

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(11): 416-420
www.biochemjournal.com
 Received: 15-09-2025
 Accepted: 19-10-2025

Dr. Ranganath GJ
 Scientist Animal Science
 ICAR-KVK, Uttara Kannada,
 Sirsi, University of
 Agricultural Sciences,
 Dharwad, Karnataka, India

Dr. Prathviraj H
 Assistant Professor,
 Department of Veterinary
 physiology and Biochemistry
 Veterinary College Bidar,
 KVAFSU, Karnataka, India

Dr. Roopa devi YS
 Assistant professor
 Department of Veterinary
 Pathology Veterinary College
 Hebbal Bengaluru, KVAFSU,
 Karnataka, India

GR Halagundegowda
 Scientist -C, Statistics section,
 Central Silk Board, Bengaluru,
 Karnataka, India

Dr. Ananth Krishna L.R
 Associate Professor
 Department of Veterinary
 physiology and Biochemistry
 Veterinary College Gadag,
 KVAFSU, Karnataka, India

Corresponding Author:
Dr. Ranganath GJ
 Scientist -Animal science
 ICAR-KVK, Uttara Kannada,
 Sirsi, University of
 Agricultural Sciences,
 Dharwad, Karnataka, India

Comparative evaluation of treatment regimens for repeat breeding in cattle and buffalos of Uttara Kannada under field condition

Ranganath GJ, Prathviraj H, Roopa devi YS, GR Halagundegowda and Ananth Krishna LR

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i11f.6304>

Abstract

Maternal factors, hereditary influences, uterine infections, genital tract abnormalities, hormonal imbalances, and several other variables contribute to the occurrence of repeat breeding (RB). Selection of an appropriate therapeutic strategy therefore depends on the underlying etiology. In the present study, RB animals were treated using four different estrus-synchronization protocols with timed artificial insemination (TAI): the CIDR-Ovsynch protocol, the Ovsynch protocol, the Double-PG protocol, and the G6G protocol. Animals diagnosed with clinical endometritis were allocated into two treatment groups. One group received an intrauterine suspension of cephalixin and serratiopeptidase combined with a single intramuscular dose of cloprostenol, while the second group was treated with the intrauterine suspension alone. Similarly, animals diagnosed with subclinical endometritis were divided into two groups: one group received a single dose of cloprostenol (75 µg/ml) following intrauterine saline lavage, whereas the other group underwent saline lavage alone.

The outcomes of the study show that the CIDR-Ovsynch protocol resulted in a significantly higher pregnancy rate (PR) of 70.96% (22/31) compared with the Ovsynch protocol, which achieved a PR of 45.71% (16/35) ($t = 2.11$, $P=0.04$). Among animals with delayed ovulation, the G6G protocol yielded the highest PR (65.62%; 21/32), followed closely by the Double-PG + GnRH protocol (62.16%; 23/37). However, this difference was not statistically significant ($t = 0.29$, $P=0.77$). In animals with clinical endometritis, treatment combining intrauterine cephalixin and serratiopeptidase with PGF_{2α} resulted in a higher PR of 46.66% (7/15) compared to treatment without PGF_{2α} (33.33%; 4/12), though this difference was not statistically significant ($t = 0.84$, $P=0.41$). Among animals with subclinical endometritis, the combination of saline lavage and PGF_{2α} produced a PR of 41.66% (5/12), which was higher than saline lavage alone (11.11%; 1/9), but again statistically non-significant ($t = 1.55$, $P=0.14$). In total, 183 animals were included in the study. These findings provide evidence-based guidance for veterinarians in selecting effective treatment strategies to enhance reproductive performance in repeat-breeding cattle and buffaloes.

