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INFLUENCE OF THE DURATION OF CIDR PLACEMENT IN CIDR-$PGF_{2α}$ PROTOCOL FOR THE TREATMENT OF ANESTRUS IN WATER BUFFALO DURING LOW BREEDING SEASON

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ABSTRACT

This study compared the effectiveness of CIDR (Controlled Internal Drug Release)-$PGF_{2α}$ protocol in terms of the duration of CIDR placement for the treatment of anestrus in buffaloes during low breeding season. Anestrous Murrah-cross buffaloes were randomly assigned to two Treatment groups. In Group 1 (T1, n = 15), a CIDR was inserted intravaginally for 7 days, while in Group 2 (T2, n = 15), the CIDR was inserted for 9 days. Each buffalo in both groups received an injection of a $PGF_{2α}$ analogue, cloprostenol (500 µg), 24 h before CIDR removal. Artificial insemination (AI) was performed twice, at 60 and 72 h after CIDR removal. Ovarian cyclicity status during CIDR insertion and the size of the corpus luteum (CL) on 7th day after AI were assessed using transrectal ultrasonography. Body condition score (BCS), endoparasitic infection, anemic score, suckling status and lactation stage were recorded. Pregnancy was diagnosed two months after AI. No significant differences were found between two Treatment groups regarding estrus induction, ovulation and pregnancy outcomes. The overall estrus induction, ovulation and pregnancy rates were 81%, 93% and 26%, respectively. The ovarian cyclicity status at CIDR insertion affected the ovulation and pregnancy outcome. Additionally, the size of CL one week after AI had an influence on pregnancy outcome (P=0.03). In conclusion, the ovarian status at CIDR insertion and the CL size one week after AI, but not the duration of CIDR placement, influenced the success of CIDR-$PGF_{2α}$ protocol in treating anestrus in buffaloes during low breeding season.

Keywords: *Bubalus bubalis*, buffaloes, low breeding season, estrus induction, ovulation, pregnancy, corpus luteum

INTRODUCTION

Buffalo plays a vital role in the agricultural economy of rural areas in South and Southeast Asia, the Mediterranean region of Europe and Latin America due to their multipurpose use in milk and meat production and as draft animals (Zicarelli, 1994; Barile, 2005). In Nepal, buffalo is the major

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livestock species contributing 57% of the total milk production and 38% of total meat production in the country (MoALD, 2022). Buffaloes exhibit better breeding performance during periods of decreasing daylight but experience anestrus during spring and early summer, which coincides with longer daylight hours (Zicarelli, 1997). Spring (March to May) was the lowest breeding season for the buffaloes in Nepal. The majority of the buffaloes during low breeding season had true anestrus characterized by inactive ovaries or the lack of ovarian cyclicity (Devkota *et al*., 2023; Gautam *et al*., 2024a).

