SRYAHWA PUBLICATIONS

Journal of Zoological Research
ISSN: 2637-5575 | Volume 8, Issue 1, 2026
https://doi.org/10.22259/2637-5575.0801001

RESEARCH ARTICLE

Ultrasonographic Determination of Follicular Dynamics, Ovulation Timing, and Endocrine Profiles in Baggara Cattle (*Bos indicus*) Following an Ovsynch Protocol

Omer Adam Ibrahim Salih, Hosameldeen Mohamed Husien, Elsir Abdelhai Babiker, Ashwag Elnour Ahmed Musaad

Faculty of Veterinary Medicine,University of Albutana, Sudan.

Received: 21 July 2026 Accepted: 05 August 2026 Published: 12 August 2026

Corresponding Author: Hosameldeen Mohamed Husien, Faculty of Veterinary Medicine,University of Albutana, Sudan.

Abstract

Background: Reproductive efficiency in cattle production systems is constrained by inefficient estrus detection, particularly in indigenous breeds with subtle behavioral signs (Mukasa-Mugerwa, 1989). Fixed-time artificial insemination (FTAI) protocols like Ovsynch offer a solution but require breed-specific validation (Baruselli et al., 2017).

Objective: This study characterized ovarian follicular dynamics, ovulation timing, and associated endocrine profiles in Baggara cattle (*Bos indicus*) subjected to the Ovsynch protocol.

Methods: Twelve mature, non-lactating, non-pregnant Baggara cows (3-6 years, 350-420 kg) received the Ovsynch protocol: 1000 µg GnRH (Day 0), 500 µg PGF2α (Day 7), and 1000 µg GnRH (Day 9) (Pursley et al., 1995). Transrectal ultrasonography was performed every 4 hours after the second GnRH injection until ovulation. Blood samples were collected concurrently for ELISA analysis of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β (E2), and progesterone (P4).

Results: The dominant follicle diameter increased progressively from 0.75±0.09 cm at 4 hours to 1.5±0.05 cm at 32 hours post-second GnRH. Ovulation occurred in 50% of cows between 32-36 hours and in 100% between 36-40 hours. Estradiol peaked at 24 hours (6.71±0.91 ng/mL), FSH at 32 hours (18.29±0.41 miu/mL), and LH at 28 hours (36.82±2.72 miu/mL). Progesterone showed a steady rise, reaching maximum concentration at 40 hours (2.72±0.23 pg/mL).

Conclusion: The Ovsynch protocol effectively synchronizes a predictable follicular wave in Baggara cattle, with ovulation occurring 36-40 hours after the second GnRH injection. These findings establish that fixed-time AI at approximately 20 hours post-second GnRH is optimally timed for this breed, providing a scientific basis for reproductive management improvement.

Keywords: Baggara Cattle, Ovsynch, Follicular Dynamics, Ovulation Synchronization, Fixed-Time AI, Reproductive Hormones.

1. Introduction

Reproductive management represents a critical determinant of profitability in both dairy and beef cattle production systems worldwide (Lucy, 2001). A persistent challenge in artificial insemination (AI) programs remains the accurate and efficient detection of estrus, which is particularly problematic in *Bos indicus*-influenced breeds that often exhibit less overt behavioral signs of standing heat compared to

Citation: Omer Adam Ibrahim Salih, Hosameldeen Mohamed Husien, Elsir Abdelhi Babeker, *et al*. Ultrasonographic Determination of Follicular Dynamics, Ovulation Timing, and Endocrine Profiles in Baggara Cattle *(Bos indicus)* Following an Ovsynch Protocol. Journal of Zoological Research. 2026; 8(1): 01-09.

their *Bos taurus*counterparts (Saini et al., 2023). The development of ovulation synchronization protocols, most notably the Ovsynch regimen (GnRH-7 days-PGF2α-2 days-GnRH), has revolutionized reproductive management by enabling fixed-time artificial insemination (FTAI) without reliance on visual estrus detection (Baruselli et al., 2017; Laven, 2019; Nowicki et al., 2017).

The physiological foundation of the Ovsynch protocol rests on precise manipulation of the bovine estrous cycle. The initial gonadotropin-releasing hormone (GnRH) administration triggers a luteinizing hormone (LH) surge, inducing ovulation of an existing dominant follicle or its luteinization, thereby synchronizing the emergence of a new follicular wave approximately 2 days later (Bó et al., 2002). The subsequent prostaglandin F2α (PGF2α) injection induces luteolysis of the corpus luteum (CL), precipitating a rapid decline in circulating progesterone concentrations. The final GnRH injection then elicits the preovulatory LH surge, culminating in synchronized ovulation approximately 24-32 hours post-injection (Wiltbank et al., 2015).