Keywords: Repeat breeder, CIDR-ovsynch, ovsynch, endometritis, synchronization

Introduction

Reproductive efficiency is a key determinant of the economic sustainability of dairy enterprises. Achieving an optimal calving interval ideally one calf per cow per year is essential for maintaining steady milk production and effective herd replacement. However, repeat breeding (RB) remains a significant challenge for both dairy farmers and veterinarians. Multiple factors contribute to RB, including maternal and hereditary influences, uterine infections, genital tract abnormalities, hormonal imbalances, and various other reproductive disorders [1, 2]. Several diagnostic tools, such as vaginoscopy, uterine cytology, recto-genital palpation, and ultrasonography, are considered valuable for identifying the underlying causes of RB. In routine field conditions, however, clinicians frequently rely predominantly on vaginoscopy and palpation due to their practicality and accessibility. Despite advancements in diagnostic methods including hormonal assays, ultrasonography, and hysteroscopy accurately determining the cause of pregnancy failure remains difficult in some cows and buffaloes, particularly when infertility presents without clear clinical abnormalities.

To improve pregnancy outcomes, advanced reproductive management strategies have been developed. A variety of estrus-synchronization protocols with timed artificial insemination (TAI) are used globally, with commonly adopted regimens including CIDR-Ovsynch, Ovsynch, Double-Ovsynch, Double-PG with GnRH, Pre-synch Ovsynch, and the G6G protocol [3, 4]. Likewise, therapeutic strategies for endometritis frequently involve intrauterine administration of antibiotics, with or without concurrent PGF $_{2\alpha}$ administration, and uterine saline lavage [5-7].

The variability in outcomes reported by different researchers following hormonal treatments may be largely attributed to factors such as nutritional status, suboptimal management practices, ovarian alterations, endocrine fluctuations, and underlying uterine infections. To improve treatment efficacy and pregnancy outcomes, the present study adopted a targeted therapeutic approach based on the presumed etiology of repeat breeding (RB). Repeat-breeding animals were subjected to four estrus-synchronization protocols with timed artificial insemination (TAI): the CIDR-Ovsynch protocol, the Ovsynch protocol, the Double-PG protocol, and the G6G protocol. In addition, treatment regimens incorporating intrauterine antibiotics or saline lavage, with or without PGF $_{2\alpha}$ administration, were evaluated for animals diagnosed with endometritis.

The findings from this study provide a comparative assessment of these reproductive management strategies in cattle and buffaloes, highlighting the relative effectiveness of each protocol for addressing RB and endometritis. These results offer valuable insights for optimizing reproductive efficiency and improving herd productivity in dairy systems.

Materials and methods

Selection of animals with reproductive disorders

Animals exhibiting repeat breeding and presented to the KVK scientists for diagnostic evaluation, as well as those examined during infertility camps conducted across various regions of the Uttara Kannada district between 2019 and 2025, were included in the present investigation. A total of 183 animals exhibiting repeat breeding at different stages of lactation were categorized into functional repeat breeders and endometritis. Classification was based on a comprehensive assessment that included reproductive history, clinical evaluation, and per-rectal examination of the uterus, ovaries, genital tract, and associated reproductive fluids.

Treatment protocols employed

Functional repeat breeder (RB) animals were further categorized into anovulators (absence detectable CL) and delayed ovulators (presence of detectable CL) and subjected to four different synchronization protocols with timed artificial insemination (TAI), including the CIDR-Ovsynch protocol, Ovsynch protocol, Double PG protocol, and G6G protocol.

Animals diagnosed with clinical endometritis were segregated in to two groups, one group treated using an

intrauterine suspension of Cephalexin and Serratiopeptidase (Uteriguard I.U., Zenex Animal Health India Pvt. Ltd.) combined with a single dose of Cloprostenol (75 mcg/ml) and second group treated with intrauterine suspension of Cephalexin and Serratiopeptidase (Uteriguard I.U.) alone. Similarly, animals diagnosed with subclinical endometritis were separated in to two groups, one groups a single dose of Cloprostenol (75 mcg/ml) along with intrauterine saline infusion and second with uterine saline lavage alone.