To induce the follicular activity in the ovaries of anestrous buffaloes, particularly during the low breeding season, progesterone or similar compounds can be administered to mimic luteal activity. Various intravaginal progesterone-releasing devices, containing different amounts of progesterone (0.5 to 1.9 g), are available commercially. Controlled Internal Drug Release (CIDR) is the widely used intravaginal progesterone-releasing device in the cattle industry worldwide (Macmillan and Peterson, 1993) and it has also been used to address postpartum anestrus in buffaloes (Andukar and Kadu, 1995; Andukar *et al*., 1997; Singh, 2003). The progesterone released from CIDR increases blood progesterone levels, exerting a negative feedback effect on the hypothalamus. The removal of CIDR causes a rapid decrease in circulating progesterone, thus removing the progesterone block on the hypothalamus, leading to GnRH release, followed by FSH and LH release, and the resumption of ovarian cyclicity (Malik, 2005; Zerbe *et al*., 1999). The CIDR along with other hormones can be applied for varying duration, with longer duration (9 to 14 days) or shorter duration (5 to 8 days) (Macmillan and Peterson, 1993). Studies on dairy cows and heifers have shown that CIDR placement for longer duration results in higher estrus expression rates but lower conception rates. Conversely, shorter duration of CIDR placement leads to lower estrus expression rates but moderate conception rates (Macmillan and Peterson, 1993; Gordon, 1999; Juan and Fahning, 2013). However, whether the duration of CIDR placement in the CIDR-PGF$_{2\alpha}$ protocol influences the estrus induction and pregnancy outcome in buffalo was not known. Furthermore, most of the estrous synchronization protocols previously used in buffaloes were often expensive (requiring various hormones), required frequent animal handling, and resulted in moderate to low pregnancy rates (Naseer *et al*., 2011; Vecchio *et al*., 2013; Zaabel *et al*., 2009; Gautam *et al*., 2024b; Gautam *et al*., 2024c; Kandel *et al*., 2024). The objective of the present study, therefore, were (i) to compare the effectiveness of 7-day versus 9-day CIDR-PGF$_{2\alpha}$ protocols for the treatment of anestrus in buffaloes during the low breeding season and (ii) to assess the effect of various factors on estrus induction, ovulation and pregnancy outcome when applying CIDR-PGF$_{2\alpha}$ protocol in those buffaloes.

MATERIALS AND METHODS

Animals

This study was conducted in the buffaloes of household farms around the vicinity of the Agriculture and Forestry University (AFU) Rampur, Chitwan, located in the mid-southern region of Nepal, which has a subtropical climatic condition. The sources for identifying anestrous buffaloes included dairy cooperatives, local veterinarians, para-veterinarians and personal contacts. Purposive sampling method was used until the number of desired anestrous buffalo reached 30. All the buffaloes were Murrah crossbreeds, although the exact blood level of the breed was not known. Buffaloes included in the study were at least three months postpartum, had not exhibited estrus since last calving, had no anatomical defects or abnormalities in their reproductive organs and had a Body Condition Score (BCS) greater than 2.0.

All buffaloes were dewormed against internal parasites by drenching with a broad-spectrum anthelmintic (oxyclozanide levamisole-Adzanide forte, Nepal Pharmaceutical Ltd., Birgunj, Nepal) at the recommended dosage of 1 ml per 3 kg body weight. Following the administration of anthelmintics, a mineral-vitamin mixture supplement (Kalvimin forte, Karnataka, India) was provided at the recommended dosage of 50 gm per day for 20 days before starting the hormonal protocols.

Clinical examination and the determination of ovarian cyclicity status

Buffaloes were assessed for their BCS on a scale of 1 to 5 with 0.25 increment (Ferguson *et al*., 1994) and anemic condition (based on eye's mucous membrane). Fecal samples were collected and examined using sedimentation and floatation methods, for determining the endoparasitic infection, if any. Presence of a suckling calf and lactation stage were also recorded. Transrectal ultrasonography was performed to determine the major structures on the ovaries and to rule out anatomical defects or abnormalities, if any.

Hormonal treatment protocols

The buffaloes (n=30) were randomly assigned to two Treatment groups based on the duration of CIDR (Controlled Internal Drug Release, Eazi-Breed, Zoetis, Australia, containing 1.9 g of progesterone) placement. In Group 1 (T1, n=15), a CIDR was inserted intravaginally for 7 days, while in Group 2 (T2, n=15), the CIDR was inserted for 9 days. Variation in the duration of CIDR placement was to examine the influence of 9-days insertion period as compared to standard shorter duration (7-days) insertion (Macmillan and Peterson, 1993).

Each buffalo in both groups received an injection of a $PGF_{2\alpha}$ analogue, cloprostenol 500 µg (Cloprochem, Interchem, Netherlands) 24 h before CIDR removal. Fixed time artificial insemination (FTAI) was performed twice, at 60 and 72 h after CIDR removal using frozen-thawed semen of Murrah buffalo produced by the National Livestock Breeding Office, Pokhara, Nepal.

