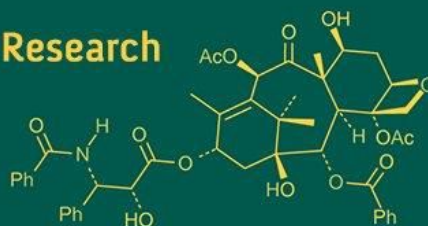
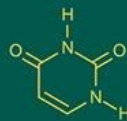
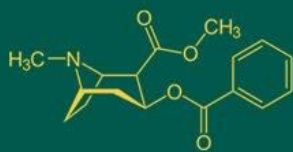


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Studies on the comparative efficacy of the Ovsynch and Clomiphene citrate on estrus induction and early conception rate in anestrus dairy cows

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Abstract

The present study evaluated and compared the efficacy of the Ovsynch protocol and Clomiphene citrate on estrus induction and early conception in postpartum anestrous dairy cows. 12 cows, each beyond 60 days post-calving, were randomly allotted into two groups. Before initiating treatments, all animals were dewormed with Mectin® (0.2 mg/kg, SQ, weekly once for three weeks) and supplemented with a mineral mixture (Alvite-M®, 50 g/day) along with supportive vitamins to improve reproductive health. Group I animals received Clomiphene citrate (300 mg orally for five consecutive days), while Group II animals were treated with the standard Ovsynch protocol consisting of Gynatrop® (10 µg, IM) on day 0, Pragma® (500 µg, IM) on day 7, and a second Gynatrop® injection (10 µg, IM) on day 9, followed by timed artificial insemination on day 10. Reproductive performance was evaluated based on estrus signs, ultrasonographic monitoring of follicular development, and conception outcomes. The estrus induction rate was 83.33% in Clomiphene citrate-treated cows and 100% in the Ovsynch group, without a significant difference between treatments. Conception rates, however, did not differ significantly, being 40.00% and 33.33% in Clomiphene citrate and Ovsynch groups, respectively ($\chi^2 = 0.0524$; $p > 0.05$). The mean onset of estrus was earliest and most synchronized in Ovsynch-treated cows (58.67 ± 0.843 h), whereas Clomiphene citrate produced a delayed response (129.60 ± 5.879 h). Preovulatory follicle size was significantly ($p < 0.05$) larger in the Ovsynch group (12.98 ± 0.750 mm) compared with Clomiphene citrate (11.93 ± 0.108 mm). Economic evaluation ranked Clomiphene citrate as the most cost-effective protocol (₹766/animal) compared with Ovsynch (₹1193/animal). It may be concluded that both protocols effectively induced estrus in postpartum anestrous cows, with Ovsynch achieving better synchronization and follicular growth, while Clomiphene citrate, being economical and orally administered, represents a farmer-friendly alternative under field conditions.

Keywords: Anestrous cows, Ovsynch, clomiphene citrate, estrus induction, conception rate, mineral supplementation, deworming

Introduction

Anestrus, defined as the absence of overt estrus signs either due to ovarian inactivity or poor estrus detection, is widespread in tropical dairy systems. Its prevalence ranges from 9.09% to 82.5% depending on breed, management, and region (Bhoraniya *et al.*, 2012; Naikoo *et al.*, 2016, 2021) [3, 18, 17]. Crossbred cows are particularly prone, with estimates between 20-50% (Khan *et al.*, 2016) [11]. Multiple factors contribute to the condition, including negative energy balance during early lactation, nutritional deficiencies, heat stress, and suboptimal reproductive management (Abraham *et al.*, 2017) [1]. The economic consequences are considerable, with losses estimated at ₹193 per day in cows (Pawshie *et al.*, 2011) [24] and ₹372.90 per day in buffaloes (Kumar *et al.*, 2013) [15].

Several therapeutic approaches have been explored to address postpartum anestrus, with hormonal protocols playing a central role. Among these, the Ovsynch protocol has been widely adopted due to its ability to synchronize follicular growth and ovulation through a strategic sequence of GnRH and PGF_{2α} injections, followed by fixed-time artificial insemination (Pursley *et al.*, 1995; Bhoraniya *et al.*, 2012) [26, 3]. However, despite its effectiveness in inducing estrus, variability in conception outcomes across management systems has limited its universal success.

