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Effectiveness of Once Used versus New CIDR in CIDR Co-synch Protocol for Resuming Reproductive Function in Anestrous Buffaloes During Low Breeding Season

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ABSTRACT

This study compared the effectiveness of once used versus new Controlled Internal Drug Release (CIDR) device in CIDR co-synch protocol for resuming reproductive function in anestrous buffaloes during low breeding season. Either a new (T1, n=19) or once used CIDR (T2, n=31) was inserted into the vagina of buffalo on a random day (d0) and removed on d7. Buffaloes in both treatment groups received GnRH at the time of CIDR insertion and $PGF_{2\alpha}$ during its removal. At 60-66 hrs after CIDR removal, fixed time artificial insemination (FTAI) was done and GnRH was injected. Ovarian status during CIDR insertion, corpus luteum (CL) size on 8-9 d after FTAI and pregnancy diagnosis were determined using transrectal ultrasonography. There was no significant difference between the two treatment groups in terms of estrus expression, ovulation, pregnancy rates and local inflammatory response into the vagina of treated buffaloes. Although FTAI pregnancy rate was 16%, the overall pregnancy from FTAI and natural breeding within 50 d after FTAI was 42%. Estrus expression was affected by the ovarian cyclicity status at the start of the protocol. The size of the CL during 8-9 d after FTAI was larger in pregnant buffaloes as compared to non-pregnant buffaloes. In conclusion, the once used CIDR was as effective as the new CIDR for resuming reproductive function in anestrous buffaloes during low breeding season, however, the efficacy of protocol depended on the ovarian cyclicity status at the start of the protocol and the size of CL during 8-9 d after FTAI.

Key words: Buffalo, CIDR, Estrus expression, Ovulation, Pregnancy, Ovarian cyclicity.

INTRODUCTION

In Nepal, the buffalo (*Bubalus bubalis*) is the major livestock species contributing 57.2% of the total milk and 36.1% of the total meat produced in the country (MoALD 2022). Buffaloes are known for having characteristics of hardiness, adaptation to humid tropical conditions and better feed efficiency compared to cattle (Peralta-Torres et al. 2020). However, the production and productivity of buffaloes in Nepal is quite low due mainly to subfertility and infertility (Gautam et al. 2017; Devkota et al. 2023). The major causes of poor fertility associated with buffaloes are delayed onset of puberty, prolonged postpartum anestrus period and longer inter-calving interval (Kaurav et al. 2020; Kumar et al. 2020; Hassaneen et al. 2021). The situation is further exaggerated by reduced ovarian activity, short estrus duration along with poor estrus expression during summer season (Abulaiti et al. 2022).

Seasonal pattern of reproduction is one of the major reasons for reduced reproductive efficiency and production output in buffaloes (Singh et al. 2000; Baruselli 2001; Nanda et al. 2003; Otava et al. 2021; Devkota et al. 2023; Gautam et al. 2024). Various environmental factors like photoperiod or day length, temperature, relative humidity and rainfall, either alone or in combination play significant roles in controlling such reproductive behavior (Das and Khan 2010; Hassaneen et al. 2021). The buffalo is a short-day breeder and shows increased reproductive activity with decreasing day length (Neglia et al. 2020; Gautam et al. 2024). In Nepal and Indo-Pakistan subcontinent, maximum breeding activity occurs during the month of July to December, with a peak during October to November, and

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the minimal breeding activity occurs during late spring and early summer months (i.e., April to June) (Devkota and Bohora 2009; Noakes et al. 2019; Gautam et al. 2024). For this reason, spring and early summer season is considered as a low breeding season for Nepalese buffaloes (Devkota and Bohora 2009; Gautam et al. 2024).

Various hormonal protocols used in cows have been tried to enhance the reproductive performance of anestrous buffaloes during low breeding season (Barile 2005; Dhami et al. 2020; Otava et al. 2021; Sharma et al. 2021). One of the most commonly used protocols is Co-synch which is a modified 7day (d) Ovsynch protocol where gonadotropin-releasing hormone (GnRH) is given as an initial dose followed by prostaglandin F2 alpha ($PGF_{2\alpha}$) after 7 days and second dose of GnRH approximately 3 days later at the time of artificial insemination (AI). The advantage of co-synch protocol is that it reduces the number of animal handling events as compared to Ovsynch protocol (Colazo and Ambrose 2011).