Scoring of vaginal discharge at CIDR removal

Immediately after the withdrawal of the CIDR from the vagina of the buffalo, the vaginal discharge attached on the surface of the CIDR was scored as: clear, mucopurulent (almost 50% pus with 50% mucus), purulent (>50% pus) or bloody.

Estrus detection, ovulation confirmation and pregnancy diagnosis

Estrus was detected based on secondary estrous signs. After the removal of CIDR until second FTAI, all the buffaloes were carefully observed for external estrous signs (mucus discharge from vulva, vulva swelling, congestion of vulval mucous membrane, teat engorgement and decrease in milk yield). Ovulation was confirmed based on the detection of corpus luteum (CL) on 7th day after second AI using transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China). Pregnancy was diagnosed two months after AI through transrectal palpation.

Data analyses

Data were analyzed using an Excel spreadsheet (Microsoft Corporation, 2007) and R-Studio (Version 3.6.1, USA, 2019). Estrus expression rate, ovulation rate and pregnancy rate between two Treatment groups were compared using the Chi-square test. Fisher's exact probability test was employed when the expected frequency was <5 in more than 20% of the cells.

The effects of various factors on estrus induction, ovulation and pregnancy outcome were also analyzed using the Chi-square test or Fisher's exact probability test, whichever applicable. For this, the buffaloes were categorized as primiparous or pluriparous, with a low BCS (<2.75) or good BCS (≥2.75), presence or absence of endoparasites, anemic or non-anemic, with or without a suckling calf, in early lactation (up to 100 days postpartum) or later lactation (more than 100 days postpartum) and the ovarian status at CIDR insertion- either true anestrus (No CL, No DF), silent estrus with DF>10 mm, or silent estrus with CL.

CL size on 7th day post AI between two treatment groups was compared using a two-sample t-test. A probability value of ≤0.05 was considered statistically significant, while a P-value from >0.05 to ≤0.1 was considered as a tendency towards significance.

RESULTS

Out of the initial 30 buffaloes, three from the 9-day group were excluded from the study due to the loss of CIDR in two buffaloes and the sale of one buffalo by its owner before the protocol was completed. As a result, there were 27 buffaloes for further analysis.

Estrus induction rate

Overall, 81% (22 out of 27) of the buffaloes exhibited at least one of the secondary signs of estrus. There was no significant difference in overall estrus induction rate between the 7-day group (86.7%) and the 9-day group (75%) (Table 1). Mucus discharge from the vulva was the most common sign of estrus, observed in 66.7% of the buffaloes in both groups. There was a tendency (P=0.10) that the proportion of buffaloes showing teat engorgement was higher in 7-day group (40%) than in 9-d group (8.3%).

Ovulation and pregnancy rates

There was no significant difference between the 7-day CIDR-$PGF_{2α}$ and 9-day CIDR-$PGF_{2α}$ groups in terms of ovulation rate (86.7% vs 100%) and pregnancy rate (20.0% vs. 33.3%) (Table 2). Overall, the ovulation rate was 92.6% (25 out of 27) and the pregnancy rate was 26.0% (7 out of 27).

Effect of various factors on estrus induction, ovulation and pregnancy outcome in buffaloes treated with CIDR-$PGF_{2α}$ protocol

Since the estrus induction, ovulation and pregnancy rates were similar between the 7-day and 9-day groups, all the buffaloes were pooled together for analyzing the effect of various factors on estrus induction, ovulation and pregnancy outcome in buffaloes treated with CIDR-$PGF_{2α}$ protocol. None of the factors included in the analysis (parity, BCS, endoparasitic infection, anemic score, presence of suckling calf, stage of lactation and the ovarian cyclicity status at CIDR insertion influenced the estrus induction rate.

Effect of various factors on ovulation induction after CIDR-$PGF_{2α}$ protocol has been shown in Table 3. Only the ovarian cyclicity status at the time of CIDR insertion affected the ovulation induction; there was a tendency (P=0.07) that the silent estrous buffaloes had higher ovulation response than the true anestrous buffaloes, whereas the other factors had no effect on ovulation.

