

Research Article



Strategic Management of Pre- and Post-Partum Anestrus in Cattle: A Problem-Based Approach

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Abstract | This study aimed to evaluate treatment protocols for pre- and post-partum anestrus in cattle under field conditions. A total of 168 anestrus cattle were selected randomly based on clinical findings, and their age, breed, parity and body condition score (BCS) were recorded. All selected animals were initially dewormed with a broad spectrum anthelmintic. The animals were then randomly (lottery) allocated to one of eight treatment groups; group-A served as the untreated control group; group-B underwent per-rectum massage of the reproductive organs (MRO); group-C was administered vitamins and minerals (VM); group-D received an injection of gonadotropin-releasing hormone (GnRH); group-E received a combination of MRO and VM; group-F received MRO and GnRH; group-G received VM and GnRH; and group-H received a combination of MRO, VM and GnRH. The overall induced estrus and pregnancy rates were observed 52.4%, and 34.5%, respectively. The estrus and pregnancy rates in group-G and H were significantly higher (84.2% and 72.7%, respectively) than those in the other groups ($p < 0.05$). Regarding age categories, animals aged 3.6–4.5 years exhibited a higher pregnancy rate (50%) than other age groups. In terms of genetics, $L \times SL$ genotypes showed a higher estrus and pregnancy rate (61.4, 42.9%, respectively) than other genotypic variations. Cows in their second parity cows demonstrated a comparatively higher estrus and pregnancy rate than those in other parities. Conversely, no significance difference was observed across different BCS categories. In conclusion, combination treatments incorporating GnRH significantly increased both estrus and pregnancy rates in anestrus cattle. Further investigations should incorporate bio-chemical and hematological analysis to better understand the underlying mechanisms.

Keywords | Cattle, Disease, Estrus, Pregnancy, Hormone, Reproduction

Received | April 09, 2026; **Accepted** | June 18, 2026; **Published** | September 16, 2026

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Citation | Mondal H, Roy A, Khanam SA, Biswas D, Paul AK (2026). Strategic management of pre- and post-partum anestrus in cattle: A problem-based approach. *J. Anim. Health Prod.* 14(4): 1506–1513.

DOI | <https://dx.doi.org/10.17582/journal.jahp/2026/14.4.1506.1513>

ISSN (Online) | 2308-2801



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INTRODUCTION

In Bangladesh, the livestock sector plays a significant role in the national economy contributing approximately 1.81% to the gross domestic product (GDP) while generating roughly 20% of direct and 50% of partial employment opportunities (BBS, 2025). Within rural households, integrated farming systems are widely practiced, functioning as a primary way for income

generation, nutritional security, and rural employment. The national domesticated animal population is estimated at 25.17 million cattle, 27.29 million goats, 3.98 million sheep, 1.53 million buffaloes (BBS, 2025). Among these livestock species, cattle represent the primary and most vital resource for milk and meat production. However, sustainable and profitable dairy farming faces critical constraints, including a high prevalence of reproductive pathologies, limiting grazing land, hike of feed and fodder

cost, and a fragmented dairy supply chain (Paul *et al.*, 2022). Breeding strategies are heavily reliant on artificial insemination (AI), which is utilized by 83.12% of farmers while 16.88% continue to rely on natural mating (Pehan *et al.*, 2022). A significant gap in technical knowledge persists, with approximately 71.3% of farmers reporting inadequate training in scientific herd management, which strongly correlates with the outbreak of reproductive disorders in animals (Paul *et al.*, 2022). Alarming, up to 97% of farmers encounter reproductive issues in their herds, which severely depress annual calf yield, cause profound reproductive infertility, and culminate in substantial economic losses nationwide (Paul *et al.*, 2017). Among these reproductive disorders, anestrus (the prolonged absence of behavioral estrus or cyclicity) remains the most pervasive challenge, with multiple epidemiological surveys estimating its prevalence between 20% and 30% (Maruf *et al.*, 2014; Paul *et al.*, 2017; Akter *et al.*, 2022).