While extensively validated in *Bos taurus*dairy breeds, particularly Holstein-Friesians, the efficacy and precise follicular dynamics of Ovsynch in indigenous African cattle breeds with *Bos indicus*ancestry, such as the Sudanese Baggara cattle, remain inadequately characterized (Galina & Arthur, 1990). Breed-specific variations in follicular turnover rates, gonadotropin sensitivity, and ovarian steroidogenesis necessitate protocol validation within specific genetic backgrounds (Rekwot et al., 2001). The Baggara cattle, a humped zebu breed predominant in Western Sudan, constitute a vital genetic resource and economic asset, yet their reproductive physiology under controlled breeding management remains understudied.

This investigation was therefore designed to comprehensively characterize the ovarian response, follicular wave synchronization, and ovulation timing following administration of the Ovsynch protocol in Baggara cattle. Specific objectives were to: (1) determine the growth pattern and preovulatory diameter of the dominant follicle, (2) establish the precise interval from the second GnRH injection to ovulation, (3) elucidate the chronological relationships between follicular diameter and concentrations of key reproductive hormones (FSH, LH, E2, P4), and (4) establish an evidence-based optimal window for fixed-time AI in this breed.

2. Materials and Methods

2.1 Study Area and Ethical Approval

The study was conducted at the El-Etegahat dairy project located 64 km north of Omdurman, Sudan (16°25’39.2”N, 31°49’03.2”E), between April 2017 and March 2018. The experimental protocol was reviewed and approved by the Animal Ethics Committee of the University of Al Butana, Faculty of Veterinary Medicine. All procedures were performed in accordance with institutional guidelines for the ethical use of animals in research.

2.2 Experimental Animals and Management

Twelve clinically healthy, non-lactating, non-pregnant mature Baggara cows aged 3-6 years, weighing 350-420 kg, were utilized *(Fig. 1)*.

Figure 1. *Experimental Animals*

All animals underwent thorough gynecological examination via transrectal palpation and ultrasonography one day prior to study commencement to confirm the absence of

reproductive abnormalities, cystic structures, or pathological conditions. Animals were identified with year tags for individual monitoring.

Cows were housed in a metal-framed building with iron sheet roofing and concrete flooring, maintained under standard management conditions. The diet consisted of *ad libitum*Rhodes grass and lucerne (*Medicago sativa*), supplemented with a concentrate mixture (sorghum grain 30%, cotton seed cake 20%, wheat bran 48%, sodium chloride 1%, limestone 1%) provided twice daily according to physiological requirements. Water was available continuously.

2.3 Estrus Synchronization Protocol

Estrus was synchronized using the Ovsynch protocol as described by Pursley et al. (1995) with modifications. Each cow received intramuscular injections of: (1) 1000 µg GnRH (Buserelin acetate, 0.0042 mg/ml equivalent to 0.004 mg Buserelin; containing 2% benzyl alcohol as preservative) on Day 0; (2) 500 µg PGF2α (cloprostenol sodium, 263 µg/ml) on Day 7; and (3) 1000 µg GnRH on Day 9. All cows were inseminated 20 hours after the second GnRH injection using frozen-thawed semen from selected Holstein Friesian bulls.

2.4 Ultrasonographic Monitoring of Ovarian Structures

Ovarian examination was performed every 4 hours after the second GnRH injection (at 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40 hours) until confirmed ovulation. Examinations were conducted using a portable B-mode ultrasound scanner (SonoStar SS-9, Pie-Medical Equipment BV, China) equipped with a 5-7.5 MHz linear array transducer designed for transrectal use *(Fig. 2)*.

For each examination, feces were evacuated from the rectum, the transducer was lubricated and covered with a plastic sleeve, and systematically moved across the reproductive tract. The diameter of the dominant follicle (largest follicle >8 mm) was measured in two perpendicular planes using electronic calipers, and the average was recorded. Ovulation was defined as the complete disappearance of a previously identified dominant follicle ≥10 mm in diameter. The interval from the second GnRH injection to ovulation was recorded for each cow. All ultrasound images were stored digitally for subsequent analysis.

