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EFFICACY OF OMITTING INITIAL GnRH IN ONCE-USED CIDR CO-SYNCH PROTOCOL FOR THE TREATMENT OF ANESTRUS IN BUFFALOES DURING LOW BREEDING SEASON

Gokarna Gautam*, Santosh Adhikari and Shatrughan Shah

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ABSTRACT

Progesterone-based hormonal protocols are effective to treat anestrus in buffaloes. However, most of those protocols are costly, thus, requiring a cost-effective protocol that minimizes handling frequencies. This study, therefore, evaluated the effectiveness of the ‘once-used CIDR co-synch protocol’ with or without initial GnRH for treating anestrus in buffaloes during low breeding season. Anestrous buffaloes (n=29) were divided into two Treatment groups. As a part of the ‘once-used CIDR co-synch protocol,’ the first group (T1, n=18) received an initial GnRH on a random day (day 0) while the second group (T2, n=11) did not receive it. On day 0, the once-used CIDR device was inserted intravaginally in both groups, with GnRH administered in T1 but omitted in T2. On day 7, during CIDR removal, each buffalo received $PGF_{2\alpha}$, and on day 10, GnRH was given, and fixed-timed artificial insemination (FTAI) was done. Ovarian status was assessed on d0 and on 8 to 9-day post-FTAI using transrectal ultrasonography. Plasma progesterone concentrations on 8 to 9-day post-FTAI were determined using ELISA. No significant differences in estrus expression, ovulation and pregnancy rates were observed between two Treatment groups, with overall rates of 93%, 69%, and 34.5%, respectively. However, the overall pregnancy outcome (from FTAI plus natural breeding of buffaloes that returned to estrus) up to 2 months post-FTAI was 48.3%. Parity affected the ovulation among treated buffaloes, with higher (P=0.04) ovulation rate in buffaloes ≤4^{th} parity (79.2%) compared to >4^{th} parity (20%). There were no differences in CL size and plasma progesterone concentrations during 8 to 9-day post-FTAI between two treatment groups. In conclusion, omitting the initial GnRH in ‘once-used CIDR Co-synch protocol’ proved equally effective as the protocol with initial GnRH for the treatment of anestrus in buffaloes during the low breeding season.

Keywords: *Bubalus bubalis*, buffaloes, once-used CIDR, GnRH, anestrous buffalo, low breeding season

INTRODUCTION

Buffalo (*Bubalus bubalis*) farming is an important sector of livestock production in Nepal, serving various purposes such as milk,

Department of Theriogenology, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Chitwan, Nepal, *E-mail: ggautam@afu.edu.np

meat, manure, and draught. Buffalo contributes 57% of the total milk production and 38% of total meat production in the country (MoALD, 2023). However, buffalo production faces constraints due to sluggish reproductive characteristics unlike that of cows (Singh *et al*., 2000; Michael *et al*., 2020). Buffaloes in the regions away from equator exhibit reproductive cyclicity mainly during late summer to early autumn because of decreasing day-length (Zicarelli, 1997). Nepalese buffaloes exhibit distinct breeding patterns across different seasons, with late monsoon, autumn, and early winter being the good breeding seasons (July to December), while spring and early summer were considered low breeding seasons, and January to March are transition breeding seasons (Devkota and Bohora, 2009; Devkota *et al*., 2012; Devkota *et al*., 2023).

Anestrus during low breeding season poses a significant challenge in buffalo production, increasing inter-calving intervals, reducing calf births per annum, and escalating daily farm costs (Kumar *et al*., 2013; Gautam *et al*., 2017; Devkota *et al*., 2023). Various hormonal protocols have been employed with varying success to treat anestrus in buffaloes. Progesterone-based treatments combined with estradiol, prostaglandin, GnRH or hCG, along with timed insemination, are considered more effective during the low-breeding season in buffaloes due to poor responses to other synchronization protocols such as prostaglandin and ovsynch (Naseer *et al*., 2011; de Carvalho *et al*., 2013; Vecchio *et al*., 2013). Among progesterone-based treatments, the CIDR co-synch protocol is one of the widely used protocols in which fixed timed artificial insemination (FTAI) and administration of a second dose of GnRH is made at the same time (Martínez *et al*., 2002), thus reducing the need for frequent animal handling. However, these protocols have been costly to the small-scale buffalo producers because of the high cost of CIDR and double doses of GnRH used in the treatment protocol. Previous studies have shown that the once-used CIDR was equally effective as new CIDR for estrous synchronization in cattle (Colazo *et al*., 2004; Cerri *et al*., 2009; El-Tarabany, 2016). Similarly, a previous study reported comparable pregnancy rates in buffaloes treated with either once used or new CIDR for anestrus treatment (Naseer *et al*., 2011). Utilizing the once-used CIDR can reduce the cost of treating anestrous buffaloes. Furthermore, studies in cattle have indicated that omitting the first GnRH in the 5-day CIDR co-synch protocol did not adversely affect pregnancy compared to the standard protocol (Helguera *et al*., 2018). Thus, utilizing the once-used CIDR and omitting the initial GnRH injection in 'CIDR co-synch protocol' in anestrous buffaloes can be cost-effective and reduce the need for frequent handling of animals. However, the efficacy of omitting the first GnRH in the CIDR co-synch protocol for treating anestrus in buffaloes during low breeding season has not been studied yet. The objectives of this study were, therefore, to evaluate the efficacy of omitting the initial GnRH in the once-used CIDR co-synch protocol for treating anestrus in buffaloes during the low breeding season and to determine the effect of various factors on the efficacy of such protocol.

