CIDR PLUS OVSYNCH-BASED FIXED TIME AI PROTOCOL AS A THERAPEUTIC STRATEGY IN POST-PARTUM ANOESTRUS COWS

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ABSTRACT
The use of biotechnological advances in breeding and reproduction is a vital tool for enhancing the reproductive efficiency of cattle production. The study investigated the efficacy of a combined controlled-internal drug release (CIDR) and ovsynch synchronisation protocol with fixed-time artificial insemination (FTAI) as a therapeutic strategy for post-partum anoestrus in Bunaji cross-bred cows. Twenty-two apparently healthy cows (3-5 years old; 250-400 kg, previous calving history, 2-4 times) suffering from post-partum anoestrus (90-120 days) were randomly assigned into control and treated groups (n=11 each). The control group received 2ml of normal saline at time of hormonal treatments while the treatment group received an ovsynch regimen (Day 0: 100 µg GnRH, Day 7: 500 µg, $PGF_2\alpha$ Day 9: 100 µg GnRH) augmented by a progesterone releasing intravaginal insert (CIDR 1.38g progesterone) between days 0 and 7. FTAI was performed 16-24 h after the second GnRH administration. Blood sampling was performed at time of each treatment and AI. There was a significant increase in progesterone level in the treated group as compared with the control. Oestrus synchronisation rate and first service conception rate (90.90% and 80%) were significantly ($P<0.05$) higher than control group (18.18% and 50%). It was concluded that the CIDR plus ovsynch based FTAI protocol is an effective management strategy for enhancing fertility in post-partum anoestrus Bunaji cross-bred cows.
Keywords: CIDR, Bunaji, Ovsynch, Post-partum, Anoestrus

INTRODUCTION
Anoestrous is a widespread reproductive disorder in dairy cattle often triggered by hormonal imbalances, nutritional deficiencies and underlying disease conditions (Islam *et al.,* 2013). Accurate diagnosis and timely treatment of this condition are crucial to the overall health and profitability of the dairy herd. Various hormonal protocols have been tried to enhance the reproductive performance of anoestrus cows (Kwate *et al.,* 2004; Ghunan *et al.,* 2011; Mehni *et al.,* 2012). One of the most commonly used protocols is the ovsynch protocol developed by Pursely *et al.* (1995). The addition of intravaginal progesterone releasing device into the ovsynch protocol further improved the oestrus induction and pregnancy outcomes in anoestrus cows (El-sayed *et al.,* 2019) and anoestrus water buffaloes (Hassanee *et al.,* 2021; Kadel *et al.,*2024). Despite the advances in reproductive technologies, post-partum anoestrus remains a persistent challenge in Nigerian Bunaji cross-bred cows, largely due to inadequate diagnostic and management strategies. Notably, the efficacy of combining CIDR and ovsynch protocols for FTAI in this specific breed has received limited research attention. This study therefore seeks to investigate the effectiveness of integrating CIDR and ovsynch protocols for improving reproductive outcomes in post-partum anoestrus Bunaji cross-bred cows.

MATERIALS AND METHODS
Ethical approval
This study was performed in accordance with the ethical guidelines of the Ethics committee for Animal experimentation of the committee on Animal use and care, Directorate of Academic planning and Monitoring, Ahmadu Bello University Zaria, Nigeria.

Study Site
The study was conducted at a commercial dairy farm in Dan Hassan, Kano, Kano State, Nigeria (11°47'4"N, 8°31'30" East) located in the Sahel Savannah Zone of Nigeria.

Experimental Design
All the selected anoestrus cows (n=22) were randomly divided into two groups of eleven animals each. Group 1 (n=11) control (c). All animals received 2 ml of normal saline at the time of hormonal treatments. Group II (n=11) CIDR plus ovsynch treated group. Animals received 1.M injection of 2 ml of GnRH (Gonabred®, Bimeda, Global, Kenya) on day 0 followed by intravaginal device CIDR containing 1.38 gm of Progesterone (Zootis®New Zealand Ltd, Australia). On day 7, the CIDR was removed and a $PGF_2\alpha$ analogue synchromate® 500 mg (Bremer, Pharma, GnRH, Germany) was injected I.M at the time of CIDR removal. On day 9, 2 ml of GnRh (Gonabred®, Bimeda, Global Kenya) was inserted 1.M followed by TAI 16-24 h after the second GnRh injection.

Collection of blood sample
Five ml blood samples were collected from the Jugular vein into vacutainer heparinised at the time of each treatment and at AI. Blood samples were centrifuged at 3000 rpm for 15 min. After being separated, plasma samples were stored at -20 ºC until further assay, progesterone concentration was measured in all samples.