Figure 2. *Ultrasound Scanner*

2.5 Blood Sample Collection and Processing

Blood samples (10 mL) were collected from the jugular vein of each cow at the same time points as ultrasonographic examinations (every 4 hours from 4 to 40 hours post-second GnRH), using sterile disposable needles and syringes. Samples were collected prior to morning feeding. Blood was allowed to clot at room temperature (19-23°C) for 1-2 hours, then centrifuged at 3000 rpm for 5 minutes (ROTINA 380 centrifuge, Germany). Serum was aliquoted into Eppendorf tubes and stored at -20°C until hormone analysis.

2.6 Hormone Assays

Concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol-17β (E2), and progesterone (P4) in serum were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits according to manufacturers' protocols. Estradiol and progesterone were measured using Biorex Diagnostic Ltd (United Kingdom) kits, while FSH and LH were measured using Fortress Diagnostic Ltd (United Kingdom) kits. All assays were performed using a microplate reader (Sunrise,

TECAN, Austria) with absorbance measured at 450 nm (reference wavelength 620-630 nm). The intra- and inter-assay coefficients of variation were <10% for all hormones.

2.7 Statistical Analysis

Data are presented as mean ± standard deviation (SD). Follicular diameter measurements and hormone concentrations over time were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. The relationship between follicular diameter and hormone concentrations was examined using Pearson's correlation coefficient. All analyses were performed using SPSS version 16.0 (IBM Corporation, USA), with statistical significance set at $P < 0.05$.

3. Results

3.1 Follicular Growth Dynamics and Ovulation Timing

All 12 cows completed the Ovsynch protocol without adverse effects. The diameter of the synchronized dominant follicle exhibited progressive, linear growth following the second GnRH injection *(Table 1).* Starting at 0.75 ± 0.09 cm at 4 hours post-injection *(Fig. 3),* follicular diameter increased significantly ($P < 0.05$) to 1.1 ± 0.09 cm at 12 hours, reaching 1.28 ± 0.10 cm by 16 hours. Maximum preovulatory diameter of 1.5 ± 0.05 cm was attained at 32 hours post-second GnRH, with no further significant increase thereafter *(Fig. 4).*

Ovulation occurred in a synchronized manner, with 6 of 12 cows (50%) ovulating between 32-36 hours after the second GnRH injection *(Fig. 5)*. The remaining 6 cows (50%) ovulated between 36-40 hours, resulting in 100% ovulation by 40 hours post-injection *(Fig. 6)*. The mean interval from second GnRH to ovulation was 36.8 ± 2.4 hours. Corpus luteum formation was confirmed via ultrasonography in all cows by 4 days post-ovulation *(Fig. 7).*

Table 1. *Chronological relationships between follicle diameter and reproductive hormones during estrus synchronization in Baggara cattle (n=12)*

Hours post-2nd GnRH	Follicle Diameter (cm)	Estradiol (ng/mL)	FSH (miu/mL)	LH (miu/mL)	Progesterone (pg/mL)
4	0.75 ± 0.09	5.77 ± 0.99	16.43 ± 1.19	31.55 ± 3.28	1.37 ± 0.33
8	0.84 ± 0.05	5.93 ± 0.78	16.45 ± 1.18	30.71 ± 3.49	1.32 ± 0.18
12	1.1 ± 0.09	6.37 ± 0.69	16.69 ± 1.0	32.88 ± 1.52	1.54 ± 0.41
16	1.28 ± 0.10	6.49 ± 0.78	17.03 ± 0.94	31.83 ± 3.12	1.95 ± 0.47
20	1.28 ± 0.05	6.53 ± 0.75	17.22 ± 0.83	34.22 ± 2.02	2.29 ± 0.35
24	1.28 ± 0.07	6.71 ± 0.91	17.40 ± 0.82	34.48 ± 2.84	2.43 ± 0.35
28	1.45 ± 0.05	6.62 ± 1.08	17.90 ± 0.77	36.82 ± 2.72	2.55 ± 0.42
32	1.5 ± 0.05	6.49 ± 1.30	18.29 ± 0.41	35.61 ± 2.82	2.56 ± 0.24
36	1.5 ± 0.05	6.10 ± 1.49	17.29 ± 1.57	32.77 ± 2.72	2.55 ± 0.18
40	-	5.53 ± 0.45	16.49 ± 1.08	32.26 ± 2.36	2.72 ± 0.23

Values are mean ± SD. Different superscript letters within columns indicate significant differences ($P < 0.05$).