MATERIALS AND METHODS

Animals

This study was conducted on 32 anestrous Murrah crossbred buffaloes from four commercial farms in the western Chitwan district, which lies in the central southern part of Nepal, having a sub-tropical climate characterized by hot and humid summer and cool, dry winter. Buffaloes in the trial were calved at least 70 days prior and had not shown estrous signs since last calving. Parity (mean±SE) of buffaloes used in the study was 3.38±0.27 (range: 1 to 8).

Examination of buffaloes was performed to determine their body condition score (BCS) (1-5 scale with 0.25 increment, Ferguson *et al*., 1994). Only the buffaloes having BCS≥2.0 were included in the study. BCS (mean±SE) of buffaloes used in the study was 2.72±0.09 (range: 2.0 to 4.0). Transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co. Ltd, China) of reproductive organs was done using 7.5 to 10 MHz transducer to determine the ovarian cyclicity status and to rule out anatomical defects or anomalies of reproductive tract, if any.

All buffaloes in the study received a broad-spectrum anthelmintic comprising oxyclonazide and levamisole (Nilzan, Virbac, India) at the recommended dosage of 1 ml per 3 kg body weight. Following deworming, a mineral-vitamin mixture supplement (Agrimin Forte, Virbac, India) was fed to each buffalo at the dose rate of 50 gm per day for three weeks prior to commencing the hormonal protocols.

Preparation of once-used CIDR

CIDR (Controlled Internal Drug Release, Eazi-Breed, Zoites, Australia, containing 1.9 gm of progesterone) devices that were previously inserted into the vagina of buffaloes for a duration of 7 days were utilized in this research trial. After removal from vagina, the devices were immersed in a bucket filled with soapy water for 5 minutes. Subsequently, each CIDR device was thoroughly washed with tap water to eliminate any attached mucus, dung, or debris. Afterward, the devices were air-dried and packed into clean plastic bags. One day before the use in buffalo, the air-dried CIDR device was autoclaved at 121^{o}C and 720 mmHg pressure for 20 minutes as described previously (Zuluaga and Williams, 2008) and a meticulous visual inspection was conducted on each device to confirm its physical integrity, as recommended by Cerri *et al.* (2009).

Hormonal treatment

Three buffaloes were excluded from the study because their owners were not interested in keeping them for the duration of the experiment. The remaining buffaloes (n=29) were divided into two Treatment groups based on whether GnRH was administered on day zero (day 0) of the 'once-used CIDR co-synch protocol' (Figure 1). Buffaloes in Treatment 1 (T1, n=18) received an intramuscular (i.m.) injection of GnRH analogue buserelin acetate 20 µg (Gynarich, Intas Pharmaceuticals, India) on day 0, while those in Treatment 2 (T2, n=11) did not receive GnRH on day 0; remaining procedures were same in both Treatment groups. Briefly, a once-used CIDR device was inserted into the vagina of each buffalo on a random day (day 0) and removed on day 7. $PGF_{2\alpha}$ analogue cloprostenol 500 µg (Cloprochem, Interchem, the Netherlands) was injected i.m. on day 7 at the time of CIDR removal. On day 10 (70±2 h after CIDR removal), FTAI was performed and simultaneously GnRH (buserelin acetate 20 µg) was injected.

