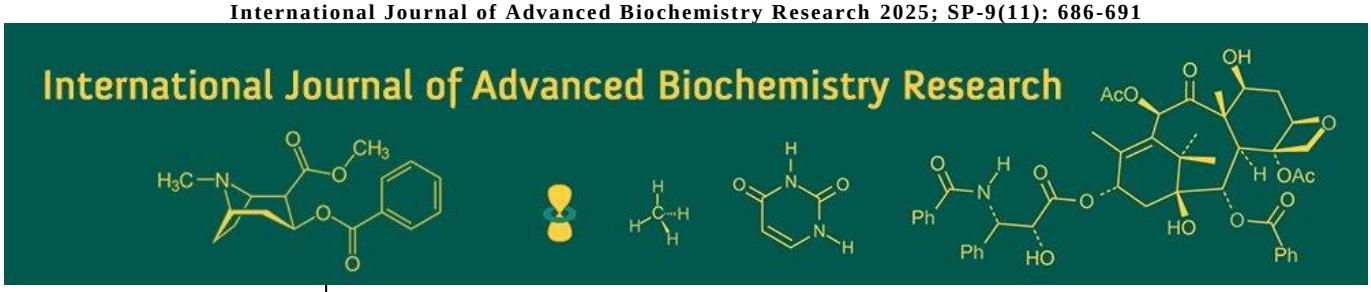


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# Field based comparative study of therapeutic protocols for anestrus and ovarian cysts in cattle and buffaloes of Uttara Kannada

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**Abstract**
The present study evaluated the efficacy of CIDR-Ovsynch, Double Ovsynch, Ovsynch, single-dose GnRH, and nutritional supplementation protocols for the management of true anestrus and ovarian cysts in cattle and buffaloes. In multiparous anestrous animals, both CIDR-Ovsynch and Double Ovsynch achieved high estrous induction rates (100% and 89.47%, respectively), with CIDR-Ovsynch producing a non-significantly higher pregnancy rate (77.27%) (t-stat- 0.98, P-0.33). Among primiparous anestrous heifers, CIDR-Ovsynch again resulted in significantly higher pregnancy rate (69.23%) compared with Double Ovsynch (58.33%), and national correction group (30.43%), despite slightly lower estrous induction rates than multiparous cows (F-4.9, P-0.02). These findings highlight the influence of progesterone supplementation in enhancing cyclicity, likely through improved modulation of hypothalamic-pituitary activity and support of oocyte and embryo development.
Nutritional supplementation alone yielded comparatively low estrous induction (58.69%) and pregnancy rates (30.43%), indicating that while nutrition is fundamental for reproductive health, it is insufficient as a standalone treatment for established anestrus.
For the treatment of ovarian cysts, CIDR-Ovsynch achieved the highest pregnancy rate (47.05%), outperforming Ovsynch (21.42%) and single-dose GnRH (28.57%). Overall, the findings indicate that CIDR-Ovsynch is the most effective protocol for managing both true anestrus and ovarian cysts, outperforming other hormonal and nutritional strategies by restoring ovarian function and improving conception rates.

**Keywords:** CIDR-Ovsynch, Ovsynch protocol, GnRH, Aestrous, Ovarian cyst, Primiparous, multiparous

## Introduction
The reproductive efficiency dairy herds is critically dependent on achieving an optimal calving interval, which is frequently compromised by reproductive disorders, with anestrus and anovulation being among the most prevalent. Anestrus absence of ovarian cyclicity, accounts for approximately 45% of all reproductive failures in both primiparous and multiparous cows [1]. The etiology of postpartum anestrus and anovulation is multifactorial and includes parity, negative energy balance and excessive body condition score loss, metabolic and uterine diseases, nutritional imbalances, and environmental or management-related stress [2]. Early identification of anovular cows and implementation of targeted interventions are essential for minimizing the voluntary waiting period and improving overall fertility of cows.
A range of therapeutic and synchronization protocols has been developed to address anestrus and induce cyclicity, with selection of the most appropriate protocol dictated by the underlying cause, herd management system. Widely adopted protocols worldwide include progesterone-based programs such as CIDR-Synch, Presynch-Ovsynch, Double-Ovsynch, and GPG (GnRH-PGF$_2\alpha$-GnRH), as well as the norgestomet ear implant, although pregnancy per AI and submission rates vary considerably depending on cow-level factors and timing of application [3, 4].
Continued research into the optimization of these protocols and the integration of precision dairy farming technologies for earlier detection of anovulatory conditions remains crucial for sustainable intensification of dairy production.