The addition of intravaginal progesterone releasing devices into the Ovsynch and co-synch protocols further improved the estrus induction and pregnancy outcomes in beef heifers (Martínez et al. 2002) and non-cyclic anestrous buffaloes (De Rensis et al. 2005; De Rensis and López-Gatius 2007; Ghuman et al. 2012). As most of the buffaloes during low breeding season are non-cyclic (Gautam et al. 2024), the progesterone-based treatment regimens either alone or in combination with other hormones like GnRH and $PGF_{2\alpha}$ were found to be effective to induce estrus (Barile et al. 2001; Neglia et al. 2003; Dhaka et al. 2019; Sharma et al. 2021). Among intra-vaginal progesterone releasing devices, the Controlled Internal Drug Release (CIDR®) device has been widely used in synchronizing estrus with acceptable pregnancy rates in cattle (Colazo et al. 2004, 2006) and to some extent in buffaloes too (Murugavel et al. 2009; Naseer et al. 2011; Kaurav et al. 2020; Presicce et al. 2022). However, the estrus response and subsequent conception rates are dependent upon various factors like reproductive status, days postpartum, ovarian follicular status, nutritional status, season of the year and the simultaneous administration of hormones like eCG, $PGF_{2\alpha}$ and GnRH in treated buffaloes (Purohit et al. 2019). Further, the use of such progesterone releasing devices (such as CIDR) in small scale farms in less developed countries like Nepal is expensive and thus, it increases the cost of treatment. Studies in cattle have shown that once-used CIDR was also effective in treating the anestrous cows (Colazo et al. 2004) and buffaloes (Zaabel et al. 2009; Naseer et al. 2011) although its re-use is not recommended by the manufacturer. However, there is sparse information regarding the effectiveness of once used CIDR in CIDR co-synch protocol to induce estrus cyclicity in anestrous buffaloes during low breeding season. The objectives of this study, therefore, were i) to compare the effectiveness of once-used versus new CIDR in CIDR co-synch protocol for the treatment of anestrous buffaloes during low breeding season, and ii) to determine the effect of various factors on estrus expression, ovulation and pregnancy outcomes in such treated buffaloes.

MATERIALS AND METHODS

This study was performed in accordance with the ethical guidelines of Institutional Review Board of AFU, Rampur, Chitwan, Nepal; and thus, the use of animals in this study complied with the ethical standards laid down in the seventh amendment (2013) of 1964 Declaration of Helsinki.

Animals

This study was conducted in 50 anestrous Murrah cross buffaloes from four commercial farms of Chitwan district, which lies in the mid southern part of Nepal, having sub-tropical climate with hot humid summer and cool dry winter. Buffaloes that were at least 2 months postpartum and in a state of anestrus condition, having body condition score (BCS) >2.0 with no anatomical defects or anomalies in their reproductive tract were included in the study. Parity of buffaloes ranged from 1 to 5. Transrectal-palpation and ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) of reproductive organs were performed to determine the ovarian cyclicity status and to rule out any anatomical defects or anomalies of the reproductive system.

Deworming and mineral-vitamin mixture supplementation

Broad-spectrum anthelmintic (oxyclozanide + levamisole-Adzanide forte, Nepal Pharmaceutical Ltd) was provided at the recommended doses to all the buffaloes in the study. After administration of anthelmintics, mineral-vitamin mixture supplement (Agrimin forte- Virbac, India) was provided at the recommended doses for at least 3 weeks before the commencement of hormonal protocols.

Hormonal treatment protocols

All the selected anestrous buffaloes (n=50) were randomly divided into two treatment groups in terms of using new CIDR containing 1.9g progesterone (Eazi-Breed CIDR®, Zoetis, Australia) (n=19) or once used CIDR (n=31) (Fig. 1). New CIDRs that were previously placed

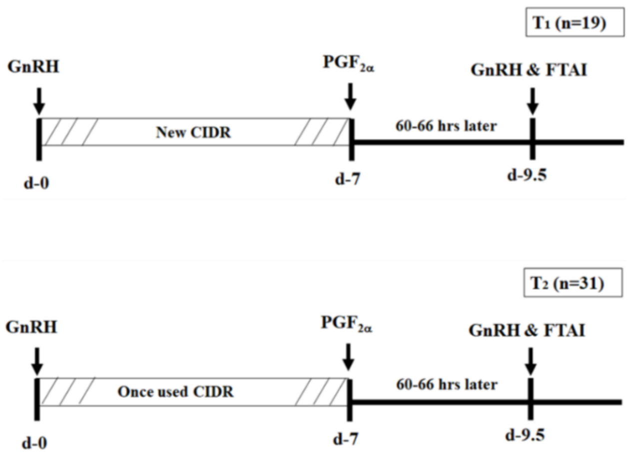

Fig. 1: CIDR co-synch protocol using new *vs* once-used CIDR (Controlled Internal Drug Release) device. Buffaloes in T1 group (n=19) received new CIDR (containing 1.9 gm progesterone) while the buffaloes in T2 group (n=31) received once-used CIDR.