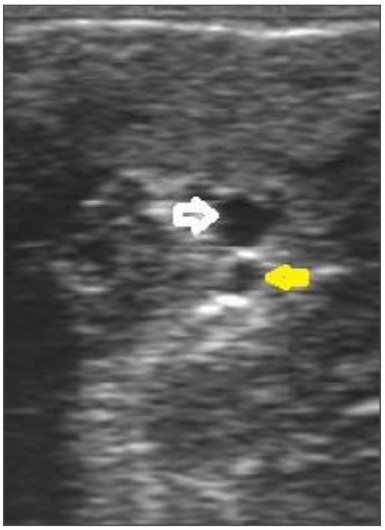

Figure 3. *Growing follicle (white arrow diameter 0.7 cm) and (yellow arrow diameter 0.4 cm) in 4 hrs.*

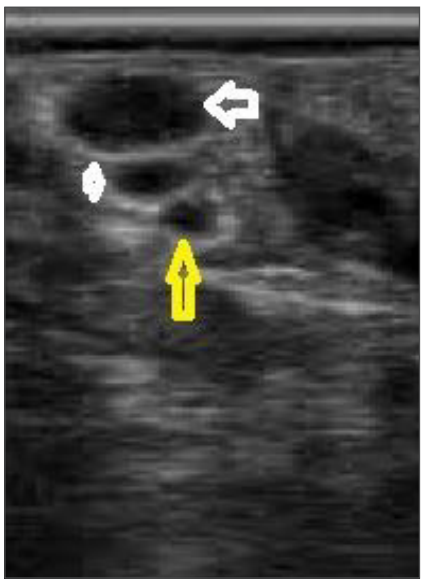

Figure 4. *Growing follicle (white arrow) diameter 1.1 cm, (white head arrow) diameter 0.6 cm and (yellow arrow) diameter 0.4 cm in 28 hrs.*