Throughout the treatment period, all animals received multivitamin and mineral supplementation along with anthelmintic therapy. Animals were systematically monitored for reproductive response, including the interval to onset of estrus post-treatment, duration of estrus, number of inseminations per conception, conception rate, and overall pregnancy rate, the pregnancy was determined on day 60 to 65 by per-rectal examination.

CIDR-Ovsynch protocol

On day 0, all cows and buffaloes were administered a CIDR device containing 1.38 g of progesterone (Eazi Breed™ CIDR® 1380, Zoetis) via intravaginal insertion, concurrently with an intramuscular injection of 10µg GnRH (Buserelin acetate, 4 µg/mL, GynarichIntas Pharmaceuticals Ltd). On day 7, the CIDR devices were removed, and each animal received a 2 ml PGF $_{2\alpha}$ analogue (Cloprostenol, 75 µg/mL; Pragma or Pragma-D, Intas Pharmaceuticals Ltd). On day 9, a second dose of GnRH (10µg) was administered, followed by fixed-time artificial insemination (FTAI) 16-18 hours later (i.e., on day 10), as illustrated in Figure 1.

Ovsynch protocol

On day 0, animals were administered GnRH (10µg Buserelin acetate; Gynarich). On day 7, 2 mL of a PGF $_{2\alpha}$ analogue (Cloprostenol, 75 µg/mL) was administered intramuscularly. A second GnRH injection was given on day 9, followed by fixed-time artificial insemination (FTAI) 16-20 hours later (day 10), as illustrated in Figure-2.

Double PG with Gynarich(PPG) protocol

Repeat-breeding animals received two intramuscular injections of PGF $_{2\alpha}$ (2 mL Cloprostenol, 75 µg/mL, Pragma) at 11 days interval, followed by an injection of GnRH (10 µgBuserelin acetate) 48 hours later (day 13). Fixed time AI was performed 16-20 hours after the GnRH injection (day 14).

G6G protocol

Animals received an intramuscular injection of PGF $_{2\alpha}$ (2mL, Cloprostenol, 75 µg/mL) on day 0, followed by GnRH (Buserelin acetate, 10 µg) on day 2. The Ovsynch protocol commenced six days later: GnRH (10 µgBuserelin acetate) on day 8, PGF $_{2\alpha}$ (2mL, Cloprostenol, 75 µg/mL) on day 15, and a second GnRH injection administered 56 hours after PGF $_{2\alpha}$ (i.e., day 17). Fixed-time artificial insemination (FTAI) was performed 16-18 hours after the second GnRH treatment (i.e., day 18), as illustrated in Figure 4.