into the vagina of buffaloes for 7 days (in another experiment) were utilized as once used CIDRs in this experiment; after removal, the CIDRs were rinsed with clean water, soaked into detergent solution for about 2 hours, individually scrubbed with a brush, thoroughly washed with water and allowed to air-dry, wrapped in aluminum foil and stored at room temperature until use. Immediately before use, the CIDR was dipped into 5% povidone iodine solution for 2-3 minutes, wiped with sterilized cotton. In both groups, CIDR was inserted on random day (d0) and GnRH analogue, buserelin acetate 20µg (Gynarich, Intas Pharmaceuticals, India) was injected intramuscular at the time of CIDR insertion (d0). The CIDR was removed on d7 and a $PGF_{2\alpha}$ analogue, cloprostenol 500µg (Cloprochem, Interchem, the Netherlands) was injected intramuscular at the time of CIDR removal. Fixed time artificial insemination (FTAI) was done at 60-66 hrs after CIDR removal; buserelin acetate 20 mcg was injected intramuscular during FTAI.

Estrus detection

Buffaloes were observed for external estrous signs at least two times a day starting from the day of CIDR removal until FTAI. Buffaloes were considered to be in estrus based on external estrous signs (teat engorgement, mucus discharge, bellowing, restlessness, vulva swelling and decrease in milk production) and the cervical relaxation and uterine contraction noted at the time of AI.

Determination of corpus luteum (CL) size

Size of CL was determined on 8-9 d after FTAI using transrectal ultrasonography (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) using 10 MHz transducer. Two diameters 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 performed during 32-35 d after FTAI using trans-rectal ultrasonography. Final pregnancy diagnosis was done at 75-90 d after FTAI using transrectal palpation. Pregnancy loss between first and second pregnancy diagnosis was documented in order to know the pregnancy losses, if any, in treated anestrous buffaloes.

Statistical analyses

The data analysis was done by using excel spreadsheet (Microsoft corporation 2007) and IBM SPSS statistical software (version 25). Estrus expression rate, ovulation rate and pregnancy rate between two treatment groups were compared using Chi-square test. If the expected frequency was <5 in more than 20% the cells, the Fisher's exact probability test was used.

For the analysis of various factors on estrus expression, ovulation and pregnancy outcomes, the buffaloes were classified as: primiparous or pluriparous; low BCS (< 2.75) or good BCS (≥2.75); presence or absence of endo-parasites; presence or absence of suckling calf; lactation stage up to 100 d postpartum (pp) or more than 100 d pp; presence or absence of CIDR impression (vaginitis) during AI; ovarian status having dominant follicle (DF), corpus luteum (CL) or inactive ovary (having no DF and CL) at the start of the protocol; and vaginal discharge scoring of CIDR having clear, mucopurulent or purulent discharge. Effect of these factors on estrus induction, ovulation and pregnancy outcome was also analyzed using Chi square-test or Fisher's exact probability test. The size of the CL was compared using two sample t-test assuming equal variance. Probability value ≤0.05 was considered as significant whereas 0.05<P≤0.1 was considered to have tendency effect.

RESULTS

Estrus expression, ovulation and pregnancy rates

There was no significant difference in estrus expression rate between new and once used CIDR groups. Overall, 80% (40/50) of buffaloes were found to be in estrus based on external signs of estrus and transrectal palpation findings. Decrease in milk production (60%) was the most frequently observed external estrous sign followed by mucus discharge from vulva (38%). Likewise, there was no significant difference in ovulation (as confirmed by presence of CL on 8-9 days after FTAI) and pregnancy rates between new and once used CIDR groups (Table 1). There was no loss of pregnancy from 32-35 d to 75-90 d post FTAI.

Local inflammatory response of new versus once used CIDR into the vagina of treated buffaloes

The occurrence of local inflammatory response into the vagina of treated buffaloes was not affected by the type (new or once used) of CIDR used. There was no significant difference between new and once used CIDR in terms of the nature of discharge attached on the surface of CIDR at its removal as well as the CIDR impression into the cranial vagina of treated buffaloes detected during the time of FTAI (Table 2). Overall, 44% of buffaloes had CIDR impression into the cranial vagina when examined trans-rectally during the time of FTAI.