Ovarian cysts in dairy cattle are defined as persistent fluid-filled structures exceeding 20 mm in diameter on one or both ovaries, in the absence of a functional corpus luteum, that fail to ovulate or regress normally [5]. These structures are classified as follicular cysts when the cyst wall thickness is <3 mm and luteal cysts when it is ≥3 mm. Reported incidence rates in lactating dairy cows range from 2.7% to 15.1%, depending on herd management, detection method, and postpartum interval examined [1, 6].

The predominant etiopathogenetic mechanism involves disruption of the preovulatory luteinizing hormone (LH) surge within the hypothalamic-pituitary-gonadal (HPG) axis. This disruption is primarily attributed to altered hypothalamic-pituitary sensitivity to estradiol feedback, resulting in inadequate pulsatile release of gonadotropin-releasing hormone (GnRH) and/or a blunted or absent LH surge [7]. Additional factors known to impair GnRH/LH secretion at the hypothalamo-pituitary level include negative energy balance, metabolic stress, endotoxemia, inflammatory mediators, and elevated cortisol concentrations, all of which can exacerbate the risk of cyst development in the early postpartum period.

The aim of this study is to evaluate the different synchronization protocols with TAI and nutritional correction strategies in management of true anestrous in multiparous and primiparous animals and Ovarian cysts. Which include, CIDR-Ovsynch, Double Ovsynch, Ovsynch and Single dose of GnRH injection protocols and nutritional corrections through supplementation. By analyzing estrous induction and pregnancy outcomes, the study seeks to provide a comprehensive understanding of how protocol selection and insemination strategy influence fertility.

## Materials and Methods

### Selection of animals with reproductive disorders

Animals exhibiting anestrum and altered estrous behaviours presented to Krishi Vigyan Kendra (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 study. A total of 157 animals were categorized into four groups: true anestrous multiparous, true anestrous primiparous, anestrous primiparous with underdeveloped ovaries, and animals with ovarian cysts. Classification was based on a comprehensive evaluation encompassing reproductive history, clinical examination, and per-rectal assessment of the uterus, ovaries, genital tract, and associated reproductive fluids.

### Treatment protocols employed

True anestrus was managed through Hormonal treatment comprised CIDR-Ovsynch and Double Ovsynch protocols for estrus induction and synchronization. Primiparous animals with under developed ovaries were provided with balanced concentrate 1.5-2.0 kg per animal per day, supplemented with probiotics containing bypass protein (15 g/day; Biobloom, Zenex Animal Health India Pvt. Ltd.), bypass fat (20 g/day; V-Fat, Varsha Multi Tech Pvt. Ltd.), and a vitamin-mineral mixture (50-60 g/day; Chelated Ultramin Forte, Neospark Drugs and Chemicals Pvt. Ltd.). The supplementation regimen was continued for 2-3 months to restore reproductive function and improve metabolic efficiency.

Animals with ovarian cysts (OC) were treated using the CIDR-Ovsynch protocol, Ovsynch protocol, and single-dose Buserelin acetate 21 µg (Receptal, MSD, Animal Health or Gynarich, Intas Pharmaceutical Ltd.).

Throughout the treatment period, all animals were supplemented with multivitamins and minerals and received anthelmintic therapy. Reproductive performance was systematically monitored by recording the interval to the onset of estrus following treatment, duration of estrus, number of inseminations per conception, conception rate, and overall pregnancy rate. Pregnancy diagnosis was performed between days 60 and 65 post-insemination via per-rectal examination.

### IDR-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 10 µg 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 (10 µg) 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 Ovsynch protocol

In this group included total 26 anestrous animals. The regimen began with an initial GnRH injection (G0) on day 0, followed by $PGF_2\alpha$ (PG1) on day 7, and a second GnRH injection (G1) on day 10 to initiate a new follicular cycle. A third GnRH injection (G2) was administered on day 17, followed by $PGF_2\alpha$ (PG2) on day 24 to induce luteolysis. The breeding GnRH was given on day 26, and fixed time AI was performed 16-18 hours later (i.e day 27), as illustrated in figure-3.