Estrus detection

Estrus detection was based on external estrous signs as well as the changes detected by transrectal palpation during FTAI. After CIDR removal until FTAI, treated buffaloes were carefully observed for external estrous signs such as bellowing, mucus discharge, teat engorgement, vulva swelling, congestion of vulval mucous membrane and decreased milk yield. During FTAI, changes in cervix and uterine horns associated with estrus such as cervical relaxation and uterine contractions were assessed.

Determination of ovulation and plasma progesterone concentrations

Ovulation was confirmed based on the presence of corpus luteum (CL) on 8 to 9 day after FTAI as detected by transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) using a 7.5 to 10MHz transducer.

On 8 to 9 day after FTAI, blood samples were collected from the jugular vein using a vacutainer and sterile needle into EDTA tubes and immediately placed in an icebox. Plasma was harvested by centrifuging blood sample 1,000 x g for 10 minutes and plasma sample was stored at -70^{o}C until ELISA. Progesterone concentration in the plasma sample was measured using a progesterone ELISA kit (Abnova, Taiwan). The sensitivity of the assay was 8.57 pg/ml. Intra-assay and iter-assay CVs (%) were 4.9 to 7.6 and 2.7 to 6.8, respectively. Cross reactivity with related steroid compounds was <1%.

Pregnancy diagnosis

Early pregnancy diagnosis (PD) was conducted during 31 to 33 day post FTAI using transrectal ultrasonography. Final PD was performed during 80-90 d post FTAI using the trans-rectal palpation. Any pregnancy loss occurring between the early PD and final PD was recorded.

Statistical analyses

Data were analyzed using Microsoft Excel (version 2010) and SPSS (version 25.0). Estrus expression, ovulation, and pregnancy rates between the two treatment groups were compared using Chi-square test; Fisher exact probability test was used if the expected frequency was less than 5 in more than 20% of cells. Effect of various factors on ovulation and pregnancy outcome was also analyzed using Chi-square test or Fisher exact probability test whichever appropriate. For the analysis of factors affecting ovulation and pregnancy outcomes, the buffaloes were stratified based on parity (≤4th parity and >4th parity), body condition score (low BCS i.e. <2.75 or good BCS i.e. ≥2.75), suckling by the calf (yes or no), lactation stage (within 100 days or beyond 100 days postpartum), endoparasitic infection (yes or no), ovarian cyclicity status at the start of the protocol (cyclic or non-cyclic), estrus expression before FTAI (yes or no). A probability value ≤0.05 was regarded as significant whereas 0.05<P≤0.1 was considered to have tendency effect.

RESULTS

Estrus expression and ovulation rates

There was no significant difference in the estrus expression rate between two Treatment groups whether GnRH was administered on day 0 or not (Table 1). The proportion of buffaloes showing each estrous sign also did not differ between the two Treatment groups. The overall estrus expression rate based on visual inspection and palpation findings was 93%. Mucus discharge at the time of FTAI was the most frequently (44.8%) and bellowing was the least frequently (6.9%) observed external sign of estrus.

Likewise, there was no significant difference (P=0.69) in the ovulation rate between the two Treatment groups; it was 72.2% (13/18) in ‘GnRH on Day 0’ group and 63.6% (7/11) in ‘no

GnRH on Day 0’ group. Overall, 69% (20/29) buffaloes had ovulation in response to once-used CIDR Co-synch protocol.

Pregnancy rate

Pregnancy rate from FTAI was not significantly different between two Treatment groups, whether GnRH was administered on day 0 (27.7%) or not (45.4%), as part of the CIDR co-synch protocol (Table 2). In total, 9 out of 19 non-pregnant buffaloes returned to estrus within two months of FTAI and were bred naturally with bull, and ultimately 4 buffaloes became pregnant. Thus, the overall pregnancy rate resulting from FTAI and natural breeding of buffaloes returning to estrus within two months of FTAI was 48.3%, which was not significantly different between GnRH on Day 0 group (38.9%) and no GnRH on Day 0 group (63.6%).

CL size and plasma progesterone concentration on 8 to 9 d post-FTAI in buffaloes treated with once-used CIDR co-synch protocol with or without initial GnRH

Total 13 buffaloes in ‘GnRH on Day 0’ group and 7 buffaloes in ‘no GnRH on Day 0’ group had corpus luteum (CL) present on 8 to 9 d following FTAI; size of the CL (mean±SE) did not differ between two Treatment groups. Furthermore, there was no difference in plasma progesterone concentrations on 8 to 9 d post-FTAI between the two Treatment groups (Table 3).

