Research Article

Incorporation of CIDR into Ovsynch Protocol Improved the Reproductive Performance of Anestrous Buffaloes during Low Breeding Season

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Abstract | A study was conducted to determine the effectiveness of CIDR (Controlled Internal Drug Release) in Ovsynch protocol for the treatment of anestrus in buffaloes during low breeding season (April to June). Buffaloes in Group T1 (Ovsynch, n=25) received GnRH on a random day (d0), $PGF_{2\alpha}$ on d7, GnRH on d9, followed by fixed timed artificial insemination (FTAI) at 16-20 hours after second GnRH. Buffaloes in Group T2 (CIDR-synch, n=30) were treated as in group T1 except that a CIDR containing 1.9 gm progesterone was inserted into the vagina on d0 and removed on d7. Ovarian cyclicity status during CIDR insertion and the presence of corpus luteum (CL) on 8-9 day after FTAI were determined using transrectal ultrasonography. Estrus expression rate (96.7% vs 68%; P=0.008) and ovulation rate (93.3% vs 76%; P=0.10) were higher in CIDR-synch than in Ovsynch group. Although pregnancy rate from FTAI did not differ between CIDR-synch (33.3%) and Ovsynch group (16%), overall pregnancy rate obtained from FTAI plus natural breeding within one month after FTAI was higher (P=0.04) in CIDR-synch (46.7%) than in Ovsynch group (20%). Pregnancy outcome from FTAI in both treatment groups was not affected by the ovarian cyclicity status at the initiation of the protocol. In conclusion, incorporation of CIDR into Ovsynch protocol improved the reproductive performance of anestrous buffaloes during low breeding season.

Keywords | Buffalo, Low breeding season, CIDR, Ovsynch, Pregnancy, Corpus luteum

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INTRODUCTION

Buffalo (*Bubalus bubalis*) can serve as a reliable living bank in Asian countries as the poor farmer can have their buffalo as an easily convertible currency to serve their immediate needs with optimum benefit through milk, meat, hide, manure, draught power and even the horn and hair (Nanda *et al*., 2003). The buffalo is the most important livestock species in Nepalese agricultural system as it contributes 57.2% of the total milk production and 36.1% of the total meat production in the nation (MoALD, 2022). However, the milk production efficiency of buffaloes is quite low mainly due to sub-fertility and infertility characterized by delayed sexual maturity, prolonged postpartum anestrous period, poor estrus expression and seasonal breeding pattern (Shah *et al*., 1990; Nanda *et al*., 2003; Barile, 2005; Devkota and Bohora, 2009; Kumar *et al*., 2012; Chaudhari *et al*., 2012).

Seasonality of reproduction was the characteristic feature of the buffaloes in the countries away from the equator, which was characterized by the active breeding activities during the months of short day length (fall and winter) and the cessation of sexual activity during the months of longer day length (summer) also known as summer anestrus (Qureshi *et al*., 1999; Neglia *et al*., 2003; Presicce *et al*., 2004; De Rensis and Lopez-Gatius, 2007; Zicarelli, 2007; Devkota and Bohora, 2009; Das and Khan, 2010; Kumar *et al*., 2012; D'Occhio *et al*., 2020). Seasonal breeding pattern was the one of the major causes of poor reproductive efficiency in Nepalese buffaloes with April to June as the low breeding season (Devkota and Bohora, 2009).