### Statistical analysis

The pregnancy rate within-group comparisons were performed using paired t-tests (in case of two treatment variables) to determine the significance of treatment.f ANOVA was employed, for studying the significance between treatment in case of more than 3 variable treatment protocols involved. The level of significance expressed at $P \leq 0.05$.

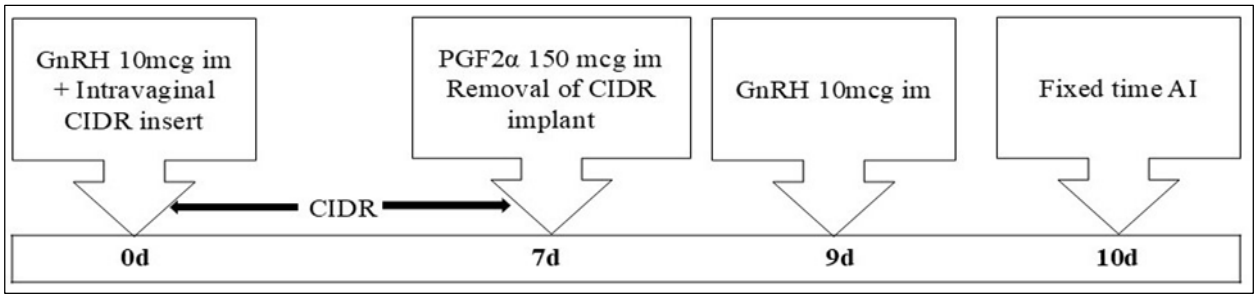


**Fig 1:** CIDR-Ovsynch protocol

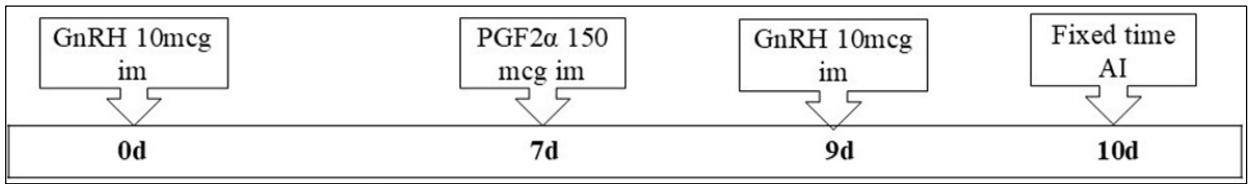


**Fig 2:** Ovsynch protocol

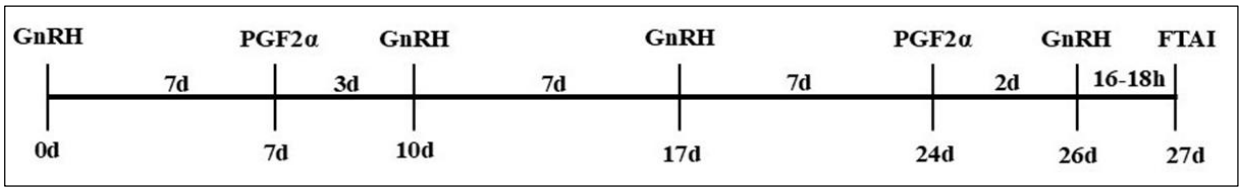


**Fig 3:** Double Ovsynch protocol

## Results

In the present investigation, a total of 157 animals with reproductive disorders were clinically examined and classified into four categories: true anestrus multiparous cows with inactive smooth ovaries, true anestrus primiparous cows with developed smooth ovaries, primiparous cows exhibiting flat or underdeveloped ovaries, and cows diagnosed with ovarian cysts (Table 1).

True anestrus multiparous cows (n=41) were randomly allocated to two treatment groups. Group 1.1 (n=22) received the CIDR-Ovsynch protocol, whereas Group 1.2 (n=19) was subjected to the Double-Ovsynch protocol. Estrus induction response was 100% and 89.47% respectively. The conception rate in the CIDR-Ovsynch group was 77.27% (17/22), which was numerically higher than the 63.63% (12/19) achieved with Double-Ovsynch. However, the difference in pregnancy rate between the two synchronization protocols did not reach statistical significance ($t = 0.98$, $P = 0.33$).