As an alternative, Clomiphene citrate, a selective estrogen receptor modulator, has shown promise as an orally administered ovulatory stimulant. By blocking estrogen receptors in the hypothalamus, it enhances gonadotropin-releasing hormone (GnRH) secretion, stimulates follicular development, and ultimately induces ovulation (Reddy *et al.*, 1990) [28]. Its non-invasive mode of administration offers added practicality for field use compared with injection-based protocols.

Although both Ovsynch and Clomiphene citrate have shown promise in improving reproductive performance, direct comparative evaluations under Indian dairy farming conditions are limited. Such studies are essential to identify protocols that not only improve estrus induction and conception but are also practical and cost-effective for field application.

In this context, the present investigation entitled “*Studies on the Comparative Efficacy of the Ovsynch protocol and Clomiphene Citrate on Estrous Induction and Early Conception Rate in Anestrous Dairy Cows*” was undertaken with the objectives of (i) assessing the effects of these two protocols on follicular growth and estrus induction in postpartum anestrous cows, and (ii) comparing their relative efficacy in enhancing estrus response and early conception rates.

Materials and Methods

The study was carried out on anestrous cows maintained at the Livestock Farm Complex, Veterinary College, Hassan, Karnataka, with due approval from the Institutional Animal Ethics Committee (Ref. No. HVC/IAEC/05/2025) Dated: 13.05.2025. A total of twelve postpartum anestrous cows (>60 days post-calving) with normal calving history were selected based on clinical and gynecological examination. All animals were initially dewormed with Ivermectin (Mectin®, Alembic; 0.2 mg/kg BW, IM, once weekly for three weeks) and supplemented with Vitamin AD₃E (VETADE®, Zenex pharma) and Butaphosphan + Cyanocobalamin (Synkomet®, Intas Pharmaceuticals) for three weeks, in addition to a daily mineral mixture (Alvite M®, Alembic; 50 g). Following this, cows were randomly allocated into two groups. Group I animals received Clomiphene citrate (Fertiboost® CC, Arjit Remedies; 300 mg/day orally for five consecutive days) 30 minutes after drenching with (1%) CuSO₄ solution, and ovarian activity was monitored from day six onwards using transrectal ultrasonography (7.5-10 MHz linear probe, IMV EXApad Mini). Artificial insemination (AI) was performed on day 10 or 11 based on the AM-PM rule. Group II animals were treated with the Ovsynch protocol, which included Gynatrop® (10 µg, IM) on day 0, Pragma® (500 µg, IM) on day 7, and Gynatrop® (10 µg, IM) on day 9, with ultrasonographic monitoring from day 7 to 10 and fixed-time AI on day 10. Data generated were analyzed using GraphPad Prism (version 10.5.0).

Result and Discussion

Estrus response rate

The estrus response rate in Group I cows treated with Clomiphene citrate was 83.33 per cent, whereas in Group II cows treated with Ovsynch protocol was 100 per cent as shown in Table 1 and Figure 1. Upon statistical analysis we found that there is no significant difference between the two groups in terms of estrus induction rate. The current results

of Group I are in accordance with the findings of Padher *et al.* (2018) [21] and Hukeri *et al.* (1979) [9], who documented estrus induction rates of 81.5 per cent in cows and 82.72 per cent in buffaloes, respectively, using a similar Clomiphene citrate treatment protocol. A slightly higher estrus induction rate was noticed by Dugwekar *et al.* (1980) [8] and Bukhari *et al.* (2016) [4] in anestrous cows and prepubertal heifers, likely due to differences in breed, animal selection, or physiological status such as ovarian activity and nutrition. In contrast, lower responses (~60%) reported by Kaikini *et al.* (1977) [10], Kodagali (1978) [12], and Pargaonkar (1978) [22] could be attributed to management differences, dosing protocols, or inclusion of animals in deeper anestrous. These findings highlight the effectiveness of Clomiphene citrate as a practical, low-cost, orally administered option for estrus induction under field conditions.

Estrus induction in Group II reached 100 per cent, in agreement with Navrange *et al.* (2012) [19] and Niaz *et al.* (2022) [20]. This superior efficacy compared to earlier reports of 70-85 per cent (Kumar *et al.*, 2010; Kumar *et al.*, 2018; Dixit *et al.*, 2023) [13, 14, 7] may be due to better nutritional status, strict protocol adherence, and favorable physiological conditions of the cows in the present study.