A number of hormonal protocols used in dairy cows have been tried to improve reproductive performance of anestrous buffaloes during low breeding season, however with poor to moderate efficacy (De Rensis *et al*., 2005; Sah and Nakao, 2010; Carvalho *et al*., 2011, 2013; Devkota *et al*., 2013; Warriach *et al*., 2015). As in cattle, the Ovsynch protocol is one of the most commonly used ovulation synchronization protocols in buffalo that allows fixed time artificial insemination (Paul and Prakash, 2005; Carvalho *et al*., 2007, 2013; Warriach *et al*., 2008, 2015) however, the effectiveness of it depends on the status of ovarian cyclicity (Pursley *et al*., 1997; Moreira *et al*., 2000; Stevenson *et al*., 2006; Souza *et al*., 2007). Ovsynch protocol was effective specially in cyclic animals (Devkota and Bohora, 2009; Nowicki *et al*., 2017) but less effective in non-cyclic animals (De Rensis *et al*., 2005; Karen and Darwish, 2010; Malik *et al*., 2011). For the treatment of anestrous buffaloes with inactive ovaries, the progesterone-releasing intravaginal devices such as CIDR (controlled internal drug release) and PRID (progesterone releasing intravaginal device) have been used in conjunction with Ovsynch regimens (Carvalho *et al*., 2011; Barile, 2012; Yendraliza *et al*., 2015). Although Ovsynch was tried in Nepalese buffaloes during good breeding season (Kharel *et al*., 2017; Shah *et al*., 2017; Devkota *et al*., 2021) and CIDR-synch (incorporation of CIDR in Ovsynch) tried during low breeding season (Devkota *et al*., 2021; Lamsal *et al*., 2017), the studies comparing the efficacy of Ovsynch vs CIDR-synch in anestrous buffaloes during low breeding season in Nepalese water buffaloes were lacking. As most of the buffaloes during low breeding season are non-cyclic (Singh *et al*., 2000; Nanda and Nakao, 2003), in the present study it was hypothesized that the incorporation of CIDR in the Ovsynch protocol would be effective to resume estrous cyclicity and to improve reproductive performance in anestrous buffaloes during low breeding season. Therefore, the objectives of the present study was to determine the effectiveness of CIDR-synch protocol as compared to the standard Ovsynch protocol for the treatment of anestrus in buffaloes during low breeding season.

MATERIALS AND METHODS

The use of animals in this study was in accordance with the ARRIVE (Animal research: Reporting of *in vivo* Experiments) guidelines.

Animals

This study was conducted in anestrous Murrah cross-bred buffaloes from six commercial buffalo farms located in Chitwan district, which is a plain region in the mid southern part of Nepal and it has a sub-tropical climatic condition with hot humid summer and cool dry winter. Only the buffaloes that were beyond 70 days postpartum with no anatomical defects or anomalies in their reproductive tract, that did not express estrus since last calving and that had body condition score (BCS) >2.25 were included in the trial. All the buffaloes were reared in similar management conditions with 24 hours tie-stall barn, hand milking twice daily and feeding seasonally available roughages and farm-prepared concentrate feed consisting of maize, rice bran, wheat bran, mustard oil-cake and commercial vitamin-mineral mixtures. The purposive sampling technique was used for selecting buffaloes until the number of buffaloes reached to 60. Parity of buffaloes (mean ± S.E.) was 3.35 ± 1.31 (Range: 1-8). Systemic recording of milk yield was not kept by the owners. Based on the approximate values provided by the buffalo owners, the milk yield of the buffaloes in the experiment ranged from 4 to 9 liters per day.

Buffaloes in the trial were clinically examined to assess their BCS (1-5 scale with 0.25 increment, Ferguson *et al*., 1994). BCS (mean ± S.E.) was 3 ± 0.45 (Range: 2.5-4). Transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) of reproductive organs was performed using 10 MHz transducer to determine the ovarian cyclicity status and to rule out the anatomical defects or anomalies of the reproductive system, if any. All the buffaloes in the study were drenched with broad-spectrum anthelmintic (oxyclozanide + levamisole, Zanide L Forte, QMED Pharmaceuticals, Nepal) at the recommended doses. After deworming, the mineral-vitamin mixture supplement (Minfa Gold- Intas Pharmaceuticals, India) was provided to all the buffaloes at the dose rate of 50 gm per animal per day for 20 days. After that the hormonal protocols were applied.

Hormonal treatment protocols

Five buffaloes were excluded before the commencement of hormonal protocols because of owners unwillingness to include those buffaloes in the trial. Remaining anestrous buffaloes (n= 55) were randomly divided into two treatment groups (Figures 1, 2). In Group T1 (Ovsynch, n= 25), Ovsynch protocol was applied in which the buffaloes were injected intramuscular (i.m.) with GnRH analogue, buserelin acetate 20 µg (Gynarich, Intas Pharmaceuticals, India) on a random day considered as day zero (d0), $PGF_{2\alpha}$ analogue, cloprostenol 500 µg (Cloprochem, Interchem, Netherlands) on d7, followed by second GnRH injection on d9 and fixed timed artificial insemination (FTAI) done at 16-20 hours after the second GnRH injection. In Group T2 (CIDR-synch, n=30), all the buffaloes were treated as in T1 except a CIDR (Controlled Internal Drug Release, Eazi-Breed, Zoites, Australia) device containing 1.9 gm of progesterone was inserted into the vagina of each buffalo on d0 and removed on d7 at the time of $PGF_{2\alpha}$ injection. All of the buffaloes in both treatment groups were inseminated artificially by the first author using frozen thawed semen of Murrah buffaloes from the National Livestock Breeding Office, Pokhara, Nepal.