Effect of various factors on pregnancy outcome after CIDR-$PGF_{2α}$ protocol has been shown in Table 4. Only the ovarian cyclicity status at CIDR insertion significantly affected the pregnancy outcome; pregnancy rate was higher in silent estrous buffaloes than in true anestrous buffaloes, whereas the other factors had no effect on pregnancy outcome.

Effect of corpus luteum (CL) size on pregnancy outcome

On the seventh day after AI, the corpus luteum (CL) was present in 92.6% (25 out of 27) of the buffaloes. Since the pregnancy rate did not differ between 7-day and 9-day groups, all the buffaloes were pooled together to determine the association of corpus luteum size with pregnancy outcome. When the CL size on seventh day post-AI was compared retrospectively between pregnant and non-pregnant buffaloes, it was significantly larger in pregnant than in non-pregnant buffaloes (Table 5).

DISCUSSIONS

This research aimed to evaluate the effectiveness of CIDR-PG protocols by varying the duration of CIDR insertion to treat anestrous buffaloes under field conditions during the low breeding season. To determine if a low-cost, simplified protocol would be effective, only CIDR and a single prostaglandin injection 24 h before CIDR removal were used, varying only with CIDR insertion durations of either 7 or 9 days. The study found that the duration of CIDR insertion did not impact estrus induction, ovulation, or pregnancy outcome.

Although the previous studies on dairy cows and heifers suggested that longer CIDR insertion durations lead to greater estrus induction (Macmillan and Peterson, 1993), the present study did not find a significant difference in estrus induction rates between the 7-day and 9-day CIDR insertion groups, possibly due to the small (only 2 days) duration difference. Estrus detection in the buffaloes, which were all kept in a 24 h tie-stall system, was based on secondary estrous signs such as teat engorgement, vulvar mucus discharge, congestion and swelling of the vulva and reduced milk yield. Mucus discharge from the vulva was the most observed heat sign, observed in 66.7% of buffaloes before or during the time of AI. Overall, 81% of treated buffaloes showed at least one estrous sign, indicating that the CIDR-$PGF_{2α}$ synchronization protocol effectively induced estrus during the low breeding season. The estrus induction rate in the present study (81%) was similar to that (80%) reported by a previous study in *Nili-Ravi* buffaloes during the low breeding season (Zahid *et al*., 2013). A higher estrus expression rate (97.4%) was noted in Indian buffaloes when the protocol was used round the year (Vikash *et al*., 2014), suggesting that estrus induction response could be higher during good season than during the low breeding season. Factors such as parity, body condition score (BCS), endoparasitic infection, anemic condition, presence of sucking calf, lactation stage, ovarian cyclicity status during CIDR insertion and vaginal discharge score at CIDR removal were analyzed for their effects on estrus expression. However, none of these factors significantly affected estrus expression in CIDR-$PGF_{2α}$ treated buffaloes.

Ovulation response was also unaffected by the duration of CIDR placement whether it was placed for 7 or 9 days. Although the exact time of ovulation was not known in the present study, 92.6% of treated buffaloes had ovulation response as indicated by the presence of corpus luteum on 7th day after AI. Ovulation response in the present study was slightly higher than the 75% in *Nili-Ravi* buffaloes in Pakistan as reported by a previous study that also used CIDR-$PGF_{2α}$ protocol (Zahid *et al*., 2013). Despite not using GnRH, the ovulation rate observed in the present study (92.6%) was comparable to the rates in previous studies involving GnRH or other treatments (Zaabel *et al*., 2009; Murugavel *et al*., 2009; Baruselli *et al*., 2007). The higher ovulation rate may be due to a higher proportion of buffaloes with silent estrus (having dominant follicle or corpus luteum). While analyzing the effect of various factors on ovulation induction, only the ovarian cyclicity status during CIDR insertion had a tendency (P=0.07) to influence the ovulation response. The probability of value in this case was in the borderline which might be due to small sample size. However, in field condition, there is biological relevancy of this finding because it can be assumed that although estrus can be induced in both true anestrous as well as silent estrous buffaloes, the ovulation response would be compromised by the ovarian cyclicity status of the animal (De Rensis and Lopez Gatius, 2007; Stevenson *et al*., 2012). The other factors such as parity, body condition score (BCS), endoparasitic infection, anemic condition, presence of sucking calf and the lactation stage had no effect on ovulation response in CIDR-$PGF_{2α}$ treated anestrous buffaloes. There was a lack of prior studies investigating the effects of these factors on ovulation rate in estrous-synchronized buffaloes.