Effect of various factors on estrus expression, ovulation and pregnancy outcomes in CIDR co-synch treated buffaloes

Since there was no difference in estrus expression, ovulation and pregnancy rates between new and once used CIDR groups, all the buffaloes were pooled together to analyze the effect of various factors on estrus expression, ovulation and pregnancy outcomes. Estrus expression was not affected by the other factors (listed in statistical analysis) except the ovarian cyclicity status at the start of the protocol. Buffaloes having either dominant follicle (follicle ≥8 mm) or CL at the initiation of protocol had significantly higher estrus expression rates (86.4 and 89.5%, respectively) than those having inactive ovaries (44.5%). Likewise, the ovulation was not affected by any of the above factors. Pregnancy outcome was also not affected by other factors except the CL size on 8-9 d after FTAI. The CL was present in 92% (46/50) of buffaloes when examined during 8-9 d after FTAI. When the size of the CL was compared between pregnant and non-pregnant buffaloes retrospectively, there was a tendency (P=0.1) that the CL size was larger in pregnant buffaloes than in non-pregnant buffaloes (Table 3).

Resumption of estrous cyclicity within 50 days after FTAI and overall pregnancy outcome

Out of 42 non-pregnant buffaloes, 25 buffaloes (9 from new and 16 from once used CIDR groups) returned to estrus within 50 d after FTAI and those were naturally bred with bull (Table 4). Thus, total 66% (33/50) of treated

Table 1: Estrus expression, ovulation and pregnancy rates in anestrous buffaloes treated with new or once used CIDR in CIDR co-synch protocol

Parameters	Overall (n=50)	Treatment group				P-value
		New CIDR (n=19)		Once used CIDR (n=31)		
		No.	%	No.	%	
Estrus expression rate (%)	80	15	78.9	25	80.6	0.82
Ovulation rate (%)	92	18	94.7	28	90.3	1.0
Pregnancy rate (%)	16	2	10.5	6	19.4	0.69

Table 2: Local inflammatory response of new versus once used CIDR into the vagina of anestrous buffaloes treated with CIDR co-synch protocol

Parameters of local inflammatory response	Levels	Overall (n=50) %	Treatment group				P-value
			New CIDR (n=19)		Once used CIDR (n=31)		
			No.	%	No.	%	
Vaginal discharge attached on the surface of CIDR at its removal	Clear	20	6	31.6	4	12.9	0.25
	Mucopurulent	28	4	21.1	10	32.3	
	Purulent	52	9	47.4	17	54.8	
CIDR impression into the vagina detected during FTAI	Yes	44	9	47.4	13	41.9	0.92
	No	56	10	52.6	18	58.1	

Table 3: Size of corpus luteum (CL) during 8-9 d post FTAI in pregnant and non-pregnant buffaloes

Pregnancy outcome	No. of buffaloes having CL during 8-9 d post FTAI	CL size (cm) (Mean±SE)	P value
Pregnant	8	1.66±0.31	0.1
Non-pregnant	38	1.53±0.06	

Table 4: Resumption of estrous cyclicity within 50 d after FTAI and pregnancy outcome in CIDR co-synch treated buffaloes

S. No.	Parameters	Overall	CIDR Treatment		P-value
			New	Once used	
1	No. of buffaloes treated	50	19	31	
2	No. of buffaloes pregnant from FTAI (A)	8	2	6	
3	FTAI pregnancy rate (%)	16	10.52	19.4	0.69
4	No. of buffaloes returned to estrus within 25 d after FTAI & bred with bull	12	4	8	
5	Pregnancy (%) among buffaloes those returned to estrus within 25 d after FTAI & bred with bull (B)	41.7 (5/12)	50 (2/4)	37.5 (3/8)	1.0
6	No. of buffaloes returned to estrus between 26 and 50 d after FTAI & bred with bull	13	5	8	
7	Pregnancy (%) among buffaloes those returned to estrus between 26 and 50 d after FTAI & bred with bull (C)	61.5 (8/13)	60 (3/5)	62.5 (5/8)	1.0
8	Overall pregnancy outcome (%) from FTAI and natural breeding within 50 d after FTAI (A+B+C)	42.0 (21/50)	36.8 (7/19)	45.2 (14/31)	0.77

buffaloes (8 pregnant from FTAI and 25 returning to estrus and bred with bull) had resumption of reproductive function. Out of 25 buffaloes returned to estrus within 50 d after FTAI, 13 became pregnant. Thus, the overall pregnancy outcome from FTAI and natural breeding within 50 d after FTAI was 42%.