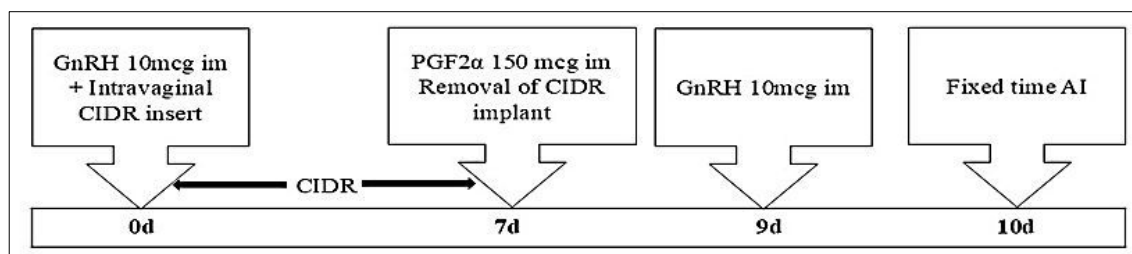


Fig 1: CIDR- Ovsynch Protocol

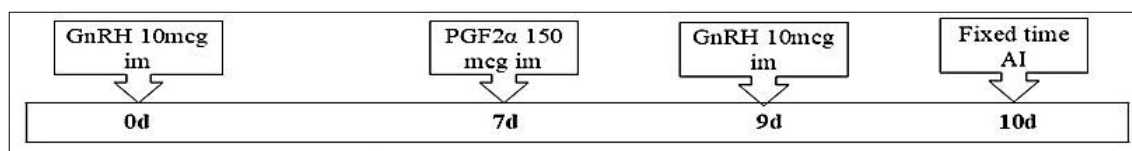


Fig 2: Ovsynch Protocol

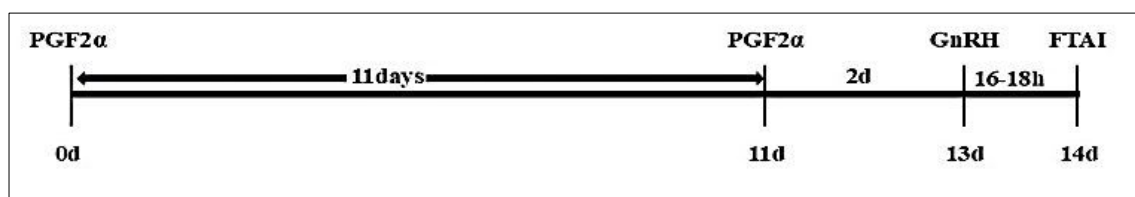


Fig 3: Double PG+GnRH Protocol

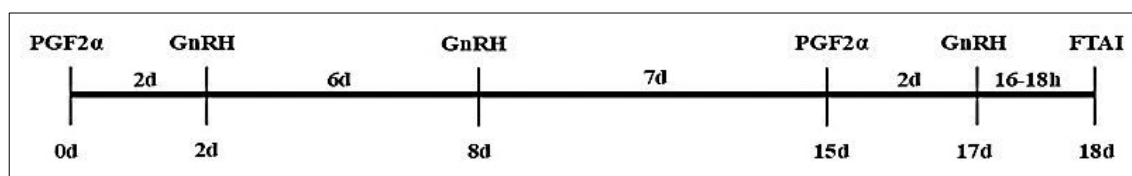


Fig 4: G6G synchron protocol

Results

In the present study, 183 repeat-breeding cattle and buffaloes at various stages of lactation were treated using different estrus-synchronization protocols, as summarized in Table 1. Based on clinical examination, functional repeat breeders were categorized into two groups: repeat breeders with anovulation, in which a functional corpus luteum (CL) was not detectable, and repeat breeders with delayed ovulation. Animals classified as repeat breeders with anovulation were treated using either the CIDR-Ovsynch protocol or the Ovsynch protocol. The pregnancy rate obtained with the CIDR-Ovsynch protocol was significantly higher than that achieved with the Ovsynch protocol ($t = 2.11$, $P=0.04$).

Similarly, repeat breeders with delayed ovulation were treated using either the double-PG + GnRH protocol or the G6G protocol. Although the pregnancy rate was higher in animals treated with the G6G protocol compared to the double-PG + GnRH protocol, the difference was not statistically significant ($t = 0.29$, $P=0.77$). Overall, one-way ANOVA revealed no statistically significant differences

among the four treatment groups. The F-value (1.67) was low, and the associated P -value (0.18) exceeded the conventional significance threshold of 0.05.

Animals diagnosed with endometritis were classified into two categories: clinical endometritis and subclinical (non-clinical) endometritis. In animals with clinical endometritis, the pregnancy rate following treatment with intrauterine cephalaxin combined with serratiopeptidase and intramuscular $PGF_{2\alpha}$ was higher than that achieved with intrauterine cephalaxin alone; however, this difference was not statistically significant ($t = 0.84$, $P=0.41$).

Similarly, in animals with subclinical endometritis, the pregnancy rate was higher in those treated with uterine lavage using saline followed by $PGF_{2\alpha}$ administration compared to those treated with saline lavage alone, but the difference did not reach statistical significance ($t = 1.55$, $P=0.14$). Overall, one-way ANOVA demonstrated no statistically significant differences among the four treatment groups. The F-value (1.11) was low, and the corresponding P -value (0.35) exceeded the conventional significance threshold of 0.05.