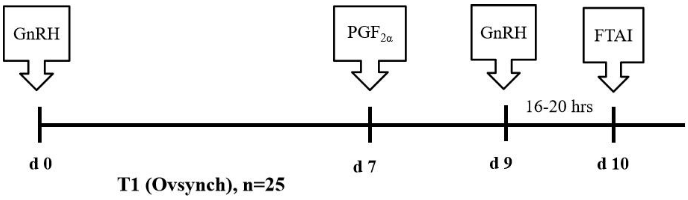

Figure 1: Hormonal protocol used in buffaloes in group T1 (Ovsynch, n=25).

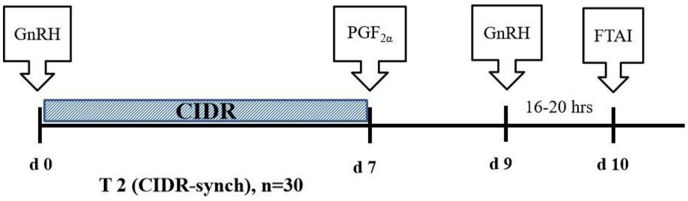

Figure 2: Hormonal protocol used in buffaloes in group T2 (CIDR-synch, n=30).

Estrus detection

Starting after $PGF_{2\alpha}$ injection until FTAI, the buffaloes in both treatment groups were observed for the external estrous signs (mucus discharge from vulva, bellowing, vulva swelling, teat engorgement and decrease in milk yield) at least three times a day. Estrus related changes in the reproductive organs (uterine contraction) were determined at the time of FTAI. Buffalo was considered to be in estrus if it showed any of the external estrous signs or uterine contraction.

Ovulation confirmation and corpus luteum (CL) size determination

Ovulation was confirmed based on the presence of CL on 8-9 days after FTAI examined by transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) using 10 MHz transducer. Two diameters of CL at perpendicular to each other were measured and the average of two measurements was taken as the diameter of CL.

Pregnancy diagnosis

Early pregnancy diagnosis was conducted during 30-35 days after FTAI using transrectal Ultrasonography. Final pregnancy was confirmed during 75-90 days after FTAI using transrectal palpation. Pregnancy loss between first and second examination if any, was recorded.

Statistical analyses

The data were analyzed using IBM SPSS statistical software (version 26). Estrus expression rate, ovulation rate and pregnancy rates between two treatment groups were compared using Chi-square test; when the expected frequency was less than 5 in more than 20% of the cells, the Fisher's exact probability test was used. Effect of ovarian status at the initiation of the protocol on pregnancy outcome from FTAI was also analyzed using Chi-square test or Fisher's exact probability test, whichever applicable. The results with P-value ≤0.05 were considered as significant whereas those with 0.05<P≤ 0.1 were considered to have tendency effect.

RESULTS

Estrus expression and ovulation rates

Table 1 shows the proportions of buffaloes showing various estrous signs. Among various estrous signs, the proportions of buffaloes showing mucus discharge (P=0.01), those having uterine contraction during FTAI (P=0.02) and those with decrease in milk yield (P=0.1) were higher in CIDR-synch than in Ovsynch groups. Ultimately, the overall estrus expression rate was higher (P=0.008) in CIDR-synch group (96.7%) than in Ovsynch group (68%).

Table 1: Proportions of buffaloes showing various estrous signs in Ovsynch and CIDR-synch groups.

Estrous signs	Total (n=55)	Treatment group		P value
		Ovsynch (n=25)	CIDR-synch (n=30)	
Mucus discharge (%)	54.5	36.0	70.0	0.01
Bellowing (%)	14.5	16.0	13.3	1
Vulva swelling (%)	32.7	28.0	36.7	0.50
Teat engorgement (%)	43.6	44.0	43.3	1
Decrease in milk yield (%)	63.6	52.0	73.3	0.10
Uterine contraction during FTAI (%)	78.2	64.0	90.0	0.02
Overall estrous expression rate (%)	83.6	68.0	96.7	0.008

Table 2: Pregnancy rate of FTAI and the overall pregnancy rate (resulting from FTAI plus natural breeding of buffaloes returning to estrus within one month of FTAI) in Ovsynch and CIDR-synch groups.