DISCUSSION

The present study indicated that the new and once used CIDR in the CIDR co-synch protocol had the similar effectiveness in terms of estrus expression (78.9 vs 80.6%), ovulation (94.7 vs 90.3%) and pregnancy outcomes (10.5 vs 19.4%) in treated anestrous buffaloes during low breeding season. This result was in agreement with the findings of previous study (Naseer et al. 2011) that indicated that estrus expression (96.6 vs 88.5%) and pregnancy rate (37.1 vs 36.6%) were similar between the new and once-used CIDR groups in CIDR-PG treated anestrous Nili-Ravi buffaloes in Pakistan. Similar findings were reported by a previous study in Holstein heifer where estrus expression (86.4 vs 84.3%) and ovulation rate (96.6 vs 94.7) did not differ between new and once used CIDR groups (Sanz et al. 2022). Previous studies in beef cows (Colazo et al. 2004; Martins et al. 2009) and dairy cows (Cerri et al. 2009; El-Tarabany 2016) also demonstrated that both the new and once used CIDR were capable of inducing estrus with a comparable fertility rate. Therefore, the results from the present study as well as previous literature support the fact that the once used CIDR was as effective as new CIDR to synchronize estrus resulting in reasonable level of pregnancy outcomes in anestrous buffaloes.

Since all the buffaloes in this study were in 24-hrs tie-stall system, the estrus induction after treatment was based on observation of secondary estrous signs (teat engorgement, mucus discharge from vulva, congestion and swelling of vulva and decrease in milk yield) as well as on trans-rectal palpation findings (cervical relaxation and uterine contraction) at the time of FTAI. Overall, 80% of treated buffaloes exhibited at least any one of the estrous signs, which is similar to the findings of previous study in which 88.5% buffaloes receiving once-used CIDR and 96.6% buffaloes receiving a new CIDR had expressed the estrus signs (Naseer et al. 2011).

In the present study, overall, 92% of treated buffaloes had development of CL during 8-9 d after FTAI, however, the exact timing of ovulation was not known as the determining the time of ovulation was beyond the scope of this study. In spite of good estrus induction and ovulation rates, the overall pregnancy rate (16%) in the present study was lower than that the findings of the previous study (Naseer et al. 2011). The exact reason behind this lower pregnancy rate was not known but might be associated with hot, humid and stressful environment during treatment period that might have been associated with embryonic mortality because it has been demonstrated that there is increased embryonic mortality during the non-breeding season (Neglia et al. 2020). Furthermore, the quality of semen might have also been attributed to the low pregnancy outcome from FTAI, however, the quality of semen was not evaluated in the present study.

There was similar local vaginal tissue response with new and once used CIDR as indicated by no difference in terms of local temporary impression/inflammation into the vagina as well as no difference in terms of nature of vaginal discharge attached on the surface of CIDR device at its removal. These results suggest that once used CIDR would have similar local inflammatory response provided those were properly disinfected. Once used CIDR, after proper cleaning, can be autoclaved (Colazo et al. 2004) or disinfected with povidone iodine (Naseer et al. 2011).

Different factors like BCS, parity, endoparasitic infection, stage of lactation, suckling status, CIDR impression into the vagina and ovarian cyclicity status at the start of the protocol were also studied to know whether these factors had any effect on the estrus expression, ovulation and pregnancy outcome. Except the ovarian cyclicity status at the initiation of protocol, there was no significant effect of other factors on estrus expression in anestrous buffaloes treated with CIDR co-synch protocol. Buffaloes having either DF or CL at the initiation of protocol had significantly higher estrus expression rate than those having inactive ovaries. Previous studies in cattle have also demonstrated that the cows having DF at the time of first GnRH injection (i.e. ovulatory response to first GnRH) was the key determinant for a successful synchronization outcome (Martinez et al. 1999; Bello et al. 2006). Similarly, none of those factors had a significant effect on the ovulation in CIDR co-synch treated anestrous buffaloes. This might be due to high proportion (92%) of buffaloes having ovulation, although determination of exact time of ovulation was beyond the scope of this study. Previous study on effect of such factors on ovulation rate in estrous synchronized buffaloes was lacking.