Table 1: Details of different treatment regimens used for management of repeat breeding, estrus induction rate and pregnancy rate

Description of the conditions	Treatment protocol employed	No. of animals treated (n)	Estrous induction (%)	PR @FTAI (%)	PR @ 2 nd estrous (%)	PR (%)
1. Repeat breeder with non-detectable CL (anovulators)	1.1 CIDR-Ovsynch	31	100.00 (31)	58.06 (18)	12.90 (4)	70.96 (22)
	1.2 Ovsynch protocol	35	91.42 (32)	40.00 (14)	5.71 (2)	45.71 (16)
2. Repeat breeder with detectable CL (delayed ovulators)	2.1 Double PG with GnRH protocol	37	91.89 (34)	59.45 (22)	2.70 (1)	62.16 (23)
	2.2 G6G protocol	32	93.75 (30)	65.62 (21)	0.0	65.62 (21)
3. Endometritis- Clinical	3.1 Cephalixin and Serrati peptidase IU + PGF2 α injection	15	93.33 (14)	20.00 (3) *	26.66(4) *	46.66 (7)
	3.2 Cephalixin and Serrati peptidase IU	12	75.00 (9)	16.66 (2) *	16.66 (2) *	33.33 (4)
4. Endometritis- Sub-clinical	4.1 Saline lavage + PGF2 α injection	12	91.66 (11)	25.00 (3) *	16.66 (2) *	41.66 (5)
	4.2. Saline lavage alone	9	77.77 (7)	0.0	11.11 (1) *	11.11 (1)
Total numbers		183				

Note: PR@FTAI: Pregnancy rate in cows artificially inseminated at fixed time during synchronization protocol

PR @ 2nd estrous: Pregnancy rate in cows that failed to conceive following fixed-time artificial insemination (FTAI) and were subsequently inseminated at the next observed natural estrus.

* Cows or heifers inseminated during first observed natural estrus

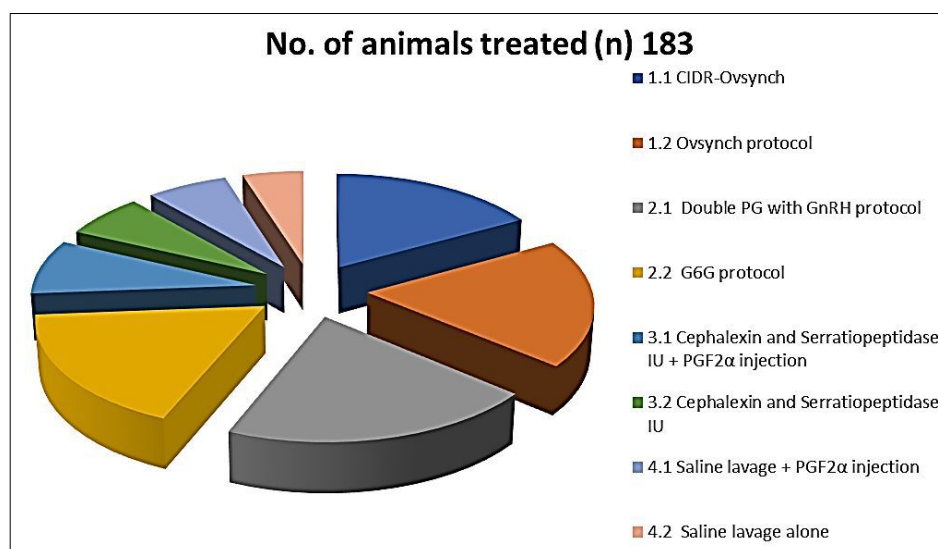


Fig 4: Picture depicting the total number of animals treated during the research period with different treatment protocol for Repeat breeding

Discussion

The results presented in the table 1. provide valuable insights into the efficacy of various therapeutic and hormonal synchronization protocols for managing different reproductive disorders in animals, specifically repeat breeders (anovulators and delayed ovulators) and those suffering from clinical or sub-clinical endometritis. The primary objective was to evaluate the pregnancy rates (PR) achieved through these interventions.