True anestrus primiparous cows possessing developed but inactive smooth ovaries (n=25) were randomly allocated to two treatment groups. Group 2.1 (n=13) received the CIDR-Ovsynch protocol, whereas Group 2.2 (n=12) was subjected to the Double-Ovsynch protocol. Estrus induction response was 100% in both groups. The conception rate was higher in the CIDR-Ovsynch group (69.23%; 9/13) than in the Double-Ovsynch group (58.33%; 7/12)

Similarly, true anestrous primiparous animals were randomly allotted in to two groups, group one (2.1) comprised of 13 animals which are treated with CIDR-Ovsynch protocol and group two (2.2) comprised of 12 animals which are treated with double ovsynch protocol. The estrous induction and pregnancy rate achieved in CIDR-Ovsynch protocol was higher (PR-69.23%) when compared to double ovsynch protocol (PR-58.33%).

Primiparous cows diagnosed with inactive flat or underdeveloped ovaries (n = 46) were subjected to a targeted nutritional intervention daily supplementation of bypass protein, bypass fat, probiotic mineral-vitamin mixture, with concentrate feeding. This management strategy resulted in an estrus induction rate of 58.69% (27/46) and a subsequent pregnancy rate of 30.43% (14/46) following the induced estrus.

One-way analysis of variance comparing pregnancy rates across the three treatment modalities applied to anestrus primiparous cows (CIDR-Ovsynch, Double-Ovsynch, and intensive nutritional supplementation) revealed a statistically significant difference among group means ($F = 4.19$, $P = 0.02$).

For the treatment of animals diagnosed with ovarian cysts, three hormonal protocols were evaluated: CIDR-Ovsynch, Ovsynch, and a single intramuscular injection of GnRH. The CIDR-Ovsynch protocol resulted in the highest estrus induction rate (76.47%), followed by Ovsynch and single-dose GnRH treatments (71.40%). Similarly, the CIDR-Ovsynch group achieved the highest pregnancy rate (47.05%, 8/17), compared with the GnRH single-dose group (28.57%, 4/14) and the Ovsynch protocol (21.42%, 3/14). A one-way ANOVA revealed no statistically significant differences among the mean responses of the three treatment groups, as reflected by an F-value of 1.22 and a corresponding p-value of 0.30.

**Table 1:** Depicting the different conditions of the anestrous animals treated with different protocol and the research outcome in terms of pregnancy rate

| Description of the conditions | Treatment protocol employed | No. of animals treated (n) | Estrous induction (%) | PR @FTAI (%) | PR @ 2nd estrous (%) | PR (%) |
|---|---|---|---|---|---|---|
| 1. True Anestrous:Multiparous animals with smooth ovaries (Non-functional) | 1.1. CIDR-Ovsynch | 22 | 100.00 (22) | 68.18(15) | 9.09 (2) | 77.27 (17) |
| | 1.2. Double Ovsynch | 19 | 89.47 (17) | 63.63(12) | 0.0 | 63.63(12) |
| 2. True Anestrous Primiparous animals | 2.1. CIDR-Ovsynch | 13 | 84.61 (11) | 61.53 (8) | 7.69 (1) | 69.23 (09) |
| | 2.2. Double Ovsynch | 12 | 75.00 (9) | 58.33 (7) | 0.0 | 58.33 (7) |
| 3. Anestrous Primiparous animals with flat, small or smooth ovaries | 4.1. Bypass Protein (15gm/day), Bypass fat (20gm/day), multivitamin mineral mix (50-60 gm/day) | 46 | 58.69 (27) | 17.39(8) | 13.04 (6) | 30.43 (14) |
| 4. Ovarian cysts | 5.1. CIDR-Ovsynch | 17 | 76.47 (13) | 43.75 (8) | -- | 47.05 (8) |
| | 5.2. Ovsynch protocol | 14 | 71.40 (10) | 21.42 (3) | - | 21.42 (3) |
| | 5.3. Single dose GnRH | 14 | 71.40 (10) | 28.57 (4)* | - | 28.57 (4) |
| Total numbers | Total numbers | 157 | | | | |