Pregnancy outcome was not affected by the other factors except the CL size on 8-9 d after FTAI. When CL size during 8-9 d post FTAI was compared retrospectively, the size of the CL was larger (P=0.1) in pregnant buffaloes than in non-pregnant buffaloes. Due to smaller size of the CL, the optimum level of progesterone would not have been maintained that might have led to embryonic death at early stage of gestation (Wathes et al. 1998; Mann and Lamming 1999) resulting in low pregnancy outcome.

Although pregnancy outcome in CIDR co-synch treated anestrous buffaloes in this study was low, this treatment was effective to resume ovarian cyclicity in 66% of treated buffaloes. As a consequence, ultimately 42% pregnancy rate was achieved considering the conception from FTAI and natural breeding of those buffaloes returning to cyclicity within 50 days after FTAI. Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting negative feedback effect on hypothalamus and pituitary for LH release. Upon withdrawal of progesterone, the normal follicular phase of the cycle is stimulated. The priming of hypothalamo-hypophyseal-gonadal (HPG) axis with adequate amounts of progesterone is beneficial for the recovery of HPG axis function (De Rensis et al. 2005; De Rensis and López-Gatius 2007; Ghuman et al. 2012). The present study clearly indicated that the intravaginal progesterone-based treatments were effective to resume estrous cyclicity in anestrous buffaloes during low breeding season.

Conclusion

Once used CIDR was as effective as new CIDR in terms of estrus expression, ovulation and pregnancy outcome as well as local inflammatory response into the vagina in CIDR co-synch treated anestrous buffaloes during low breeding season; however, the efficacy of the protocol depended on the ovarian cyclicity status at the start of the protocol and the size of CL on 8-9 d after FTAI.

Author's contribution

Saurav Kandel: Methodology; Data curation; Formal analysis; Writing - original draft. Gokarna Gautam: Conceptualization; Funding acquisition; Project administration; Resources; Investigation; Methodology; Formal analysis; Supervision; Writing -original draft; Writing - review & editing. Bhuminand Devkota: Methodology; Investigation; Supervision, Writing – review & editing. Subir Singh: Methodology; Investigation; Supervision.

Declaration of competing interest

The authors declare no conflicts of interest.

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REFERENCES

Abulaiti A, Naseer Z, Ahmed Z, Wang D, Hua G and Yang L, 2022. Effect of different synchronization regimens on reproductive variables of crossbred (swamp × riverine) nulliparous and multiparous buffaloes during peak and low breeding seasons. Animals 12: 415. https://doi.org/10.3390/ani12040415

Barile VL, 2005. Improving reproductive efficiency in female buffaloes. Livestock Production Science 92: 183–194. https://doi.org/10.1016/j.livprodsci.2004.06.014

Barile VL, Galasso A, Marchiori E, Pacelli C, Montemurro N and Borghese A, 2001. Effect of PRID treatment on conception rate in mediterranean buffalo heifers. Livestock Production Science 68: 283–287. https://doi.org/10.1016/S0301-6226(00)00228-1

Baruselli P, 2001. Control of follicular development applied to reproduction biotechnologies in buffalo. Proceedings of the 1st Congresso Nazionale Sull'Allevamento Del Bufalo, pp: 128–146.

Bello NM, Steibel JP and Pursley JR, 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. Journal of Dairy Science 89: 3413–3424. https://doi.org/10.3168/jds.S0022-0302(06)72378-5

Cerri RLA, Rutigliano HM, Bruno RGS and Santos JEP, 2009. Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Animal Reproduction Science 110: 56–70. https://doi.org/10.1016/j.anireprosci.2007.12.005

Colazo MG and Ambrose DJ, 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: 578–588. https://doi.org/10.1016/j.theriogenology.2011.03.013

Colazo MG, Kastelic JP, Mainar-Jaime RC, Gavaga QA, Whittaker PR, Small JA, Martinez MF, Wilde RE, Veira DM and Mapletoft RJ, 2006. Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 65: 557–572. https://doi.org/10.1016/j.theriogenology.2005.06.001

Colazo MG, Kastelic JP, Whittaker PR, Gavaga QA, Wilde R and Mapletoft RJ, 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. Animal Reproduction Science 81: 25–34. https://doi.org/10.1016/j.anireprosci.2003.09.003

Das GK and Khan FA, 2010. Summer anoestrus in buffalo–a review. Reproduction in Domestic Animals 45(6): 483-494.