Overall, the present study highlights Clomiphene citrate as a practical, low-cost, orally administered alternative for estrus induction in cows, while reaffirming the superior efficacy of the Ovsynch protocol under field conditions. The consistent 100 per cent response with Ovsynch underscores its reliability compared to Clomiphene citrate, the latter showing comparatively lower effectiveness that may require further refinement. These findings emphasize the importance of selecting appropriate protocols based on animal condition and management practices to optimize reproductive performance.

Conception rate

In the present study, the conception rate was 40 per cent in Group I cows and 33.33 per cent in Group II cows as shown in Table 1 and Figure 1, with no statistically significant difference observed between the groups. In Group I (Clomiphene citrate), the conception rate was 40 per cent, comparable to Nagendra *et al.* (2022) [16] who reported 46 per cent in anestrous Sahiwal heifers, and higher than the 25 per cent and 33.33 per cent reported by Kodagali *et al.* (1978) [12] and Wankar *et al.* (2017) [31], respectively. However, it remained lower than the 50-80 per cent reported by Purohit and Bishnoi (1993) [25], Rahman *et al.* (2024) [27], and Hukeri *et al.* (1979) [9]. In Group II (Ovsynch protocol), conception rate was 33.33 per cent, consistent with Niaz *et al.* (2022) [20], though slightly lower than the 50-60 per cent reported by Kumar *et al.* (2010, 2018) [13], Angadi *et al.* (2024) [2], and Dixit *et al.* (2023) [7]. A similar rate (30%) was also noted by Patel *et al.* (2013) in true anestrous cows. Variations in conception across studies may be influenced by estrus detection efficiency, physiological status, parity, nutrition and management factors.

Average time of onset of estrus

The average time to estrus onset was 129.60±5.87 hrs in Group I (Clomiphene citrate) and 58.67±0.84 Hrs in Group II (Ovsynch), with the difference being statistically significant ($p < 0.005$) as represented in Table 1 and Figure 2. The onset observed in Group I is comparable to reports by Wankar *et al.* (2017) [31] and Rahman *et al.* (2024) [27],

though longer intervals (180 ± 36 hrs) have been noted when Clomiphene was used alone. In contrast, the shorter interval in Group II aligns with findings of Dhimi *et al.* (2015) [5], Kumar *et al.* (2010) [13], and Salman *et al.* (2023) [29]. The delay with Clomiphene may be explained by its indirect mode of action via estrogen receptor blockade, requiring time for gonadotropin release and follicular development, whereas Ovsynch exerts direct hormonal control over follicular dynamics and ovulation, ensuring a more synchronized and predictable estrus response.

Dominant Follicular diameter

In Group I, the mean dominant follicle diameter increased steadily from 7.54 ± 0.394 mm on day 0 to a maximum of 11.23 ± 0.596 mm on day 11 (Table 2 and Figure 3). Statistical analysis revealed that the differences in follicular diameter between days were significant ($p < 0.05$). Values on day 11 were significantly higher than those on day 0 and day 5, confirming the stimulatory effect of Clomiphene citrate on follicular development.

In Group II, the mean dominant follicle diameter increased significantly ($p < 0.05$) from 6.57 ± 0.461 mm on day 0 to 11.08 ± 0.600 mm on day 7, reaching 11.73 ± 0.668 mm and 12.28 ± 0.889 mm on days 8 and 9, respectively (Table 3 and Figure 4). Statistical analysis revealed no significant difference ($p > 0.05$) in follicle diameter among days 7, 8, and 9. However, a significant increase ($p < 0.05$) was

observed when these values were compared with the diameter recorded on day 0.

The preovulatory follicle in Group I (11.23 ± 0.596 mm on day 11) was smaller than that reported by Patil *et al.* (2016; 16.12 ± 0.56 mm) [23], though the growth trajectory was comparable. In Group II, the follicular diameter (12.28 ± 0.889 mm) was consistent with Udin *et al.* (2017; 10.8 ± 2.44 mm) [30] but much lower than the values reported by Dirandeh *et al.* (2018; 19.20 ± 0.52 mm). Such variations may be attributed to differences in reproductive status, with anestrus cows in the present study showing reduced ovarian responsiveness compared to cyclic cows in earlier reports. Breed also plays a role, as exotic breeds like Holsteins exhibit larger ovarian structures than indigenous or crossbred cattle. Nutritional status, particularly body condition score, further influences follicular growth through its effects on metabolic hormones such as insulin and IGF-1. Differences in hormonal protocols (Clomiphene citrate vs. Ovsynch) and environmental factors including stress and herd management likely contributed to the variability observed across studies.