The etiology of anestrus in this region is multifactorial and heavily lined to deficient herd management. Key factors include poor body condition scores (BCS), post-partum negative energy balance (NEB), environmental stress on high yielding exotic crossbred, and neuroendocrine disruptions caused by direct freely milk suckling and micronutrient deficiencies. Field data show that 87.4% of farmers are practiced uninhibited calf suckling, 50% fail to dewormed their herds at regular intervals or omit deworming entirely, and 95.5% do not provide vitamin-mineral supplementation (Paul *et al.*, 2022). Gastrointestinal parasitism acts as a chronic stressor, progressively deteriorating the overall health and reproductive performance of these livestock species (Amin *et al.*, 2016). Furthermore, prolonged suckling significantly contributes to post-partum anestrus in *Bos indicus* and crossbred cows, severely exacerbating reproductive inefficiencies (Islam *et al.*, 2022). Pathophysiologically, the synergy between an imbalanced diet, nutrient deficiencies, parasitism, and unrestricted calf suckling accelerates the onset of a profound NEB. This energetic deficit suppresses the hypothalamic secretion of gonadotropin-releasing hormones (GnRH), thereby decreasing luteinizing hormone (LH) pulse frequency. Consequently, dominant follicle maturation and ovulation are impaired, resulting in a prolonged anestrus period (Washaya *et al.*, 2025). This metabolic state also interacts with concurrent post-partum stressors such as suckling, subclinical uterine infections, and ambient heat stress to further exacerbating ovarian dysfunction (Rhodes *et al.*, 2003). Suckling, in particular, inhibits LH secretion via complex neural and hormonal reflex pathways. Therefore, delaying the resumption of post-partum ovarian cycles. Notably, suckled cows exhibit a 2.82-fold higher likelihood of remaining anestrus compared

to non-suckled counterparts. Thus, aggressive nutritional intervention and balanced energy supplementation are crucial for restoring ovarian cyclicity following parturition (Taher *et al.*, 2026).

In veterinary practice, the GnRH and prostaglandin F2 α hormone (PGF2 α) with or without nutritional supplementation, are commonly deployed to resolve anestrus in Bangladesh. For instance, therapeutic regimens combining supportive vitamin-mineral therapy with an Ovsynch protocol yielded an estrus response rate of 100% and a pregnancy rate of 85.7% in post-partum cows, compared to lower efficacies when using either supportive therapy (85.7% estrus, 83.3% pregnancy) or Ovsynch alone (57.1% estrus, 50% pregnancy; Srilatha and Phaneendra, 2024). Similarly, treating anestrus cows with nutritional supplements, GnRH alone, and GnRH combine with PGF2 α resulted in estrus induction rates of 71.4%, 75%, and 75%, respectively (Akhter *et al.*, 2022). However, while fixed-time synchronization protocols consistently enhance estrus expression rates in post-partum anestrus crossbred cows, significant improvements in overall pregnancy rates are not always achieved (Shrestha *et al.*, 2025). To date, the majority of regional investigations have focused exclusively on post-partum anestrus while approaching its underlying etiology through single-factor treatments. Comprehensively, strategic management strategies that simultaneously address metabolic, parasitic, and hormonal deficits across both pre- and post-partum anestrus cattle remain sparse. Therefore, this study was designed to evaluate a multifaceted strategic management approach to induce estrus and optimize pregnancy rate under typical field conditions.

MATERIALS AND METHODS

MATERIALS

Gonadotropin-releasing hormone (GnRH; Gonavet[®] injection, 5 mL vial; Gonadorelin Acetate BP 0.105 mg/mL) and a vitamin AD₃E and B complex (Vita-S Plus[®] injection, 10 mL vial) were purchased from Chemist Laboratories Limited (Barishal, Bangladesh). A combination anthelmintic formulation comprising Triclabendazole BP (900 mg) and Levamisole BP (600 mg) (Navadex-vet[®]) were purchased from Navana Pharmaceuticals Limited (Dhaka, Bangladesh). Furthermore, a selenium and vitamin E combination syrup (Rena Sel-E[®] syrup, 100 mL bottle; Vitamin E, 80 mg/mL; Selenium, 0.6 mg/mL) along with a multivitamin-mineral premix (Rena DB Plus[®] powder, 1 kg bucket) were sourced from Renata Animal Health PLC (Dhaka, Bangladesh). All therapeutic and nutritional agents were stored according to the manufacturers' instructions.