Effect of various factors on ovulation response in anestrous buffaloes subjected to once-used CIDR co-synch protocol

As there was no significant difference in ovulation rate between two treatment groups, for analyzing the effect of various factors on ovulation, all the buffaloes were pooled together (Table 4). Among the various factors analyzed, only the parity had a significant effect on ovulation. The ovulation rate was higher (P=0.04) in buffaloes with ≤4th parity than in those with >4th parity.

Effect of various factors on pregnancy outcome in anestrous buffaloes subjected to once-used CIDR co-synch protocol

Since there was no significant difference in pregnancy rate from FTAI between two Treatment groups, therefore, for analyzing the effect of various factors on pregnancy outcome, all the buffaloes were pooled together (Table 5). None of the factors considered in the analysis had a significant effect on pregnancy outcome from FTAI.

DISCUSSION

Implementing an estrous synchronization protocol that reduces the need for frequent animal handling and treatment expenses, while maintaining optimal pregnancy outcomes, is crucial for efficient buffalo reproductive management. Previous studies in both cattle (Cerri *et al*., 2009) and buffaloes (Naseer *et al*., 2011; Carvalho *et al*., 2014) have shown that once-used CIDR was as effective as new CIDR. Therefore, to reduce the cost of treatment, the present study utilized the once-used CIDR and assessed the efficacy of omitting initial GnRH in once-used CIDR co-synch protocol for the treatment of anestrus in buffaloes during low breeding season. Because of co-synch protocol, each buffalo was handled only three times. Buffaloes in the CIDR co-synch protocol were divided into two groups: one group received GnRH on day 0 while the other group did not receive GnRH on d0.

In the present study, there was no significant difference in estrus expression rate between the two treatment groups. The overall estrus expression was based on manifestation of any of the external or internal heat signs. The most observed visual indication of estrus was vaginal mucus discharge (44.8%), followed by vulva congestion (27.6%). To our knowledge, there were no previous studies regarding the observation of various estrous signs in buffaloes under this protocol. Overall estrus expression rate among the treated buffaloes was 93%, which was almost similar to that (73.1% and 88.5%, respectively) demonstrated by previous studies (Shah *et al*., 2017; Naseer *et al*., 2011) that applied CIDR co-synch protocol in buffaloes. However, the overall estrus expression rate in the present study was higher than that (16.7%) reported by a previous study (Zaabel *et al*., 2018) that applied used-CIDR 7 days + $PGF_{2α}$ + GnRH protocol. From this study, it can be inferred that the omitting the initial GnRH in CIDR co-synch protocol was as effective as including initial GnRH for inducing estrus in anestrous buffaloes during low breeding season.

The ovulation rate also did not differ between two Treatment groups. The overall ovulation rate among treated buffaloes was 69%, comparable to the 75% reported by Carvalho *et al.* (2007) using a progesterone device inserted for 9 days during the low breeding season. However, it was slightly lower as compared to the 81.8% ovulation rate as reported by a previous study that used a once-used CIDR device for 9 days (Carvalho *et al*., 2014) and lower than the 85% ovulation rate as reported by Haider *et al.* (2021) that used CIDR co-synch protocol. In the present study, ovulation was confirmed by the presence of the corpus luteum during 8 to 9 d post-FTAI, while in the aforementioned studies, it was confirmed using daily ultrasound monitoring from the day of CIDR removal until ovulation. This difference may explain the higher ovulation rate in those studies. The absence of significant differences between the two Treatment groups in terms of ovulation rate suggests that the omitting GnRH on day 0 was equally effective as the use of GnRH on day 0 for inducing ovulation in anestrous buffaloes.

There was no significant difference in pregnancy rate whether the GnRH was used on day 0. This result was similar to the finding of a previous study in dairy heifers in which the pregnancy rate did not differ regardless of whether using (67.9%) or not using (71.4%) GnRH on day 0 at PRID insertion in a 5-day Co-synch PRID protocol (Colazo and Ambrose, 2011). The authors in that study justified the similar pregnancy rate with or without the use of GnRH on day 0 due to the ovulatory response to the first GnRH treatment; 25% of heifers ovulated with the first GnRH treatment, and those ovulated heifers had a lower pregnancy rate than those that did not ovulate with the first GnRH. The 20% of ovulatory animals after the first GnRH injection showed a tendency to have partial regression of CL in response to $PGF_{2α}$ (Colazo and Ambrose, 2011). Determining the ovulatory response to the first GnRH was beyond the scope of the present study. However, the ovulatory response to the second GnRH was similar between the two Treatment groups. Furthermore, the corpus luteum size and the plasma progesterone concentrations 8 to 9 day post FTAI did not differ between two Treatment groups. Thus, it can be speculated that no difference in pregnancy rates between two treatment groups might be attributed to the similarities between two groups in terms of estrus expression rate, ovulation rate, corpus luteum size and the plasma progesterone concentrations during early diestrus. There were no previous studies

Table 1. Estrus expression rate in buffaloes treated with once-used CIDR co-synch protocol with or without initial GnRH.