There was no difference in pregnancy outcomes between the 7-day and 9-day treatment groups. This was in agreement with the findings of a previous study, in which the duration of progesterone insert (5-day vs 7-day) as well as the initial GnRH did not affect pregnancy per timed-insemination in dairy heifers subjected to a Co-synch protocol (Colazo and Ambrose, 2011). The overall pregnancy rate obtained in the present study (26%) was close to that (33.3%) reported by a previous study in which anestrous buffaloes were treated with CIDR for seven days and $PGF_{2α}$ was given one day before CIDR removal (Zaabel *et al*., 2009). However, it was lower than the 60% conception rate as reported by Zahid *et al*. (2013) using heat detection and artificial insemination (AI) technique. The lower pregnancy rate in this study could be attributed to the absence of GnRH in the protocol. As the study aimed to assess the effectiveness of a low-cost estrous synchronization protocol, the GnRH was intentionally omitted, which may have led to unsynchronized ovulation. In order to catch the opportunity of ovulations at different times (i.e. un-synchronized ovulation), AI was performed two times at 12 h interval i.e. at 60 and 72 h following CIDR removal. Despite conducting AI twice at 12 h intervals, the pregnancy rate remained low. This underscores the importance of incorporating fixed-time AI (FTAI) with GnRH to enhance pregnancy success because the GnRH plays a crucial role in stimulating the anterior pituitary gland to release FSH and LH, with the LH surge triggering the ovulation of dominant follicles (Bo *et al*., 2003; Mapletoft and Bo, 2018).

Ovarian cyclicity status at the time of CIDR insertion was a significant factor that influenced

Table 1. Estrus induction rate in buffaloes treated with 7-d versus 9-d CIDR-$PGF_{2\alpha}$ protocols.

Estrous signs	**Proportion of buffaloes showing particular estrous sign (%)**		**P-value**
	7-d CIDR-$PGF_{2\alpha}$ group (n=15)	**9-d CIDR-$PGF_{2\alpha}$ group (n=12)**	
Mucus discharge	66.7	66.7	1.00
Vulva swelling	20.0	41.7	0.40
Vulva congestion	13.0	33.3	0.40
Teat engorgement	40.0	8.3	0.10
Decrease in milk yield	20.0	8.3	0.70
Overall estrus expression rate	86.7	75.0	0.70

Table 2. Ovulation and pregnancy rates in buffaloes treated with 7-d versus 9-d CIDR-$PGF_{2\alpha}$ protocols.

Parameters	**Treatment group**		**P-value**
	7-d CIDR-$PGF_{2\alpha}$ (n=15)	**9-d CIDR-$PGF_{2\alpha}$ (n=12)**	
Ovulation rate (%)	86.7	100.0	0.56
Pregnancy rate (%)	20.0	33.3	0.70

Table 3. Effect of various factors on ovulation in buffaloes treated with CIDR-$PGF_{2\alpha}$ protocol.