STUDY AREA AND PERIOD

The field-based component of this study was conducted under commercial small-holder farm conditions. Farms were selected based on accessibility, farmers cooperation, and the availability of anestrus cattle, during the period from January to December 2025.

ANIMALS AND MANAGEMENT

A total of 168 anestrus cattle were selected based on a clinical history of anestrus. Clinical diagnosis was confirmed via transrectal palpation, which revealed smooth-surfaced ovaries and a flabby uterus. Cattle presenting with anestrus stemming from premature age, congenital complications, or infectious reproductive pathologies (e.g. pyometra, endometritis, and fetal mummification) were excluded from the study, as were animals displaying silent heat. Thorough reproduction records, including age, breed, parity, and body condition score (BCS), were documented using a structured record sheet. All selected animals were dewormed using a broad-spectrum anthelmintics (Navadex-vet®; 1 bolus/75 kg body weight), with the treatment repeated after a 7-day interval. Dietary intake was supplemented with standard concentrated feed. For lactating cows, calf suckling was permitted three times daily coinciding with regular milking sessions. The experimental animals were reared under the existing farm management systems.

GROUPING OF ANIMALS

The enrolled cohort was categorized based on demographic and physiological parameters. The study population ranged in age from 1.5 to ≥ 5.6 years and were subdivided into five age groups: 1.5-2.5 years ($n=59$), 2.6-3.5 years ($n=27$), 3.6-4.5 years ($n=22$), 4.6-5.5 years ($n=17$), and ≥ 5.6 years ($n=43$). In terms of genetic background, the study population included indigenous/local (L; $n=67$), Sahiwal crossbred (L $\times$ SL; $n=70$) and Holstein-Friesian crossbred (L $\times$ HF; $n=31$) genotypes. Based on parity status, the animals were classified as nulliparous heifers ($n=69$), primiparous cows (P1; $n=22$), or multiparous cows [P2 ($n=24$), P3 ($n=18$), and $\geq P4$ ($n=35$)]. Regarding physical condition assessment via BCS (evaluated on a standard 1-5 scale), the distribution consisted of 2.5 ($n=49$), 3.0 ($n=86$) and ≥ 3.5 ($n=33$).

EXPERIMENTAL DESIGN

This study was performed under field conditions. Following the clinical confirmation of anestrus, a total of 168 anestrus cattle were allocated to one of the eight treatment regimens (A to H) using a simple randomization method (Lottery system). The treatment efficacy was evaluated by estrus and pregnancy rates. Pregnancy rates were calculated based on a single service per estrus. The experimental groups were

defined and executed as follows:

- Group A ($n = 31$; Control): Animals received no therapeutic intervention.
- Group B ($n = 23$; MRO): Per-rectum massage of the reproductive organs (cervix, uterus and ovaries) for 3 minutes.
- Group C ($n = 28$; VM): Intramuscular injection of vitamin ADE (15 mL/animal) at 7-day interval for three doses, combined with oral administration of vitamin E-Selenium (20 mL/animal) once daily for 30 days, and a multivitamin supplemented (20 gm/animal) mixed with feed for 45 days.
- Group D ($n = 23$; GnRH): Intramuscular injection of GnRH (Gonavet®, 2.5 mL/animal) administered twice at an 11-day interval.
- Group E ($n = 20$; MRO+VM): Combination of per-rectum massage of reproductive organs (3 min) and the VM regimen (vitamin ADE injection, oral vitamin E-Selenium, and multivitamins feed premix at identical doses and duration as group C).
- Group F ($n = 13$; MRO + GnRH): Combination of per rectum massage of reproductive organs (3 min) and the GnRH regimen (identical doses and intervals as group D).
- Group G ($n = 19$; VM + GnRH): Combination of the VM regimen (identical doses and duration as group C) and the GnRH regimen ((identical doses and intervals as group D).
- Group H ($n = 11$; MRO + VM + GnRH): Combined intervention comprising per-rectum massage of reproductive organs (3 min), the VM regimen, and the GnRH regimen at identical individual doses, intervals, and durations.