Estrous signs Overall (n=29)		**Proportion of buffaloes showing particular estrous sign (%)**			**P-value**
		Overall (n=29)	**GnRH on d0 (n=18)**	**No GnRH on d0 (n=11)**	
External estrous signs	Bellowing	6.9	5.6	9.1	0.69
	Mucus discharge	44.8	44.4	45.5	0.96
	Teat engorgement	13.8	16.7	9.1	0.98
	Vulva swelling	24.1	27.8	18.2	0.9
	Vulva congestion	27.6	33.3	18.2	0.66
	Decrease in milk yield	20.7	27.8	9.1	0.47
Estrus-related transrectal palpation findings during FTAI	Relaxation of cervix	86.2	88.9	81.8	0.60
	Uterine contraction	69.0	66.7	72.7	0.99
Overall estrus expression rate*		93.0	94.4	90.9	0.70

*Buffalo showing any one of the above signs was considered to be in estrus.

Table 2. Pregnancy rate in buffaloes treated with once-used CIDR co-synch protocol with or without initial GnRH.

Parameters	**Treatment group**			**P-value**
	Overall (n=29)	**GnRH on d0 (n=18)**	**No GnRH on d0 (n=11)**	
No. of buffaloes treated	29	18	11	
No. of buffaloes pregnant from FTAI (A)	10	5	5	
Pregnancy rate from FTAI (%)	34.5	27.7	45.4	0.43
No. of non-pregnant buffaloes from FTAI	19	13	6	
No. of buffaloes returned to estrus and bred with bull within 2 months of FTAI	9	6	3	
No. of buffaloes returned to estrus, bred with bull within 2 months of FTAI and became pregnant (B)	4	2	2	
Overall pregnancy rate from FTAI+natural breeding within 2 months of FTAI (A+B) (%)	48.3 (14/29)	38.9 (7/18)	63.6 (7/11)	0.20

Table 3. CL size and plasma progesterone concentration on 8 to 9 d post-FTAI in buffaloes treated with once-used CIDR co-synch protocol with or without initial GnRH.

Parameters on 8 to 9 d post FTAI	Treatment group		P-value
	GnRH on d0 (n=13)	No GnRH on d0 (n=7)	
CL size, cm (mean±SE)	1.66±0.06	1.85±0.11	0.12
P4 concentration (ng/ml) (mean±SE)	3.54±0.53	3.13±0.52	0.40

Table 4. Effect of various factors on ovulation response in anestrous buffaloes subjected to the once-used CIDR co-synch protocol.

Factors	Level	No. of buffaloes (n=29)	Ovulation rate (%)	P-value
Parity	≤4th	24	79.2 (19/24)	0.04
	>4th	5	20.0 (1/5)	
BCS	Good (≥2.75)	18	72.2 (13/18)	0.93
	Poor (<2.75)	11	63.6 (7/11)	
Suckling by the calf	Yes	20	65.0 (13/20)	0.81
	No	9	77.7 (7/9)	
Stage of lactation	Within 100 d pp	8	62.5 (5/8)	0.67
	Beyond 100 d pp	21	71.4 (15/21)	
Endoparasitic infection	Yes	10	60.0 (6/10)	0.73
	No	19	73.7 (14/19)	
Ovarian status at the start of protocol	Cyclic (CL present)	10	90.0 (9/10)	0.17
	Non-cyclic (No CL)	19	57.9 (11/19)	
Estrus expression before FTAI	Yes	18	66.7 (12/18)	0.99
	No	11	72.7 (8/11)	

Table 5. Effect of various factors on pregnancy outcome in anestrous buffaloes subjected to the once-used CIDR co-synch protocol.