Repeat Breeders

For repeat breeders classified as anovulators (non-detectable CL), both the CIDR-Ovsynch and standard Ovsynch protocols were employed. The CIDR-Ovsynch protocol demonstrated significantly superior efficacy with a final PR of 70.96% (22/31) compared to the Ovsynch protocol's 45.71% (16/35). However similar findings were also been reported in the recent research on beef cattle [8]. The high estrous induction rate (100%) with CIDR-Ovsynch indicates excellent synchronization success, which translated into a high PR at fixed-time artificial insemination (FTAI) (58.06%). Further the outcome of the current research is

found to be similar to the findings of the above protocol in repeat breeding crossbred jersey cows [9]. This suggests the inclusion of a CIDR insert is critical for effective synchronization in anovulatory animals, likely due to better progesterone supplementation and subsequent luteal regression synchronization. In addition the above protocol on repeat breeder is effective as reported by the recent research on estrous synchronization protocol in recipient cow for embryo transfer [10]. However, a contradicting result was reported wherein the pregnancy rate was non-significantly differing when lactating cows synchronized with ovsynch, double ovsynch and CIDR-Ovsynch protocol [4].

In repeat breeders with a detectable CL (delayed ovulators), both the Double PG with GnRH and the G6G protocols performed well. The G6G protocol achieved the highest overall PR in this category at 65.62% (21/32), closely followed by the Double PG with GnRH protocol at 62.16% (23/37) which is also comparable and found similar outcome as reported earlier studies [11]. However, a comparatively lower pregnancy rate (36.69%) reported when HF and Simmental breeds of cows synchronized with G6G protocol

^[12]. These rates are slightly lower than the best outcome for anovulators but still indicate effective management strategies for managing delayed ovulation. Notably, the PR @ 2nd estrus was zero for the G6G group, suggesting that most successful pregnancies occurred at the FTAI as reported recently ^[13].

Endometritis: The treatments for endometritis, both clinical and sub-clinical, showed considerably lower total pregnancy rates compared to the repeat breeder protocols. For clinical endometritis, the protocol combining Cephalixin and Serratiopeptidase with a PGF2 α injection resulted in a higher PR of 46.66% (7/15) compared to the protocol without PGF2 α , which yielded a PR of 33.33% (4/12). Which is also reported recently with similar findings in cross bred cattle endometritis treatment in India ^[5]. This outcome emphasizes the importance of PGF2 α in inducing luteolysis and promoting uterine clearance, which is a crucial aspect of managing uterine infections. The higher estrous induction rate (93.33%) in the PGF2 α group supports this interpretation these findings in the current research work is comparable with the outcome of the recent reports on cows ^[6].

A similar trend was observed for sub-clinical endometritis treatments. The saline lavage protocol combined with a PGF2 α injection resulted in a 41.66% PR (5/12), which was significantly higher than the 11.11% PR (1/9) observed when saline lavage was used alone. The inclusion of PGF2 α appears to be a critical factor in improving pregnancy outcomes for both clinical and sub-clinical cases of endometritis by enhancing the uterine environment for conception. Recent reports with similar protocol using uterine lavage along with Levamisole or PGF2 α can be used effectively to manage endometritic buffaloes ^[7].

Overall, the data suggests that the treatments for repeat breeder conditions generally yield higher pregnancy success rates compared to the treatments for endometritis. Further Hormonal synchronization protocols, especially those involving P4 supplementation, offer high efficacy for repeat breeders. For endometritis, incorporating PGF2 α appears essential for improving uterine health and subsequent pregnancy rates, a result supported by literature suggesting PGF2 α aids in uterine clearance. These results can guide veterinarians in selecting optimal, evidence-based interventions to improve reproductive efficiency in affected animals.

