours after CIDR removal, the value of VER might have decreased on following day. Unfortunately, we could not measure the VER after 48 hours because of technical problems. Therefore, it is recommended to measure the VER at least three times a day until 5 days after CIDR removal to correlate with estrus expression; the manufacturer also recommends to take vaginal electrical resistance measurements at least two times a day for correct timing of artificial insemination. From our study, at least it can be concluded that too early (i.e. 48 hours) decline of vaginal electrical resistance values after CIDR removal was not good for pregnancy outcome.

### CONCLUSION

In conclusion, 7-day CIDR-PG protocol was more effective than 11-day protocol for inducing estrus in postpartum anestrus cows while pregnancy rate was not different between two treatment protocols. In addition, estrus response was also influenced by the parity. Likewise, early decline in vaginal electrical resistance values after CIDR removal was not conducive for pregnancy outcome in CIDR-PG protocols.

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