**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

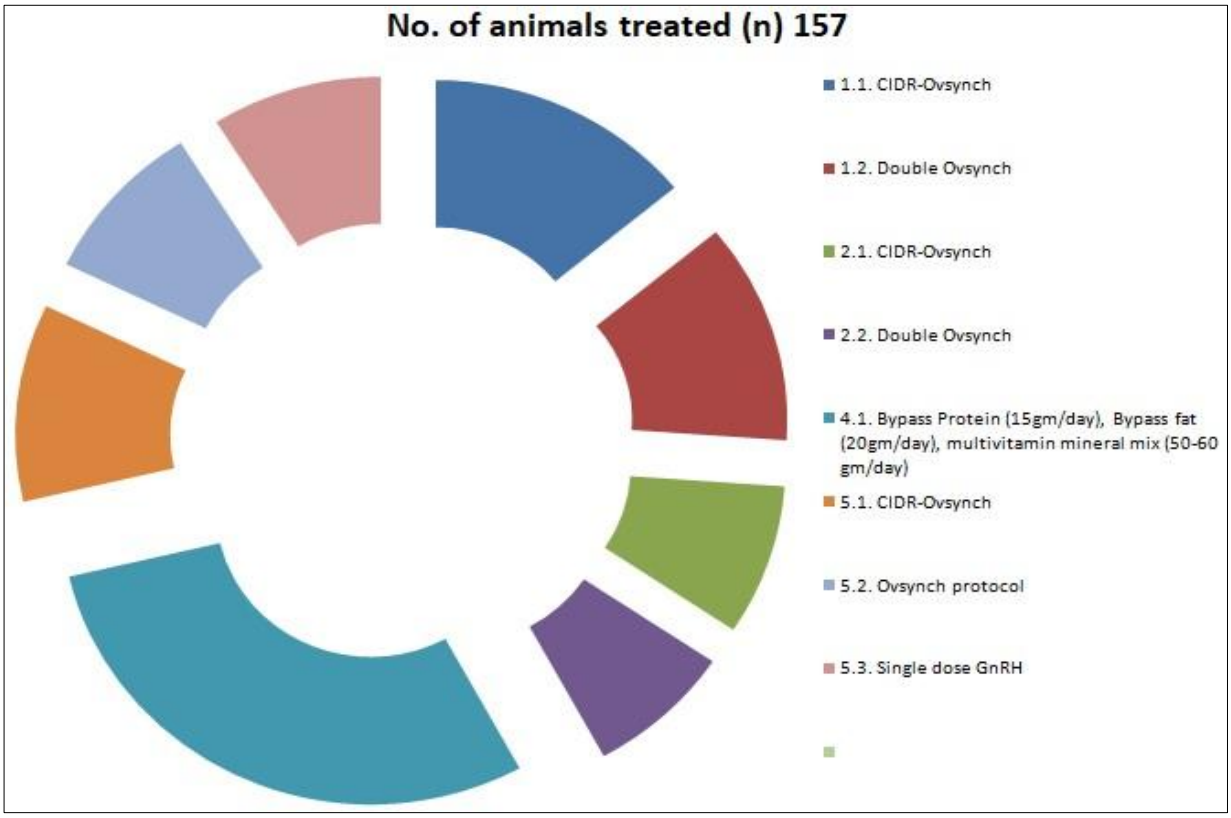


**Fig 1:** Depicting the different conditions of the anestrous animals treated with different protocol.

## Discussion

In multiparous animals exhibiting true anestrus, both the CIDR-Ovsynch and Double Ovsynch protocols achieved high estrous induction rates (100% and 89.47%, respectively), demonstrating their strong efficacy in reactivating ovarian function in animals with inactive ovaries. The CIDR-Ovsynch protocol yielded a numerically superior final pregnancy rate (77.27%) relative to the Double Ovsynch protocol (63.63%), a difference largely attributable to the higher conception rate at FTAI in the CIDR-treated group (68.18% vs. 63.63%).

These findings align with previous reports in anestrous cattle and buffalo, where slightly higher pregnancy rates were observed with CIDR-synchronization (66.66%) compared with Double Ovsynch (64.8%) [4, 8]. However, contrasting outcomes have been documented in anestrous Bos indicus cattle, in which the Ovsynch protocol produced higher pregnancy rates than CIDR-based treatments [9]. Such discrepancies may indicate species- and breed-specific variations in responsiveness to hormonal synchronization protocols.