De Rensis F and López-Gatius F, 2007. Protocols for synchronizing estrus and ovulation in buffalo (Bubalus bubalis): A review. Theriogenology 67: 209–216. https://doi.org/10.1016/j.theriogenology.2006.09.039

De Rensis F, Ronci G, Guarneri P, Nguyen BX, Presicce GA, Huszenicza G and Scaramuzzi RJ, 2005. Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (Bubalus bubalis). Theriogenology 63: 1824–1831. https://doi.org/10.1016/j.theriogenology.2004.07.024

Devkota B, Shah S and Gautam G, 2023. Reproduction and Fertility of Buffaloes in Nepal. Animals 13: Article 1. https://doi.org/10.3390/ani13010070

Devkota B and Bohora TP, 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. Pakistan Journal of Zoology 9: 763–770.

Dhaka AP, Phogat JB, Singh S, Pandey AK, Sharma K and Ghadwal S, 2019. Efficacy of modified Co-synch plus protocol with or without progesterone device for estrus induction and conception rate in Murrah buffaloes under field condition during summer season. Buffalo Bulletin 38: 353-361. https://kuojs.lib.ku.ac.th/index.php/BufBu/article/view/2556

Dhami AJ, Hadiya KK, Patel JA, Parmar SC and Chaudhari DV, 2020. Impact of different estrus induction and ovulation synchronization protocols in addressing infertility in buffaloes. Buffalo Bulletin 39: Article 3. https://kuojs.lib.ku.ac.th/index.php/BufBu/article/view/1082

El-Tarabany MS, 2016. The efficiency of new CIDR and once-used CIDR to synchronize ovulation in primiparous and multiparous Holstein cows. Animal Reproduction Science 173: 29–34. https://doi.org/10.1016/j.anireprosci.2016.08.006

Gautam G, Gyawali RR, Nath B and Pant S, 2017. Characterization and treatment of infertility in buffaloes of hills and Terai regions of mid Nepal. Proceedings of the International Buffalo Symposium, pp: 98–102. https://afu.edu.np/sites/default/files/IBS_2017_Proceedings.pdf

Gautam G, Rokaya S, Devkota B and Sapkota S, 2024. Seasonality of reproduction in buffaloes in subtropical regions of southern Nepal. Advances in Animal and Veterinary Sciences 12(5): 895-901. https://dx.doi.org/10.17582/journal.aavs/2024/12.5.895.901

Ghuman S, Honparkhe M, Singh J, Dhami DS, Kumar A, Nazir G and Ahuja C, 2012. Fertility response using three estrus synchronization regimens in lactating anestrous buffaloes. Indian Journal of Animal Sciences 162–166.

Hassaneen AS, Ibrahim AM, Yousef NA and Ahmed AE, 2021. Combined controlled-internal drug release and ovsynch synchronization protocol does not improve fertility outcome in buffaloes. Advances in Animal and Veterinary Sciences 9(12): 2027-2035. https://doi.org/10.17582/journal.aavs/2021/9.12.2027.2035

Kaurav PS, Shukla SP, Bajaj NK, Singh AK, Manjhi VK and Thakur RK, 2020. Conception rate in postpartum anoestrus buffaloes treated with different synchronization protocols. Buffalo Bulletin 39: 229–236.

Kumar TVC, Sharma D, Surla GN, Vedamurthy GV, Singh D and Onteru SK, 2020. Body condition score, parity, shelter cleanliness and male proximity: Highly associated non-genetic factors with post-partum anestrus in Murrah buffalo in field conditions. Animal Reproduction Science 214: https://doi.org/10.1016/j.anireprosci.2020.106282

Mann GE and Lamming GE, 1999. The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals 34: 269–274. https://doi.org/10.1111/j.1439-0531.1999.tb01250.x

Martinez MF, Adams GP, Bergfelt DR, Kastelic JP and Mapletoft RJ, 1999. Effect of LH or GnRH on the dominant follicle of the first follicular wave in beef heifers. Animal Reproduction Science 57: 23–33. https://doi.org/10.1016/S0378-4320(99)00057-3

Martínez MF, Kastelic JP, Adams GP, Cook B, Olson WO and Mapletoft RJ, 2002. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. Theriogenology 57: 1049–1059. https://doi.org/10.1016/s0093-691x(01)00682-3

Martins JH, Silva MIS, Crepaldi GA, Melo WO and Torres-Júnior JRS, 2009. Comparison of new vs. Previously used intravaginal device for a progesterone-based timed artificial insemination protocol in Nelore (Bos indicus) cows. Reproduction Fertility and Development 22:171-172 (Abstract). https://doi.org/10.1071/RDv22n1Ab28

MoALD, 2022. Statistical Information on Nepalese Agriculture. Ministry of Agriculture and Livestock Development, Government of Nepal.