Factors	Level	No. of buffaloes (n=29)	Pregnancy rate (%)	P-value
Parity	≤4th	24	37.5 (9/24)	0.63
	>4th	5	20.0 (1/5)	
BCS	Good (≥2.75)	18	27.8 (5/18)	0.43
	Poor (<2.75)	11	45.5 (5/11)	
Suckling by the calf	Yes	20	40.0 (8/20)	0.43
	No	9	22.2 (2/9)	
Stage of lactation	Within 100 d pp	8	25.0 (2/8)	0.67
	Beyond 100 d pp	21	38.1 (8/21)	
Endoparasitic infection	Yes	10	40.0 (4/10)	0.70
	No	19	31.6 (6/19)	
Ovarian status at the start of protocol	Cyclic (CL present)	10	40.0 (4/10)	0.70
	Non-cyclic (No CL)	19	31.6 (6/19)	
Estrus expression before FTAI	Yes	18	38.9 (7/18)	0.69
	No	11	27.3 (3/11)	

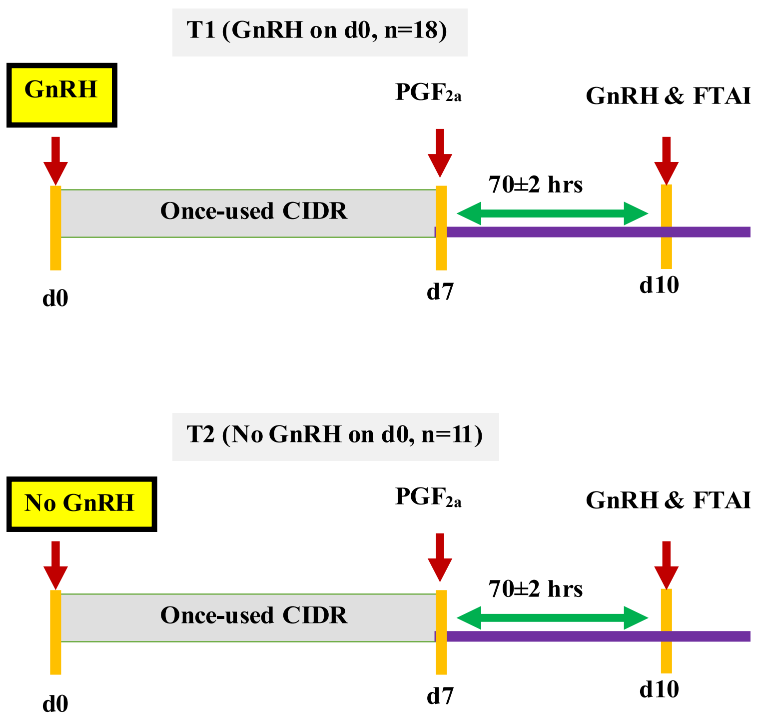

Figure 1. Once-used CIDR co-synch protocol with (T1, n=18) or without (T2, n=11) the use of initial GnRH for the treatment of anestrus in buffaloes during low breeding season.

in buffaloes under the CIDR co-synch protocol omitting the first GnRH injection. The overall pregnancy rate of FTAI using once-used CIDR was 34.5%, which was higher than that (0% and 19%, respectively) reported by previous studies that used once-used CIDR for seven days (Zaabel *et al*., 2009; Kandel *et al*., 2022), but lower than that (49.1%) obtained during the low breeding season in buffaloes (Carvalho *et al*., 2007). Among 19 buffaloes not pregnant from FTAI, nine returned to estrus within two months of FTAI and four became pregnant from natural breeding. Consequently, the overall pregnancy rate from FTAI and natural breeding during subsequent estrus within two months of FTAI was 48.3% (14/29), again showing the similarity between the two Treatment groups.

This study also assessed the effect of various factors on ovulation and pregnancy outcomes among the treated buffaloes. Since there were no differences between the two treatment groups in terms of the ovulation rate and pregnancy rate, for analyzing the effect of various on ovulation and pregnancy outcome, all buffaloes were pooled together. Among the various factors examined, only the parity had significant effect on ovulation rate; younger (≤4th parity) buffaloes had significantly higher ovulation rate than the older (>4th parity) buffaloes. This could be attributed to the lower levels of progesterone in older animals during CIDR treatment compared to younger animals (Cerri *et al*., 2009). The sudden decline in systemic progesterone concentration stimulates the growth and maturation of the dominant follicle and induction of LH receptors, resulting in a higher ovulation rate (de Carvalho *et al*., 2016). However, none of the factors examined in the present study had significant effect on pregnancy outcomes among the treated buffaloes, which was similar to the findings of previous studies that demonstrated no significant effect of parity, BCS, suckling status, stage of lactation, endoparasitic infection and ovarian cyclicity status at the initiation of protocol on pregnancy outcome from FTAI using CIDR-co-synch, CIDR-synch and ovsynch protocols during low breeding season (Kandel *et al*., 2022; Gautam *et al*., 2023).