Conclusion: The data clearly indicates that the treatment protocols for repeat breeders are more successful in achieving pregnancy than those for endometritis. The highest overall pregnancy rates were observed in the protocols for repeat breeders with a detectable CL. This could be due to the nature of the conditions, as endometritis involves inflammation of the uterus, which may be a more significant barrier to conception than the hormonal imbalances in repeat breeders.

Acknowledgement: Authors are thankful to the Vice chancellor UAS Dharwad, Director of Extension, Director of research UAS Dharwad for accomplishing the research work in time.

References

1. Singh M, Sharma A, Kumar P. Repeat breeding and its treatment in dairy cattle of Himachal Pradesh (India) - a review. *Indian J Anim Reprod.* 2017;38(2):
2. Ranganath GJ, Prathviraj, Chandrashekara N, Harish DK, Ramesh D. Study on reproductive issues in dairy cattle. *The Pharma Innovation J.* 2022;11(SP-10):1351-1355.
3. Dhami AJ, Nakrani BB, Hadiya KK, Patel JA, Shah RG, *et al.* Comparative efficacy of estrus synchronization protocols on estrus induction response, fertility, and plasma progesterone and biochemical profile in crossbred anestrous cows. *Vet World.* 2015;8.
4. Vazquez Blandria R, Randi F, Viora L, Denholm K, Pepler PT, *et al.* Comparison of three reproductive management strategies for lactating dairy cows using estrus detection or ovulation synchronization and fixed-timed artificial insemination. *Anim Reprod Sci.* 2023;257:107331:1.
5. Panigrahi SK, Behera H, Dhar S, Mishra M, Sethy K, *et al.* Evaluating therapeutic efficacy of cephalixin and PGF2 α in the management of endometritis in crossbred cattle. *Indian J Vet Sci Biotechnol.* 2025;21(5):119-123.
6. Barański W, Zduńczyk S, Tobolski D, Krupa M, Zduńczyk S, *et al.* Fertility outcomes in cows with subclinical endometritis after clinical cure of clinical endometritis. *Ir Vet J.* 2024;77(1):20:1-10.
7. Kumar R, Singh B, Singh N, *et al.* Effect of uterine lavage, levamisole, PGF2 α and its combinations on haematological indices and bacterial load in estrual mucus of endometritic buffaloes. *Indian J Vet Sci Biotechnol.* 2023;19(2):28-33.
8. Ha JG, Kim TG, Kim SH, Lee SY, Kim SB, *et al.* Effects of synchronized ovulation protocols on reproductive performance of beef cattle in Korea: a retrospective study of 755 cases. *Vet Sci.* 2025;12(10):1001:1.
9. Jayaganthan PV, Vijayarajan A, Vaiyapuri P, Prabakaran R, Rajkumar A, *et al.* Effect of Ovsynch plus CIDR protocol in management of repeat breeding crossbred Jersey cows. *Int J Cogn Technol.* 2016;5:3707-3712.
10. Salman M, Reddy KR, Venkataramana K, Aruna Kumari G, Swathi B, *et al.* Evaluation of estrus synchronization protocols (Ovsynch vs CIDR) in recipient cows in embryo transfer programme. *The Pharma Innovation J.* 2023;12(SP-9):931-935.
11. Patron R, López-Helguera I, Pesantez J, Villalobos N, Heras J, *et al.* Resynchronization with the G6G protocol: a retrospective study of second and later timed artificial inseminations on commercial dairy farms. *Reprod Domest Anim.* 2018;54.
12. Khalil AAYA. Fertility response of lactating dairy cows subjected to three different breeding programs under subtropical conditions. *Beni-Suef Univ J Basic Appl Sci.* 2019;8(6):1-10.
13. Pérez-Marín CC, Quintela LA, *et al.* Current insights in repeat breeder cow syndrome. *Animals.* 2023;13(13):2187:1