Hormonal analysis
The plasma progesterone was evaluated by a competitive progesterone ELiSA kit (Accubind Elisa, Monobind Inc)

Determination of oestrus and artificial insemination
Experimental animals were observed for external signs of oestrus at least two times a day starting from the day of CIDR withdrawal until FTAI. The oestrus intensity was categorised as follows:

i) Strong: When the animal exhibited mounting behavior and bellowing sounds along with discharge of mucus from the vagina, congested vaginal mucus membrane and frequent urination.
ii) Moderate: When there was mucus discharge from the vagina, swollen and oedematous vulva and vagina, congestion of vaginal mucus membrane and frequent urination.
iii) Weak: When there was congestion of vaginal mucus membrane

Artificial insemination was performed by one inseminator 18 h after the second GnRH with frozen thawed semen obtained from Livestock Genetic Africa Ltd, Zaria, Nigeria.

Pregnancy diagnosis
For evaluation of the post-synchronisation fertility parameter, pregnancy was diagnosed twice. Early pregnancy diagnosis was performed during 30-35 days after FTAI using transrectal ultrasonography. Final pregnancy diagnosis was done at 75-90 days post FTAI using rectal palpation. Pregnancy loss between first and second pregnancy diagnosis if any, was documented in control and treated anoestrous cows.

Statistical analysis
Data generated were presented as the mean ±SEM and analysed by one-way analysis of variance. Comparism of oestrus response and conception rates between the groups was determined by student t-test. All data were analysed using Graph pad prism (Graph Pad Software, SEM Diega CA, USA). Results were considered significant at the $P<0.05$ level.

RESULTS
Oestrus, conception and pregnancy rates
In this study, the modified ovsynch protocol was used to synchronise oestrus for FTAI in post-partum anoestrus Bunaji cross bred cows. There was a significant difference ($P\leq0.05$) in oestrus expression rate between the treated group (90.90 %) and control (18.18 %) groups based on external signs of oestrus. Conception and pregnancy rates also differed significantly among the control and treated groups,Table I. Mucus discharge from vulva was the most observed sign (65%) followed by mounting and standing to be mounted (25%) Table II. Likewise, there was a significant difference in ovulation as confirmed by disappearance of dominant follicle on day of AI by ultrasonography. There was no loss of pregnancy from day 35 to day 90 post FTAI.

Table 1: Oestrus Express, Conception and Pregnancy Rates in Post-partum AnoestrusBunaji Cross-bred Cows treated with CIDR PlusOvsynch Protocol and Control.

Parameters	Control (n = 11)	CIDR + Ovsynch
Oestrus expression rate (%)	2/11 (18.18%)	10/11 (90.90%)
Conception rate (%)	1/2 (50%)	8/10 (80%)
Pregnancy rate (%)	1/11 (0.09%)	8/11 (72.7%)

Table 2: Effect of CIDR PlusOvsynch Protocol on Intensity of Oestrus in Post-partum Anoestrus Bunaji Cross-bred Cows.

Treatment	Control (n = 11)	CIDR + Ovsynch
Oestrus response (%)	18.18%	90.90%
Intensity of Oestrus		
Strong %	18.11%	25%
Moderate %	-	65%
Weak %	-	-

Plasma progesterone concentration

The results showed that the level of progesterone was significantly higher ($P<0.05$) after treatment with progesterone device on days 0, 7, 9 and AI when compared with the control, Table 3.

Table 3: Mean Plasma Progesterone Concentration (ng/ml) of Control and Treated Groups on Different Days of Sampling in Post-partum Anoestrus Bunaji Cows.

Experimental Groups	0	7	9	At AI
Control	1.01 ± 0.04	0.9±0.02	0.85±0.03	0.74±0.39
CIDR + Ovsynch	1.75±1.00	3.65 ± 0.78	1.65±0.04	1.42±0.35

DISCUSSION

The present study describes the reproductive response of anoestrus post-partum Bunaji crossbred cows to modified ovsynch regimes. A significantly higher oestrus and conception rates were reported in the treatment group than in the control group. The high efficacy of modified ovsynch protocol to synchronise oestrus in 100 % of Muturu breed of cattle (Uchechukwu, 2017), 88.88 % in Thari cows (Wagan *et al.,* 2023) and 100% ovulation rate in dairy cattle (Kim *et al.,* 2006) were previously reported. Similar findings were reported in modified ovsynch treated anoestrus buffaloes in Egypt where oestrus expression and conception rates were significantly improved (Hassaneen *et al.,* 2021). However, other studies reported a lower efficacy when the modified CIDR ovsynch regimen was used with a conception rate about 25% - 40% (Martinez *et al.,* 2009; Cerri *et al.,* 2004; Safilho *et al.,* 2009).Various factors such as breed, condition of the ovaries, the quality of straw used in the AI, sources of the GnRH, $PGF_2\alpha$ and environmental conditions either alone or in synergy play significant roles in controlling such a reproductive outcome (Kadel *et al.,* 2024; Hassaneen *et al.,* 2021).

The present study showed a significant increase in progesterone level after treatment with progesterone device compared to the control group on days 0, 7, 9 and the day of AI respectively. Similar findings have been previously reported (Jena *et al.,* 2016; El-sayed *et al.,* 2019; Ghumen *et al.,* 2011).