CONCLUSION

This study revealed that omitting the initial GnRH in 'once-used CIDR Co-synch protocol' proved equally effective as the protocol with initial GnRH for the treatment of anestrus in buffaloes during the low breeding season. Rates of estrus expression, ovulation and pregnancy were affected neither by the protocols used nor the other various factors except that the ovulation was affected by the parity of the buffaloes.

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REFERENCES

Carvalho, N.A.T., E.M. Nagasaku, F.S. Vannucci, L.M. Toledo and P.S. Baruselli. 2007. Ovulation and conception rates according intravaginal progesterone device and hCG or GnRH to induce ovulation in buffalo during the off breeding season. *Ital. J. Anim.*

Sci., **6**(Sup. 2): 646-648. DOI: 10.4081/ijas.2007.s2.646

Carvalho, N.A.T., J.G. Soares, D.C. Souza, F.S. Vannucci, R. Amaral, J.R.G. Maio, J.N.S. Sales, M.F. Sá Filho and P.S. Baruselli. 2014. Different circulating progesterone concentrations during synchronization of ovulation protocol did not affect ovarian follicular and pregnancy responses in seasonal anestrous buffalo cows. *Theriogenology*, **81**(3): 490-495. DOI: 10.1016/j.theriogenology.2013.11.004

Cerri, R.L., H.M. Rutigliano, R.G.S. Bruno and J.E. Santos. 2009. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. *Anim. Reprod. Sci*., **110**(1-2): 56-70. DOI: 10.1016/j.anireprosci.2007.12.005

Colazo, M.G. and D.J. Ambrose. 2011. Neither duration of progesterone insert nor initial GnRH treatment affected pregnancy per timed-insemination in dairy heifers subjected to a Co-synch protocol. *Theriogenology*, **76**(3): 578-588. DOI: 10.1016/j.theriogenology.2011.03.013

Colazo, M.G., J.P. Kastelic, P.R. Whittaker, Q.A. Gavaga, R. Wilde and R.J. Mapletoft. 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. *Anim. Reprod. Sci*., **81**(1-2): 25-34. DOI: 10.1016/j.anireprosci.2003.09.003

De Carvalho, N.A.T., J.G. Soares, D.C. de Souza, J.R.G. Maio, J.N.S. Sales, B.M. Júnior, R.C. Macari and P.S. Baruselli. 2013. Use of intravaginal progesterone devices during eight or nine days in the ovulation synchronization protocol for TAI in buffaloes during the non breeding season. *Buffalo Bull*., **32**(SI2): 532-536. Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/286579

De Carvalho, N.A.T., J.G. Soares and P.S. Baruselli. 2016. Strategies to overcome seasonal anestrus in water buffalo. *Theriogenology*, **86**(1): 200-206. DOI: 10.1016/j.theriogenology.2016.04.032

Devkota, B. and T.P. Bohora. 2009. Effects of season on pregnancy rates in water buffaloes of southern Nepal evaluated by using different estrus synchronization protocols during active season and low breeding season. *Pak. J. Zool*., **9**: 763-770.

Devkota, B., S. Shah and G. Gautam. 2023. Reproduction and fertility of buffaloes in Nepal. *Animals*, **13**(1): 70. DOI: 10.3390/ani13010070

Devkota, B., T.P. Bohora and N. Yamagishi. 2012. Seasonal variation of anestrus conditions in buffaloes (*Bubalus bubalis*) in southern Nepal. *Asian J. Anim. Vet. Adv*., **7**(9): 910-914. DOI: 10.3923/ajava.2012.910.914

El-Tarabany, M.S. 2016. The efficiency of new CIDR and once-used CIDR to synchronize ovulation in primiparous and multiparous Holstein cows. *Anim. Reprod. Sci*., **173**: 29-34. DOI: 10.1016/j.anireprosci.2016.08.006

Ferguson, J.D., D.T. Galligan and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci*., **77**(9): 2695-2703. DOI: 10.3168/jds.S0022-0302(94)77212-X

Gautam, G., H. Adhikari, B. Devkota and S. Singh. 2023. Effectiveness of progesterone-based estrous synchronization protocol for the treatment of anestrous buffaloes during low breeding season. *In The Proceedings of 1st National Biotechnology Conference*, Kathmandu University, Nepal.