Factors	Level	No. of buffaloes	Ovulation rate (%)	P-value
Parity	Primiparous	10	100	0.71
	Pluriparous	17	88.2	
BCS	Low (<2.75)	11	100.0	0.63
	Good (≥2.75)	16	87.5	
Endoparasitic infection	Positive	5	80.0	0.8
	Negative	22	95.5	
Anemic condition	Normal	26	92.3	1
	Anemic	1	100.0	
Presence of suckling calf	Yes	14	92.9	1
	No	13	92.3	
Stage of lactation	Up to 100 d postpartum	22	90.9	1
	More than 100 d postpartum	5	100.0	
Ovarian cyclicity status at CIDR insertion	True anestrus (No CL, No DF)	6	66.7	0.07
	Silent estrus with DF>10 mm	19	100.0	
	Silent estrus with CL	2	100.0	

Table 4. Effect of various factors on pregnancy rate in buffaloes treated with CIDR-$PGF_{2\alpha}$ protocol.

Factors	**Level**	**No. of buffaloes**	**Pregnancy rate (%)**	**P-value**
Parity	Primiparous	10	30.0	1
	Pluriparous	17	23.5	
BCS	Low (<2.75)	11	27.3	1
	Good (≥2.75)	16	25.0	
Endoparasitic infection	Positive	5	40.0	0.82
	Negative	22	29.4	
Anemic condition	Normal	26	23.1	0.58
	Anemic	1	100.0	
Presence of suckling calf	Yes	14	21.4	0.90
	No	13	30.8	
Stage of lactation	Upto 100 d postpartum	22	27.3	1
	More than 100 d postpartum	5	20.0	
Ovarian cyclicity status at CIDR insertion	True anestrus (No CL, No DF)	6	0.0	0.02
	Silent estrus (with DF> 10mm)	19	26.3	
	Silent estrus with CL	2	100.0	
Vaginal discharge score at CIDR removal	Clear	8	25.0	0.82
	Mucopurulent	18	27.8	
	Purulent	1	0.0	

Table 5. Size of corpus luteum (CL) on seventh day post-AI in pregnant and non-pregnant buffaloes.

Pregnancy outcome	**No. of buffaloes**	**Mean (±SE) CL size on 7th day post-AI (mm)**	**P-value**
Positive	7	19.6±2.4	0.03
Negative	18	14.9±0.8	

the pregnancy outcome in CIDR-$PGF_{2α}$ treated anestrous buffaloes. This result was supported by the findings of the previous studies that reported that the presence of CL or dominant follicle affected the success of the estrus synchronization protocol (De Rensis and Lopez Gatius, 2007; Stevenson *et al*., 2012). Thus, from the findings of the present study it can be stated that although the estrus can be induced in anestrous buffaloes during low breeding season using CIDR-$PGF_{2α}$ protocol irrespective of ovarian cyclicity status, the ovulation response and the pregnancy outcome depend mainly on the ovarian cyclicity status at the start of the protocol. Other factors such as parity, body condition score, endoparasitic infection, anemic condition, presence of sucking calf and the lactation stage had no significant effect on pregnancy outcome. This lack of effect agreed with findings of a previous study that demonstrated no relationship between BCS and pregnancy outcomes in Italian Mediterranean buffaloes (Giuseppe *et al*., 2006).

Pregnancy outcome among the treated buffaloes was also influenced by the size of the corpus luteum on the seventh day after AI, with larger size of corpus luteum in pregnant buffaloes than in non-pregnant buffaloes. Smaller corpus luteum at one week after AI may not maintain optimal progesterone levels, potentially leading to embryonic loss (Wathes *et al*., 1998; Mann and Lamming, 1999).

CONCLUSION

The duration of the CIDR placement (7-day versus 9-day) had no effect on estrus induction, ovulatory response or pregnancy outcomes in CIDR-$PGF_{2α}$ treated anestrous buffaloes during the low breeding season. Although the estrus was induced in the treated buffaloes irrespective of ovarian cyclicity status at the start of the protocol, the ovulatory response and pregnancy outcome depended on the ovarian cyclicity status at the start of the protocol. Furthermore, the size of the corpus luteum one week after insemination had influence on pregnancy outcomes, with larger size of corpus luteum in pregnant buffaloes than in non-pregnant buffaloes.

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