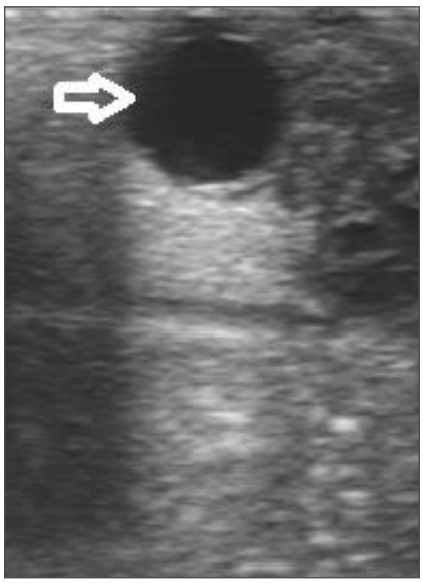

Figure 5. *Preovulatory follicle (white arrow) diameter 1.5 cm in 36 hrs.*

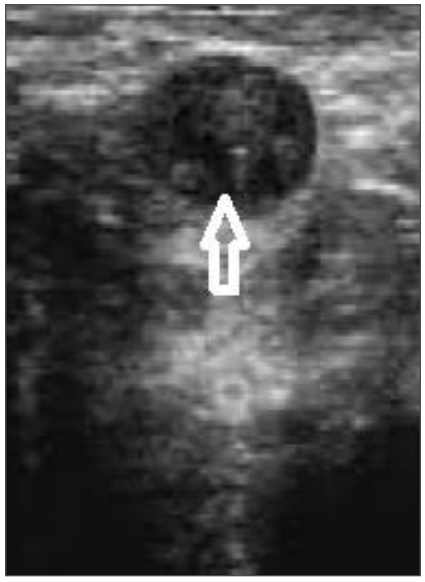

Figure 6. *Follicle after ovulation (white arrow) in 40 hrs.*

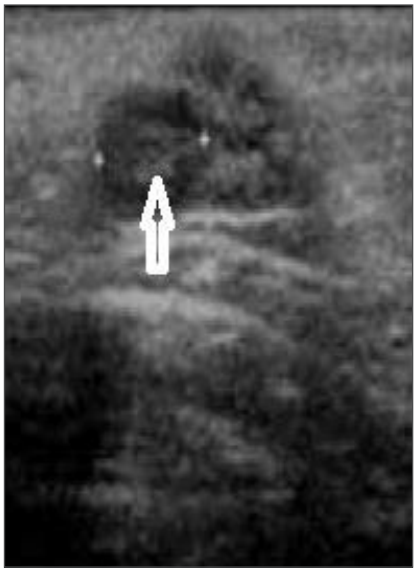

Figure 7. *Corpus luteum (white arrow) after 4 days of ovulation.*

3.2 Reproductive Hormone Profiles

3.2.1 Estradiol-17β (E2)

Serum estradiol concentrations increased progressively from 5.77 ± 0.99 ng/mL at 4 hours to a peak of 6.71 ± 0.91 ng/mL at 24 hours post-second GnRH ($P < 0.05$). Following this peak, estradiol declined significantly to 5.53 ± 0.45 ng/mL by 40 hours. A significant positive correlation was observed between follicular diameter and estradiol concentration from 4 to 24 hours ($r = 0.87$, $P < 0.01$).

3.2.2 Follicle-Stimulating Hormone (FSH)

FSH concentrations showed a gradual increase from 16.43 ± 1.19 miu/mL at 4 hours to a maximum of 18.29 ± 0.41 miu/mL at 32 hours ($P < 0.05$), coinciding with attainment of maximum follicular diameter. Subsequently, FSH decreased to 16.49 ± 1.08 miu/mL by 40 hours.

3.2.3 Luteinizing Hormone (LH)

LH concentrations displayed a distinct pattern, with levels remaining relatively stable between 30.71-34.48 miu/mL from 4 to 24 hours, then rising sharply to a peak of 36.82 ± 2.72 miu/mL at 28 hours ($P < 0.01$ compared to earlier time points). This LH surge preceded ovulation by 8-12 hours. Following the peak, LH concentrations declined to 32.26 ± 2.36 miu/mL by 40 hours.

3.2.4 Progesterone (P4)

Progesterone concentrations demonstrated a steady, progressive increase throughout the monitoring period, from 1.37 ± 0.33 pg/mL at 4 hours to 2.72 ± 0.23 pg/mL at 40 hours ($P < 0.01$). The rise in progesterone was particularly notable following the LH surge at 28 hours, consistent with initial luteinization of the preovulatory follicle.

3.3 Correlation Analysis

Significant positive correlations were observed between follicular diameter and both FSH ($r = 0.79$, $P < 0.01$) and estradiol ($r = 0.87$, $P < 0.01$) concentrations during the follicular growth phase (4-32 hours). A negative correlation was found between estradiol and progesterone concentrations ($r = -0.65$, $P < 0.05$) during the periovulatory period (24-40 hours). The LH peak at 28 hours showed a strong positive correlation with subsequent progesterone increase ($r = 0.82$, $P < 0.01$).

4. Discussion

This study provides the first comprehensive characterization of follicular dynamics and endocrine profiles in Baggara cattle subjected to the Ovsynch protocol. The findings demonstrate that this indigenous *Bos indicus*breed exhibits a predictable ovarian response to hormonal synchronization, with ovulation occurring within a consistent 36-40 hour window following the second GnRH injection.

The observed preovulatory follicular diameter of 1.5 cm in Baggara cattle is notably smaller than the 1.8-2.2 cm typically reported for *Bos taurus*dairy breeds under similar synchronization protocols (Vasconcelos et al., 1999). This size discrepancy aligns with established breed differences, as *Bos indicus*cattle generally possess smaller ovarian structures and follicles compared to *Bos taurus*counterparts (Galina & Arthur, 1990). The smaller follicular size in Baggara cattle may be attributed to genetic factors influencing follicular fluid dynamics, granulosa cell proliferation, or gonadotropin receptor density and

sensitivity. Despite the smaller diameter, these follicles demonstrated complete ovulatory competence, as evidenced by the 100% ovulation rate observed.

The ovulation timing of 36-40 hours post-second GnRH in Baggara cattle is slightly delayed compared to the 24-32 hour window commonly reported for *Bos taurus*breeds (Wiltbank et al., 2015). This extended interval may reflect differences in the kinetics of the GnRH-induced LH surge, follicular maturation processes, or the cascade of enzymatic events leading to follicular rupture. Importantly, the tight synchrony observed (all animals ovulating within an 8-hour window) validates the effectiveness of the Ovsynch protocol for fixed-time AI in this breed. Based on these findings, insemination at 20 hours after the second GnRH injection, as implemented in this study, appears well-timed to ensure adequate sperm capacitation and oviductal transport prior to ovulation.