HEAT DETECTION, INSEMINATION, AND PREGNANCY DIAGNOSIS

Estrus detection was performed by farm personnel via visual observation three times (early morning, late evening, and before midnight). Detection relied on primary estrus signs (standing to be mounted) and secondary signs (restlessness, clear vulvar mucus discharge, and vulvar hyperemia) following treatment. Farmers were instructed to report the onset of estrus immediately. A single artificial insemination (AI) was performed by an experienced technician using the standard AM-PM rule. Animals failing to return to estrus 20-25 days post-AI, were provisionally considered as pregnant. Confirmatory pregnancy diagnosis was performed between days 60 and 90 post-AI via transrectal palpation of the reproductive tract, assessing for uterine horn asymmetry, the membrane slip, and the presence of a persistent of corpus luteum. To ensure a blind assessment and bias, examinations were conducted independently by two trained investigators who were completely unaware of the treatment allocations and did not perform the AI.

DATA ANALYSIS

Data were tabulated and encoded in Microsoft Excel to ensure data integrity, duplication and missing values. Statistical analyses were performed using the Statistical Package for the Social Science (SPSS, Version 26.0; IBM Corp., Armonk, NY, USA). Estrus response and pregnancy rates were expressed as percentage. Binary logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the influence of treatment regimens and categorical covariates (age, breed, parity, and BCS) on estrus and pregnancy outcomes. Statistically significance was set at $p \leq 0.05$.

RESULTS

In this study, the overall rates of estrus rate and pregnancy were 52.4% (88/168) and 34.5% (58/168), respectively. The pregnancy rate per estrus was 65.9% (58/88).

EFFICACY OF THE TREATMENT REGIMEN ON ESTRUS AND PREGNANCY RATES

Following the treatment of anestrus animals, the estrus rates for groups A (control), B (MRO), C (VM), D (GnRH), E (MRO+VM), F (MRO+GnRH), G (VM+GnRH), and H (MRO+VM+GnRH) were 16.1%, 26.1%, 53.6%, 65.2%, 70%, 69.2%, 84.2%, and 72.7%, respectively (Table 1). The estrus and pregnancy responses in treatment groups G (VM+GnRH) and H (MRO+VM+GnRH) were significantly higher (84.2% and 72.7%, respectively) than those of the other groups ($p < 0.05$). Logistic regression analysis revealed that groups G and H had significantly higher odds of expressing estrus than the control group [$p < 0.05$; OR =27.3; 95% CI (5.82 – 132.11) and OR =13.87; 95% CI (2.7-71.2), respectively]. Furthermore, per-rectum massage of the reproductive organs (group B) resulted in a non-significant, 1.8-fold higher chances of expressing estrus compared to control group [$p > 0.05$; OR =1.84; 95% CI (0.48-6.97)]. The pregnancy rates for groups A (control), B, C, D, E, F, G, and H were 6.5%, 13%, 28.6%, 43.5%, 45%, 53.8%, 63.2%, and 63.6%, respectively (Table 2). The pregnancy rate in group G and H were also significantly higher (63.2% and 63.6%, respectively) than that of the control group ($p < 0.05$). Animals treated with a combination of reproductive organs massage, vitamin-minerals, and GnRH (group H) exhibited a significantly 25.4-fold higher probability of becoming pregnant than control animals [$p < 0.05$; OR =25.38; 95% CI (3.84-167.53)]. Additionally, two injections of GnRH at an 11-day interval significantly increased the pregnancy rate 11.2-fold [$P < 0.05$; OR =11.15; 95% CI (2.14-58.26)].