Overall, these findings underscore the multifactorial nature of follicular development and highlight the need to consider reproductive status, breed, nutrition, and management when interpreting treatment outcomes and optimizing estrus induction protocols in catt.

Table 1: Effect of Clomiphene citrate and Ovsynch on estrus induction rate, conception rate and Average time of onset of estrus

Group	Number of animals treated	Estrus induction rate	Conception rate	Average time of onset of estrus
I	6	5 (83.33%)	2 (40.00%)	129.60 ± 5.879^b
II	6	6 (100.00%)	2 (33.33%)	058.67 ± 0.843^a

Note: Mean $\pm$ SE bearing different superscripts are statistically significant at $p < 0.05$

Table 2: Dominant follicle diameter of Group I

Parameter	0 th day	5 th day	6 th day	7 th day	8 th day	9 th day	10 th day	11 th day
Dominant follicle Diameter (mm)	7.54 ± 0.394^a	7.82 ± 0.287^a	8.01 ± 0.266^a	8.51 ± 0.459^{ab}	8.35 ± 0.689^{ab}	9.19 ± 0.955^{ab}	10.40 ± 1.085^{ab}	11.23 ± 0.596^b

Note: Mean $\pm$ SE bearing different superscripts are statistically significant at $p < 0.05$

Table 3: Dominant follicle diameter of Group II

Parameter	0 th day	7 th day	8 th day	9 th day
Dominant follicle Diameter (mm)	6.57 ± 0.461^a	11.08 ± 0.600^b	11.73 ± 0.668^b	12.28 ± 0.889^b

Note: Mean $\pm$ SE bearing different superscripts are statistically significant at $p < 0.05$

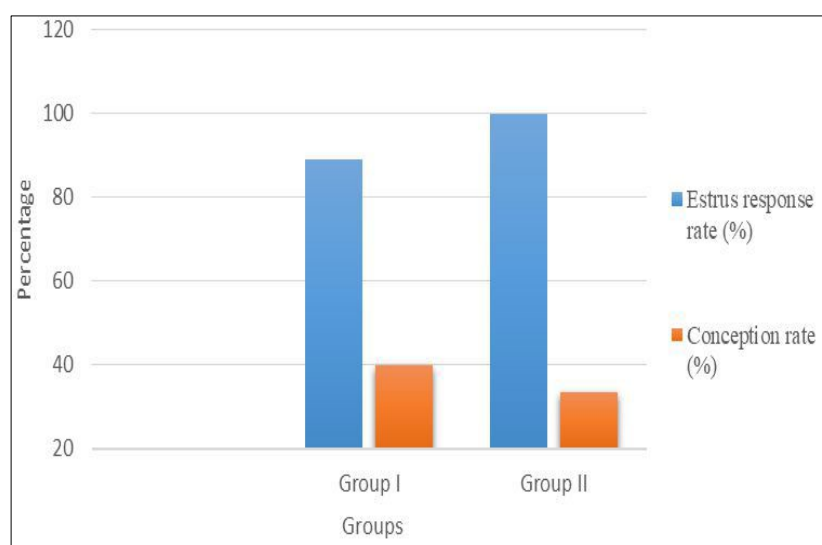


Fig 1: Graphical representation of Estrus response rate (%) and Conception rate (%) in treatment groups

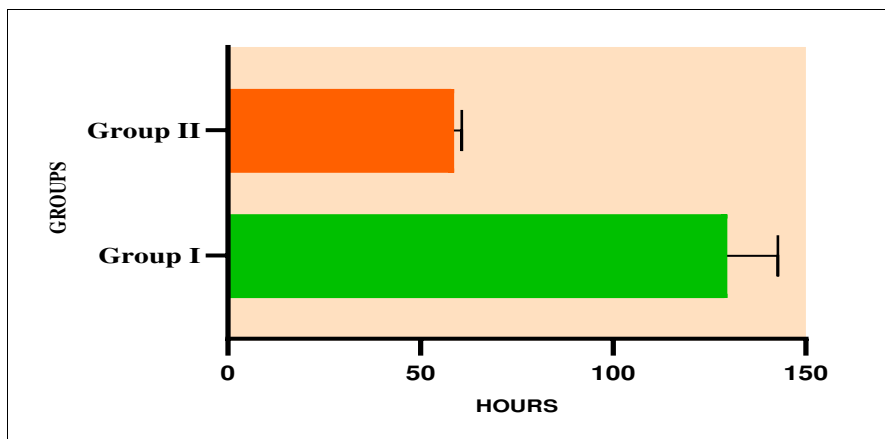


Fig 2: Graphical representation of Average time of onset of estrus in treatment groups