The endocrine profiles documented in this study reveal both consistencies and subtle variations compared to *Bos taurus* cattle. The progressive rise in estradiol concomitant with follicular growth, followed by a preovulatory decline, mirrors the classic pattern observed in spontaneous estrous cycles (Norman et al., 2009). The estradiol peak at 24 hours, approximately 12-16 hours before ovulation, is consistent with its role in inducing the GnRH/LH surge and behavioral estrus. The magnitude of the LH surge (36.82 miu/mL) is comparable to that reported in synchronized *Bos taurus* heifers, suggesting similar pituitary responsiveness to GnRH in Baggara cattle.

The steady increase in progesterone following the LH surge reflects early luteinization of granulosa and theca cells, a process essential for subsequent corpus luteum formation and function. The positive correlation between LH peak magnitude and subsequent progesterone increase supports the well-established luteotrophic role of LH in cattle (Kharel et al., 2017).

The 100% ovulation response to the second GnRH injection in this study exceeds the 70-90% response rates typically reported in *Bos taurus* herds (Silva et al., 2018; Martins and Pursley, 2016). This high response rate may be attributed to the non-lactating status of experimental animals, optimal body condition, and absence of metabolic stressors that often impair ovarian responsiveness in high-producing dairy cows. Additionally, indigenous breeds like the Baggara may possess greater ovarian follicular reserve or sensitivity to gonadotropins compared to highly selected dairy breeds (Rekwot et al., 2001).

From a practical standpoint, these findings have immediate application for reproductive management of Baggara cattle. The demonstrated efficacy of Ovsynch enables reliable fixed-time AI, eliminating dependence on estrus detection—a particular advantage in pastoral systems where continuous animal observation is challenging (Kasimanickam, 2015; Andersen et al., 2021). Furthermore, the precise ovulation timing data facilitate optimization of insemination schedules to maximize conception rates.

Limitations of this study include the relatively small sample size (n=12) and the use of non-lactating cows, which may not fully represent the reproductive physiology of lactating animals under production conditions. Future research should validate these findings in lactating Baggara cows and investigate conception rates following fixed-time AI based on the established ovulation window. Additionally, exploring presynchronization protocols like Double-Ovsynch (Herlihy et al., 2012; Carvalho et al., 2018) could further optimize reproductive efficiency in production systems.

5. Conclusion

This study establishes that the Ovsynch protocol effectively synchronizes follicular wave emergence and induces precisely timed ovulation in Baggara cattle. Ovulation occurs consistently 36-40 hours after the second GnRH injection, with a preovulatory follicle diameter of approximately 1.5 cm. The documented endocrine profiles reveal coordinated hormonal dynamics that support normal follicular maturation, ovulation, and early luteal formation. These findings provide a scientific foundation for implementing fixed-time artificial insemination in Baggara cattle, with insemination recommended at approximately 20 hours after the second GnRH injection. This protocol offers a practical tool to enhance reproductive efficiency and genetic improvement in this important indigenous breed.

Acknowledgements

The authors gratefully acknowledge the staff of Al Etegahat dairy project for providing experimental animals, and to Al-Rayada laboratory for technical assistance with biochemical and hormonal analyses. Special thanks to Dr. Mohamed Fatthy, Dr. Abdulla Hassan, Dr. Muzamel atta Ali, Dr. Mohamed Abdulla, and Mr. Mohamed Awad for their support in data analysis and figure preparation.

Funding

This research received no specific grant from any

funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflicts of interest.