FACTORS INFLUENCING THE ESTRUS AND PREGNANCY OUTCOMES

The estrus and first service pregnancy rates according to

age, breed, parity, body condition score (BCS) are presented in Tables 3 and 4, respectively.

Table 1: Efficacy of the treatment regimen on the estrus response rate.

Group	An- imal (N)	Estrus rate % (N)	P value	OR	95% CI of OR	
					Lower	Upper
A (Control)	31	16.1 (5)	0.000	-	-	-
B (MRO)	23	26.1 (6)	0.373	1.84	0.48	6.97
C (VM)	28	53.6 (15)	0.004	6.00	1.79	20.15
D (GnRH)	23	65.2 (15)	0.001	9.75	2.70	35.26
E (MRO + VM)	20	70.0 (14)	0.000	12.13	3.14	46.95
F (MRO+GnRH)	13	69.2 (9)	0.001	11.70	2.56	53.37
G (VM+GnRH)	19	84.2 (16)	0.000	27.73	5.82	132.11
H (MRO+VM+ GnRH)	11	72.7 (8)	0.002	13.87	2.70	71.20

N: number; OR: odd ratio; CI: confidence interval.

Table 2: Efficacy of the treatment regimen on the pregnancy rate.

Group	An- imal (N)	Pregnan- cy rate % (N)	P value	OR	95% CI of OR	
					Lower	Upper
A (Control)	31	6.5 (2)	0.001	-	-	-
B (MRO)	23	13.0 (3)	0.417	2.18	0.33	14.22
C (VM)	28	28.6 (8)	0.037	5.80	1.11	30.23
D (GnRH)	23	43.5 (10)	0.004	11.15	2.14	58.26
E (MRO + VM)	20	45.0 (9)	0.004	11.86	2.21	63.79
F (MRO+GnRH)	13	53.8 (7)	0.002	16.92	2.80	102.40
G (VM + GnRH)	19	63.2 (12)	0.000	24.86	4.50	137.35
H (MRO + VM + GnRH)	11	63.6 (7)	0.001	25.38	3.84	167.53

N: number; OR: odd ratio; CI: confidence interval

AGE

The overall estrus rates for animals aged 1.5-2.5, 2.6-3.5, 3.6-4.5, 4.6-5.5, and ≥ 5.6 years were 52.5%, 63.3%, 59.1%, 47.1%, and 44.2%, respectively. No significant differences were found across the age groups when compared to the reference variable. However, animals in the 2.6-3.5 years age range exhibited approximately 2-fold higher odds of estrus than in the reference category ($p > 0.05$; OR =1.99; 95% CI =0.69-5.76). The overall pregnancy rates for these same age cohorts were 30.5%, 40.7%, 50%, 29.4%, and 30.2%, respectively. The highest pregnancy rate was observed in the 3.6-4.5 years cohort (50%), which showed a non-significantly, 2.9-fold higher odds of pregnancy compared to the reference category ($p > 0.05$; OR =2.90; 95% CI =0.73-11.54).

Table 3: Factors influencing the estrus rate in anestrus animals.

Factors	Category	Animal (N)	Estrus rate % (N)	P Value	OR	95% CI of OR	
						Lower	Upper
Age (Year)	1.5 to 2.5	59	52.5 (31)	0.673	-	-	-
	2.6 to 3.5	27	63.3 (17)	0.202	1.99	0.69	5.76
	3.6 to 4.5	22	59.1 (13)	0.533	1.54	0.40	5.94
	4.6 to 5.5	17	47.1 (8)	0.772	1.28	0.25	6.61
	≥ 5.6	43	44.2 (19)	0.691	0.69	0.11	4.33
Breed	Local	67	50.7 (34)	0.092	-	-	-
	L× SL	70	61.4 (43)	0.429	1.34	0.65	2.74
	L×HF	31	35.5 (11)	0.047	0.41	0.17	0.99
Parity (N)	Nulliparous	69	58.0 (40)	0.924	-	-	-
	P1	22	50.0 (11)	0.557	0.71	0.22	2.24
	P2	24	58.3 (14)	0.913	0.92	0.23	3.79
	P3	18	38.9 (7)	0.477	0.55	0.11	2.87
	≥ P4	35	45.7 (16)	0.995	0.99	0.16	6.35
BCS (1-5)	2.5	49	49.0 (24)	0.167	-	-	-
	3.0	86	48.8 (42)	0.953	1.02	0.48	2.18
	≥3.5	33	66.7 (22)	0.062	2.34	0.96	5.70

N: number; L: local; SL: Sahiwal; HF: Holstein Friesian; OR: odd ratio; CI: confidence interval.