Parameters	Overall	Treatment groups		P value
		Ovsynch	CIDR-synch	
No. of buffaloes treated	55	25	30	
No. of buffaloes pregnant from FTAI (A)	14	4	10	
FTAI pregnancy rate (%)	25.5	16.0	33.3	0.14
No. of buffaloes returned to estrus within one month of FTAI and bred with bull	14	3	11	
No. of buffaloes pregnant from natural breeding within one month of FTAI (B)	5	1	4	
Overall pregnancy rate within one month of FTAI (A+B) (%)	34.5 (19/55)	20.0 (5/25)	46.7 (14/30)	0.04

Table 3: Effect of ovarian status at the initiation of the protocol on pregnancy outcome from FTAI in Ovsynch and CIDR-synch protocol treated buffaloes.

Protocol	Ovarian status at the initiation of the protocol	No. of buffaloes (n=55)	Pregnancy rate (%)	P value
Ovsynch	Cyclic (CL present)	9	1/9 (11.1)	1
	Non-cyclic (CL absent)	16	3/16 (18.8)	
CIDR-synch	Cyclic (CL present)	10	3/10 (30.0)	1
	Non-cyclic (CL absent)	20	7/20 (35.0)	

Overall, 85.5% (47/55) buffaloes had ovulation (presence of CL on 8-9 day after FTAI). There was a tendency (P=0.1) that the ovulation rate (93.3% vs 76.0%) was higher in CIDR-synch group (28/30) than in Ovsynch group (19/25).

Pregnancy rates

There was no pregnancy loss during a period between 30-35 days and 75-90 days post FTAI. Table 2 shows the pregnancy outcomes and the resumption of estrous cyclicity within 40 days of treatment in Ovsynch and CIDR-synch treated buffaloes. Pregnancy rates from FTAI did not differ (P= 0.14) between Ovsynch (16%) and CIDR-synch (33.3%) groups. However, the overall pregnancy rate within one month after FTAI (resulting from FTAI + natural breeding within one month of FTAI) was significantly (P= 0.04) higher in CIDR-synch (46.7%) than in Ovsynch (20%) groups.

Effect of ovarian status at the initiation of the protocol on pregnancy outcome

Effect of ovarian status at the initiation of the protocol on pregnancy outcome from FTAI has been shown in Table 3. There was no significant effect of ovarian status at the initiation of the protocol on pregnancy outcome from FTAI both in Ovysnch and CIDR-synch groups.

DISCUSSION

This study was conducted to determine the effectiveness of incorporation of CIDR in Ovsynch protocol for the treatment of anestrous buffaloes during low breeding season (April to June). One group of anestrous buffaloes received standard Ovsynch protocol while in another group (CIDR-synch) of anestrous buffaloes, in addition to Ovsynch protocol, a CIDR was inserted for seven days (from d0 to d7). There was significant difference between two treatment groups in terms of estrus expression. Since all the buffaloes in this experiment were in 24 hrs tie-stall system, the estrus expression after treatment was based on the observation of secondary estrous signs (mucus discharge from vulva, bellowing, vulva swelling, teat engorgement, decrease in milk yield and uterine contraction during FTAI). In total, the highest proportion of buffaloes showed the uterine contraction during FTAI (78.2%) followed by the decrease in milk yield (63.6%), mucus discharge (54.5%), teat engorgement (43.6%), swelling of vulva (32.7%) and bellowing (14.5%). The proportions of buffaloes showing mucus discharge, uterine contraction during FTAI, decrease in milk yield and ultimately the overall estrus expression rate were higher in CIDR-synch group than in Ovsynch group. The proportions of buffaloes showing estrus in CIDR-synch group in the present study (96.7%) was almost similar to the findings of previous studies that used CIDR-synch protocol in buffaloes: 92% (Naseer *et al*., 2011) and 100% (Kajaysri *et al*., 2015). Likewise, the estrous expression in the Ovsynch group in the present study (68%) was also similar to the findings of a previous study (73.9%) in which the buffaloes were subjected to Ovsynch protocol during low breeding season (Abulaiti *et al*., 2022). Although expression of estrous signs is generally not mandatory in FTAI protocols, previous studies in cattle have shown that the pregnancy outcome in FTAI was better in cows displaying estrus than in cows without estrous signs (Loeffler *et al.*, 1999; Pereira *et al.*, 2016).