6. References

1. Andersen, K.J., Ketchum, J.N., Mercadante, V.R.G., 2021. Pre-synchronization improves pregnancy rates in fixed-time AI protocols in beef cows. Animal Reproduction Science 225, 106672.
2. Baruselli, P.S., Reis, E.L., Marques, M.O., Nasser, L.F., Bó, G.A., 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Theriogenology* 62, 499–509.
3. Baruselli, P.S., Ferreira, R.M., Colli, M.H.A., Elliff, F.M., Sá Filho, M.F., Vieira, L.M., Freitas, B.G., 2017. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. *Animal Reproduction* 14, 558–571.
4. Bó, G.A., Baruselli, P.S., Moreno, D., Cutaia, L., Caccia, M., Tríbulo, R., Tríbulo, H., Mapletoft, R.J., 2002. The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology* 57, 53–72.
5. Carvalho, P.D., Santos, V.G., Giordano, J.O., Wiltbank, M.C., Fricke, P.M., 2018. Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. Animal Reproduction 15(3), 257-267.
6. Dobos, A., Kovács, L., Kézér, F.L., 2022. Ovsynch-based protocols in reproductive management and infertility treatment in dairy cows – when and why? Croatian Veterinary Journal 62(2), 85-97.
7. Galina, C.S., Arthur, G.H., 1990. Review of cattle reproduction in the tropics: part 4. Oestrous cycles. *Animal Breeding Abstracts* 58, 697–707.
8. Herlihy, M.M., Giordano, J.O., Souza, A.H., Ayres, H., Ferreira, R.M., Keskin, A., Nascimento, A.B., Guenther, J.N., Gaska, J.M., Kacuba, S.J., Wiltbank, M.C., 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. Journal of Dairy Science 95(12), 7001-7009.
9. Kharel, C., Devkota, B., Sah, S., Karki, R., 2017. Evaluation of Ovsynch Protocol on Reproductive Performance of Anestrous Buffaloes during Breeding Season in Chitwan, Nepal. *Nepalese Veterinary Journal* 34, 41–50.
10. Kasimanickam, R., 2015. Timed artificial insemination in beef cattle: current status and future perspective. Animal Reproduction 12(3), 472-476.
11. Laven, R.A., 2019. An overview of the Ovsynch system for synchronization of oestrus and ovulation in cattle. Livestock 24(3), 124-129.
12. Lucy, M.C., 2001. Reproductive loss in high-producing dairy cattle: where will it end? *Journal of Dairy Science* 84, 1277–1293.
13. Martins, J.P.N., Pursley, J.R., 2016. The effect of presynchronization on the response to Ovsynch in high-producing dairy cows. Journal of Dairy Science 99(11), 8847-8855.
14. Mukasa-Mugerwa, E., 1989. A review of reproductive performance of female *Bos indicus*(zebu) cattle. *ILCA Monograph No. 6*. International Livestock Centre for Africa, Addis Ababa, Ethiopia.
15. Norman, H.D., Wright, J.R., Kuhn, M.T., Hubbard, S.M., Cole, J.B., VanRaden, P.M., 2009. Genetic and environmental factors that affect gestation length in dairy cattle. *Journal of Dairy Science* 92, 2259–2269.
16. Nowicki, A., Barański, W., Baryczka, A., Janowski, T., 2017. Ovulation synchronization in cattle – a review. Journal of Veterinary Research 61(3), 329-336.
17. Pal, P., Dar, M.R., 2019. Induction and synchronization of estrus. In: Veterinary Medicine and Pharmaceuticals. IntechOpen, London, pp. 1-20.
18. Pursley, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology* 44, 915–923.
19. Rekwot, P.I., Ogwu, D., Oyedipe, E.O., Sekoni, V.O., 2001. The effect of human chorionic gonadotrophin on the synchronization of oestrus and fertility in zebu cattle. *Animal Reproduction Science* 65, 51–60.
20. Saini, G., Kumar, S., Pandey, A.K., Yadav, V., Sharma, S., 2023. Intensity of estrus expression – valuable obvious determinant of fertility in *Bos indicus*cows. *Animal Biotechnology* 34(7), 1–10.
21. Sanz, A., Macmillan, K., Colazo, M.G., 2019. A review of ovarian synchronization programs based on GnRH and PGF2α for dairy and beef heifers. ITEA – Información Técnica Económica Agraria 115(4), 326-341.
22. Silva, L.O.E., Rodrigues, J.P., Ghedini, C.P., Vieira, F.M., Sales, J.N.S., 2018. Ovulation synchronization with P4 device prior to Ovsynch improves fertility in crossbred dairy cows. *Animal Reproduction* 15, 1187–1192.
23. Souza, A.H., Ayres, H., Ferreira, R.M., Wiltbank, M.C., 2008. A new presynchronization system (Double-

Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 70, 208–215.

24. Vasconcelos, J.L.M., Silcox, R.W., Rosa, G.J.M., Pursley, J.R., Wiltbank, M.C., 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52, 1067–1078.
25. Wiltbank, M.C., Souza, A.H., Carvalho, P.D., Bender, R.W., Nascimento, A.B., 2015. Improving fertility through timely AI based on ovulation time. *Animal Reproduction* 12, 257–263.