The significant increase in circulating progesterone value on day 7 in treated group could be due to the contributions from CIDR as well as the action of the first GnRH injection which causes release of enough LH, to ovulate the largest follicle on the ovary and formation of corpus luteum.

It is evident that the prerequisite for optimal timing of FTAI is the cow's ability to exhibit behavioural oestrus leading to successful ovulation and pregnancy. The synchronisation protocol used in this study was able to induce and synchronise oestrus which culminated into a better endocrine environment leading to higher ovulation and pregnancy rates in the treated group than the control.

CONCLUSION

It was concluded that the CIDR plus ovsynch, protocol was beneficial for synchronisation of oestrus behavior and improved the reproductive outcomes in post-partum anoestrus Bunaji cross-bred cows. Further studies with more animal units are necessary to validate present findings.

REFERENCES

Cerri, R.L., Santos, J.E., and Juchem, S.O. (2004). Synchronization of ovulation with CIDR and GnRH in lactating dairy cows. Journal of Dairy Science, 87(10): 3328-3336

Cornwell, D., Hentges, J. and Fields, M. (1985).Lutalyse as a synchroniser of estrus in Brahman heifers. *Journal of Animal Science,* 61 (Suppl. 1) 416.

El-sayed, M.G., El-Diastry, M. and Magdy, H. A. (2019).Effect of progesterone on some reproductive performances in cattle. *Mansonia Veterinary Medical Journal,* 20:2. 52 – 57.

Ghumen, S. P. S., Hornparkhe, M., Singh, J. and Gandotra, V. K. (2011). Ovysnch plus CIDR-based fixed time AI protocol as a therapeutic strategy in repeat breeder crossbred dairy cattle. *Indian Journal of Animal Sciences,* 81 (3): 257 - 259.

Hassaneen, A. S. A., Ibrahim, A. M., Yousef, N. A. M. and Ahmed, A. E. (2021). Combine controlled-internal drug release and ovsynch synchronization prot… does not improve fertility outcome in Buffaloes. *Advances in Animal and Veterinary Sciences,* 9.12: 2027 – 2035.

Islam, M. R., Juyena, N. S., Bhuiyan, M. M. U., Rahman, M. M. and Fendonsy, R. N. (2013).Treatment outcomes in post-partum anoestrus cows guided by transrectalultrasonagraphy.*Progress Agric,* 24 (1 and 2): 93 – 100.

Jena, D., Das, S., Patra, B. K., Biswal, S. S., Mohanty, D. N. and Samal, P. (2016). Certain hormonal profiles of post-partum anoestrus jersey crossbred cows treated with controlled internal drug release and ovsynch protocol. *Veterinary World,* 9 (10): 1102 – 1106.

Kim, I., Suh, G., Kim, U. and Kang, H. (2006). A CIDR based timed AI protocol can be effectively used for dairy cows with follicular cysts. *Animal Reproduction Science,* 95: 206 - 213.

Kwate, N., Itami, T., Choushi, T., Saitoh, T., Wada, T., Matsuoka, K., Ueneka, K., Tanaka, N., Yamanak, A., Sakase, M., Tamada, H., Inaba, T. and Sawada, T. (2004). Improved conception in timed-artificial insemination using a progesterone-releasing intravaginal device and ovsynch protocol in postpartum suckled Japanese black beef cows . *Theriogenology*: 61, 399-406

Martinez, M.F., Kastelic, J.P., and Adams, G.P. (2002). Effects of CIDR and GnRH on ovulation and fertility in beef heifers. *Theriogenology*, 58(6), 1039-1049.

Mehni, S., Shabankareh, H., Kazemi-Bonchenari, M. and Eghbali, M. (2012). The comparism of treating Holstein dairy cows with Progesterone, CIDR, and GnRH after insemination on serum progesterone and pregnancy rates. *Reproduction in Domestic animals*, 47: 131-134

Pursely, J.R., Mee, M.O., and Wiltbank, M.C. (1995). Synchronozation of ovulation in dairy cows with $PGF_2\alpha$ and GnRH. *Theriogenology*, 44: 915-913.

Rahman, M. M. (2010). Management and effective treatment of post-partum anoestrus cows guided by ultrasonography, M.Sc Thesis.Department of Surgery and Obstertrics, Faculty of Veterinary Scence, Bangledesh Agricultural University, Mymensign.

Safilho, O., Meneghetti, M., Peres, R., Lamb, G. and Vasconcelos, J. L. M. (2009). Fixed time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: Strategies and factors affecting fertility. *Theriogenology,* 72 (2): 210 – 218.

Uchechukwu, O. (2017). Improving the reproductive performance of the Muturu breed of cattle in Nigeria using modified ovsynch and progesterone synchronization protocol.M.Sc Thesis.Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand.

Wagan, S. A., Memon, A. A., Kalwar, Q., Kaka, A., Hassan, M. F., Soomro, H., Kadhro, S., Sethar, A. and Jehangeer, M. (2023). Assessment of Estrus Response and Fertility Rate in THari cows following estrus synchronization during peak and low breeding season. *Pakistan Journal of Zoology,* 55(2): 843 – 848.