Ovulation was determined based on the presence of corpus luteum during 8-9 days after FTAI. Overall ovulation rate was 85.5% though it was not known when the ovulation actually took place; determination of exact time of ovulation was beyond the scope of this study. There was a tendency that the ovulation rate was higher in buffaloes treated with CIDR-synch than in buffaloes treated with Ovsynch protocols. Ovulation rate in CIDR-synch group in the present study (93.3%) was almost similar to that (89%) reported by a previous study in which buffaloes were treated with CIDR (7 days)-PG-GnRH protocol (Naseer *et al.*, 2011). Likewise, the ovulation rate in Ovsynch group in the present study (76%) was almost similar to the findings (60.9%) of Abulaiti *et al.* (2022). Rapid drop in circulatory progesterone concentration following CIDR removal might be the cause for higher estrous expression rate and ovulation rate in CIDR-synch group as compared to Ovsynch group. This rapid drop in progesterone concentration removes the negative feedback effect of progesterone on hypothalamus, as a result there is release of GnRH from hypothalamus followed by the release of FSH and LH from anterior pituitary, and the subsequent restoration of ovarian cyclicity (Zerbe *et al.*, 1999; Zabeel *et al.*, 2009; Azawi *et al.*, 2012).

Pregnancy rate of FTAI in CIDR-synch group (33.3%) in the present study was almost similar to the findings of previous studies that used CIDR-synch [31.8% (Azawi *et al.*, 2012), 32.8% (El-Tarabany, 2016)] and CIDR co-synch protocols (37% (Naseer *et al.*, 2011)). Pregnancy outcomes of FTAI in Ovsynch protocol during low breeding season in buffaloes were inconsistent in previous studies that ranged from 0% (Jabeen *et al.*, 2013) to 26.1% (Abulaiti *et al.*, 2022) and 29.4% (Warriach *et al.*, 2008). Although the pregnancy rate from FTAI in the present study did not differ between Ovsynch and CIDR-synch groups, the overall pregnancy rate (resulting from FTAI + natural breeding within one month of FTAI) was significantly higher in CIDR-synch group (46.7%) than in Ovsynch group (20%). This increase in overall pregnancy rate within one month of FTAI in CIDR-synch treated buffaloes might be attributed to the higher proportion of buffaloes that resumed estrous cyclicity due to hormonal protocol. It has been shown that the progesterone concentration in previous luteal phase can affect follicle development and subsequent fertility along with the sufficient priming of the endometrium (Stevenson *et al.*, 2006; Madureira *et al.*, 2021). It has been also demonstrated that progesterone increases the expression of estradiol receptors in the hypothalamus (Van Eerdenburg *et al.*, 2000; Gumen and Wiltbank, 2002) and thus, primes the hypothalamus to be more responsive to estradiol (Woelders *et al.*, 2014) leading to the resumption of estrous cyclicity. Thus, the present study clearly indicated that the intravaginal progesterone based treatments were effective to resume estrous cyclicity and to enhance the subsequent reproductive performance in anestrous buffaloes during low breeding season.

Although previous study demonstrated that the pregnancy outcome from estrus synchronization protocol in cattle was affected by the presence of CL or dominant follicle at the initiation of the protocol (Stevenson *et al.*, 2012), in the present study, there was no effect of ovarian cyclicity status at the initiation of the protocol on pregnancy outcome from FTAI in both treatment groups of buffaloes. This might be due to small number of buffaloes used in this study. Thus, further detailed study might be needed to rule out the effect of ovarian cyclicity status, if any, on the efficacy of estrous synchronization protocol in anestrous buffaloes during low breeding season.

CONCLUSIONS

Incorporation of CIDR into Ovsynch protocol improved the resumption of estrous cyclicity, ovulation and overall pregnancy outcome within one month in anestrous buffaloes during low breeding season. During the revision of our manuscript (addressing the reviewer's comments), we forgot to delete this sentence, although we removed it from ABSTRACT. As the results related to this statement have been deleted, it is not logical to keep this statement in conclusion.

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NOVELTY STATEMENT

This study demonstrated that the inclusion of CIDR in Ovsynch protocol improved the reproductive performance of anestrous buffaloes during low breeding season through the resumption of estrous cyclicity and ovulation.

AUTHOR'S CONTRIBUTION

GG: Conceptualization, funding acquisition, project administration, resources, investigation, methodology, formal analysis, supervision, writing-original draft, writing review and editing. HA: Methodology, data curation, formal analysis, writing original draft. BD: Methodology, investigation, supervision, writing review and editing. SS: Methodology, investigation, supervision.

Conflict of interest

The authors have declared no conflict of interest.

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