Gautam, G., R.R. Gyawali, B. Nath and S. Pant. 2017. Characterization and treatment of infertility in buffaloes of hills and terai regions of mid Nepal, p. 98. *In The Proceedings of International Buffalo Symposium 2017,* Chitwan, Nepal.

Haider, S., G.A. Chishti, M.U. Mehmood, M.A. Jamal, K. Mehmood, M. Shahzad and M.Z. Tahir. 2021. The effect of GnRH administration/insemination time on follicular growth rate, ovulation intervals, and conception rate of *Nili Ravi* buffalo heifers in 7-day-CIDR Co-synch. *Trop. Anim. Health Pro.*, **53**(6): 558. DOI: 10.1007/s11250-021-03003-8

Helguera, I.L., P. Whittaker, A. Behrouzi, R.J. Mapletoft and M.G. Colazo. 2018. Effect of initial GnRH and time of insemination on reproductive performance in cyclic and acyclic beef heifers subjected to a 5-d Co-synch plus progesterone protocol. *Theriogenology*, **106**: 39-45. DOI: 10.1016/j.theriogenology.2017.10.001

Kandel, S., G. Gautam, B. Devkota and S. Singh. 2022. Comparative effectiveness of new and once used CIDR for the treatment of anestrus buffaloes during low breeding season using CIDR co-synch protocol. *In The Proceedings of the Souvenir of 14th National Veterinary Conference (VETCON 22),* Pokhara, Nepal.

Kumar, P.R., S.N. Shukla and R.D. Purkayastha. 2013. Economical analysis of the estimated cost of management of anestrus buffaloes under field conditions using different hormonal and non-hormonal strategies. *J. Anim. Health Pro.*, **1**(4): 39-41.

Martínez, M.F., J.P. Kastelic, G.P. Adams, R.B. Cook, W.O. Olson and R.J. Mapletoft. 2002. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. *Theriogenology*, **57**(3): 1049-1059. DOI: 10.1016/S0093-691X(01)00682-3

Michael, J.D., S.S. Ghuman, G. Neglia, G.V. Della, P.S. Baruselli, L. Zicarelli, J.A. Visintin, M. Sarkar and G. Campanile. 2020. Exogenous and endogenous factors in seasonality of reproduction in buffalo: A review. *Theriogenology*, **150**(1): 186-192. DOI: 10.1016/j.theriogenology.2020.01.044

MALD. 2022. *Statistical Information on Nepalese Agriculture 2020/21.* Ministry of Agriculture and Livestock Development, Government of Nepal, Kathmandu, Nepal. p. 3-4.

Naseer, Z., E. Ahmad, J. Singh and N. Ahmad. 2011. Fertility following CIDR based synchronization regimens in anoestrous *Nili-Ravi* buffaloes: CIDR based synchronization regimens in anestrous *Nili-Ravi* buffaloes. *Reprod. Domest. Anim.*, **46**(5): 814-817. DOI: 10.1111/j.1439-0531.2010.01746.x.

Singh, J., A.S. Nanda and G.P. Adams. 2000. The reproductive pattern and efficiency of female buffaloes. *Anim. Reprod. Sci.*, **60**(60-61): 593-604. DOI: 10.1016/s0378-4320(00)00109-3

Vecchio, D., P. Rossi, G. Neglia, V. Longobardi, A. Salzano, G. Bifulco and G. Campanile. 2013. Comparison of two synchronization protocols for timed artificial insemination in acyclic Italian Mediterranean buffalo cows out of the breeding season. *Buffalo Bull.*, **32**(SI2): 479-479.

Available on: https://kukrdb.lib.ku.ac.th/journal/BuffaloBulletin/search_detail/result/286563

Zaabel, S.M., A.O. Hegab, A.E. Montasser and H. El-Sheikh. 2009. Reproductive performance of anestrous buffaloes treated with CIDR. *Anim. Reprod.*, **6**(3): 460-464.

Zicarelli, L. 1997. Reproductive seasonality in buffalo. p. 29-52. *In The Proceedings of the 3rd Course on Biotechnology of Reproduction in Buffaloes*, Caserta, Italy.

Zuluaga, J.F. and G.L. Williams. 2008. High-pressure steam sterilization of previously used CIDR inserts enhances the magnitude of the acute increase in circulating progesterone after insertion in cows. *Anim. Reprod. Sci.*, **107**(1-2): 30-35. DOI: 10.1016/j.anireprosci.2007.06.006