Murugavel K, Antoine D, Raju MS and López-Gatius F, 2009. The effect of addition of equine chorionic gonadotropin to a progesterone-based estrous synchronization protocol in buffaloes (Bubalus bubalis) under tropical conditions. Theriogenology 71: 1120–1126. https://doi.org/10.1016/j.theriogenology.2008.12.012

Nanda AS, Brar PS and Prabhakar S, 2003. Enhancing reproductive performance in dairy buffalo: Major constraints and achievements. Reproduction (Cambridge, England). Supplement 61. https://pubmed.ncbi.nlm.nih.gov/14635924/

Naseer Z, Ahmad E, Singh J and Ahmad N, 2011. Fertility following CIDR based synchronization regimens in anoestrous Nili-Ravi buffaloes. Reproduction in Domestic Animals 46: 814–817. https://doi.org/10.1111/j.1439-0531.2010.01746.x

Neglia G, De Nicola D, Esposito L, Salzano A, D'Occhio MJ and Fatone G, 2020. Reproductive management in buffalo by artificial insemination. Theriogenology 150: 166–172. https://doi.org/10.1016/j.theriogenology.2020.01.016

Neglia G, Gasparrini B, Di Palo R, De Rosa C, Zicarelli L and Campanile G, 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian

Mediterranean Buffalo cows. Theriogenology 60: 125–133. https://doi.org/10.1016/S0093-691X(02)01328-6

Noakes DE, Parkinson TJ and England GCW, 2019. Veterinary Reproduction & Obstetrics—10th Edition. https://www.elsevier.com/books/veterinary-reproduction-and-obstetrics/noakes/978-0-7020-7233-8

Otava G, Squicciarini S, Marc S, Suici T, William OG, Hutu I, Torda I and Mircu C, 2021. Effects of age and season on conception rate of Mediterranean Italian Dairy Buffalo (Bubalus bubalis) following oestrus synchronization and fixed-time artificial insemination. Reproduction in Domestic Animals 56: 1511–1518. https://doi.org/10.1111/rda.14013

Peralta-Torres JA, Torres-Chablé OM, Segura-Correa JC, Ojeda-Robertos NF, Chay-Canul AJ, Luna-Palomera C, Severino-Lendechy VH and Aké-Villanueva JR, 2020. Ovarian dynamics of buffalo (Bubalus bubalis) synchronized with different hormonal protocols. Tropical Animal Health and Production 52: 3475–3480. https://doi.org/10.1007/s11250-020-02381-9

Presicce GA, Vistocco D, Capuano M, Navas L, Salzano A, Bifulco G, Campanile G and Neglia G, 2022. Pregnancies following protocols for repetitive synchronization of ovulation in primiparous buffaloes in different seasons. Veterinary Sciences 9: 616. https://doi.org/10.3390/vetsci9110616s

Purohit GN, Thanvi P, Pushp M, Gaur M, Saraswat C, Arora A, Prakash S, Gocher T, Atul S, Arora S and Prakash P, 2019. Estrus synchronization in buffaloes: Prospects, approaches and limitations. The Pharma Innovation Journal 8: 54-62. https://doi.org/10.13140/RG.2.2.33173.78563

Sanz A, Gobikrushanth M and Colazo MG, 2022. Type of CIDR but not eCG administration affected pregnancy per AI in Holstein heifers subjected to a 5-day estrus synchronization protocol. Journal of Applied Animal Research 50: 504–510. https://doi.org/10.1080/09712119.2022.2106989

Sharma K, Phogat JB, Pandey AK, Dhaka AP, Singh S and Ghadwal S, 2021. Role of progesterone supplementation in estrus induction in Murrah buffaloes under field conditions in non-breeding season. Buffalo Bulletin 40: Article 1.

Singh J, Nanda AS and Adams GP, 2000. The reproductive pattern and efficiency of female buffaloes. Animal Reproduction Science 60–61: 593–604. https://doi.org/10.1016/s0378-4320(00)00109-3

Wathes DC, Robinson RS, Mann GE and Lamming GE, 1998. The establishment of early pregnancy in cows. Reproduction in Domestic Animals 33: 279–284. https://doi.org/10.1111/j.1439-0531.1998.tb01358.x

Zaabel SM, Hegab AO, Montasser AE and El-Sheikh H, 2009. Reproductive performance of anestrous buffaloes treated with CIDR. Animal Reproduction 6: 460–464.