In primiparous anestrous heifers, the CIDR-Ovsynch protocol produced significantly higher pregnancy rate (69.23%) compared with the Double Ovsynch protocol (58.33%). Although estrous induction rates were slightly lower than those observed in multiparous animals, they remained comparatively high for both protocols (84.61% and 75.00%, respectively). These findings suggest that primiparous animals may exhibit marginally reduced responsiveness to synchronization treatments, potentially

due to their ongoing growth, increased nutritional demands, or differences in postpartum physiological recovery. The present results are consistent with earlier reports indicating the effectiveness of CIDR-based synchronization strategies in inducing estrus and improving pregnancy outcomes in primiparous animals [10].

It is well established that low basal progesterone concentrations exert insufficient inhibitory feedback on the hypothalamus, thereby disrupting the normal surge of gonadotropins and contributing to impaired ovarian activity and anestrus. Providing exogenous progesterone at the onset of a synchronization protocol can help modulate this endocrine environment; the abrupt withdrawal of progesterone subsequently removes the negative feedback, facilitating the induction of estrus. Adequate circulating progesterone levels are also essential for supporting optimal oocyte maturation, embryo development, and reducing early embryonic losses [11].

The nutritional supplementation group for primiparous anestrous animals (Group 4.1) exhibited considerably lower effectiveness than the hormonal treatment protocols, achieving an estrous induction rate of only 58.69% and a final pregnancy rate of 30.43%. Although adequate nutrition is fundamental to reproductive function, nutritional intervention alone appears insufficient to reverse established anestrus, particularly when compared with the targeted endocrine modulation achieved through Ovsynch-based protocols. The comparatively low pregnancy rate indicates that, despite partial estrous expression, underlying ovarian activity or uterine receptivity may have remained suboptimal due to persistent nutritional deficiencies.

Recent studies on the management of true anestrus in crossbred Jersey cows similarly reported that supplementation with minerals and vitamins plays an important role in establishing estrus and improving conception, complementing but not surpassing the effects of hormonal therapies such as Ovsynch [12]. Additionally, earlier findings in swamp buffaloes demonstrated that dietary modification using bypass fat enriched with minerals and vitamins effectively restored estrus in anestrous cows and heifers, further supporting the role of targeted nutritional correction in improving reproductive outcomes [13].

**Management of Ovarian Cyst**

In animals with ovarian cysts, the CIDR-Ovsynch protocol produced a higher pregnancy rate (47.05%) compared with the Ovsynch protocol (21.42%), indicating its relative superiority for managing cystic ovarian conditions. The pregnancy rate achieved with a single GnRH injection (28.57%) was slightly better than that of Ovsynch. These outcomes are consistent with earlier reports demonstrating significantly higher conception rates with CIDR-based synchronization (52.3%) compared with a single GnRH treatment (26.9%) [14]. Likewise, previous studies have documented lower conception rates with the Ovsynch protocol than with single-dose GnRH administration [15].

Notably, other investigations have reported substantially higher conception rates (67.7%) in dairy cows with ovarian cysts when treated with either GnRH or dinoprost, likely attributable to the differentiation of cysts into follicular versus luteal types and the application of targeted therapy accordingly [16]. Furthermore, treatment protocols involving GnRH followed by prostaglandin administration 7 days later have been evaluated in buffaloes, achieving a cyst resolution rate of 60% [17].

The superior performance of the CIDR-Ovsynch protocol indicates that the addition of progesterone may facilitate cyst resolution and promote the re-establishment of normal cyclicity, likely through more effective modulation of GnRH and $PGF_2\alpha$ release. The comparatively lower efficacy observed with the standard Ovsynch protocol and a single GnRH injection for cystic ovaries has been documented previously, potentially because a lone GnRH dose commonly induces cyst luteinization, which then necessitates subsequent $PGF_2\alpha$ administration for complete resolution a sequence that may not be fully reflected in the initial pregnancy rate at FTAI.

**Conclusion**

CIDR-Ovsynch proved to be the most effective protocol for managing both true anestrus and ovarian cysts in cattle and buffaloes under field conditions. It consistently achieved higher estrous induction and pregnancy rates than Double Ovsynch, Ovsynch, single-dose GnRH, and nutritional supplementation, with significant advantages observed particularly in primiparous animals. Nutritional support alone improved reproductive responses but was insufficient to resolve established anoestrus. Overall, progesterone-based synchronization offers a superior strategy for restoring ovarian function and enhancing conception outcomes followed by double ovsynch protocol in bovine reproductive disorders.

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