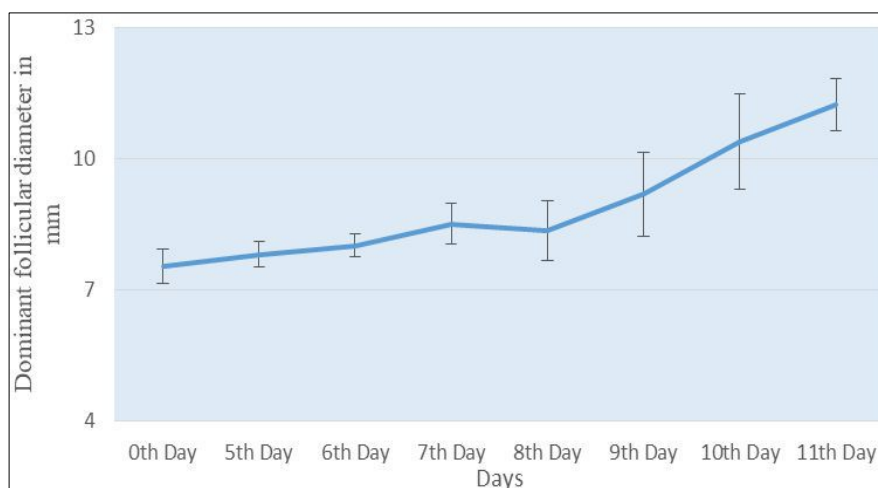


Fig 3: Graphical representation of dominant follicle of Group I

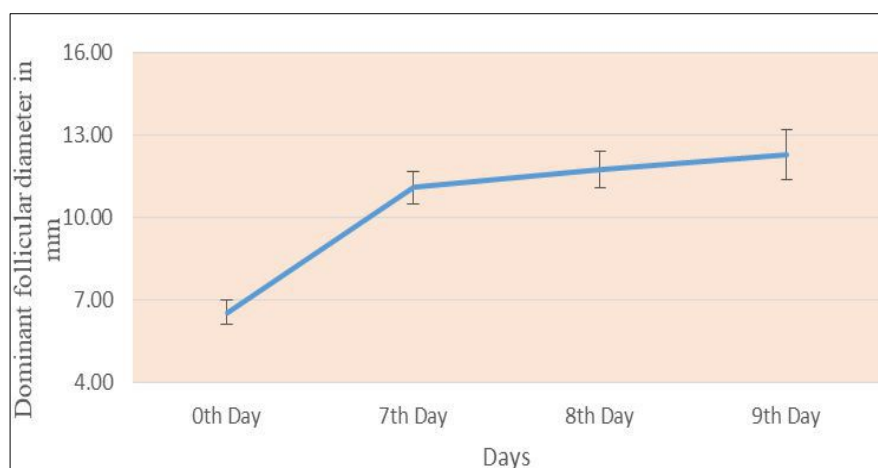


Fig 4: Graphical representation of dominant follicle of Group II

Conclusion

The present study compared the efficacy of Clomiphene citrate and the Ovsynch protocol for estrus induction and conception rate in anestrus cows under field conditions. Both hormonal and non-hormonal approaches proved effective in inducing estrus in anestrus cows. The non-hormonal treatment, Clomiphene citrate, achieved an 83.33% response rate with a delayed onset of estrus but remains a practical, low-cost, orally administered option under field conditions. In contrast, the hormonal Ovsynch protocol ensured 100% estrus induction with rapid and synchronized onset, though conception rates between the

groups were comparable. Thus, Clomiphene provides a viable alternative where hormonal interventions are limited, while Ovsynch remains the protocol of choice for reliable synchronization in timed breeding programs.

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