Table 4: Factors influencing the pregnancy rate in anestrus animals.

Factors	Category	Animal (N)	Pregnancy rate % (N)	P value	OR	95% C.I. of OR	
						Lower	Upper
Age (years)	1.5 to 2.5	59	30.5 (18)	0.279	-	-	-
	2.6 to 3.5	27	40.7 (11)	0.199	2.02	0.69	5.92
	3.6 to 4.5	22	50.0 (11)	0.131	2.90	0.73	11.54
	4.6 to 5.5	17	29.4 (5)	0.785	1.27	0.23	7.10
	≥ 5.6	43	30.2 (13)	0.407	0.37	0.03	3.96
Breed	Local	67	31.3 (21)	0.152	-	-	-
	L× SL	70	42.9 (30)	0.263	1.53	0.73	3.22
	L×HF	31	22.6 (7)	0.136	0.47	0.18	1.26
Parity	Nulliparous	69	36.2 (25)	0.724	-	-	-
	P1	22	31.8 (7)	0.483	0.64	0.19	2.21
	P2	24	41.7 (10)	0.799	0.83	0.20	3.47
	P3	18	22.2 (5)	0.470	0.52	0.09	3.09
	≥ P4	35	34.3 (12)	0.427	2.64	0.24	28.78
BCS (1-5)	2.5	49	32.7 (16)	0.475	-	-	-
	3.0	86	32.6 (28)	0.900	1.05	0.47	2.36
	≥3.5	33	42.4 (14)	0.224	1.72	0.72	4.12

N: number; L: local; SL: Sahiwal; HF: Holstein Friesian; OR: odd ratio; CI: confidence interval

BREED

Local, L×SL, and L×HF cattle breeds exhibited estrus rates of 50.7%, 61.4%, and 35.5%, respectively, and pregnancy rates of 31.3%, 42.9%, and 22.6%, respectively. Although the estrus rate of the L×HF genotype was significantly lower (35.5%) than genotypes, no significant difference was found in the pregnancy rates. The L×SL genotype was observed the highest estrus and pregnancy rates (61.4% and 42.9%, respectively), showing non-significantly higher odds (1.3-fold and 1.53-fold, respectively) than the local breed [estrus: OR =1.34; 95% CI (0.65-2.74); pregnancy: OR =1.53; 95% CI (0.73-3.22); $p > 0.05$].

PARITY

The estrus rates for nulliparous heifers and cows of parities 1, 2, 3, and ≥4 were 58%, 50%, 58.3%, 38.9%, and 45.7%, respectively, whereas the pregnancy rates were 36.2%, 31.8%, 41.7%, 22.2%, and 34.3%, respectively. Although parity did not exert a significant effect ($p > 0.05$; OR <1) on either the estrus or pregnancy rates in anestrus cattle, nulliparous heifers and parity 2 (P2) cows showed numerically higher estrus and pregnancy rates compared to the other parity categories.

BODY CONDITION SCORE (BCS)

The estrus rates for the BCS categories 2.5, 3.0, and ≥3.5 were 49%, 48.8%, and 66.7%, respectively, whereas the overall pregnancy rates were observed 32.7%, 32.6%, and 42.4%, respectively. Cattle with a BCS ≥3.5 exhibited numerically higher estrus and pregnancy rates, corresponding to a 2.3-fold and 1.7-fold increase in odds, respectively compared to the reference category [estrus: OR =2.34; 95% CI (0.96-5.70); pregnancy: OR =1.72; 95% CI (0.72-4.12); $p > 0.05$].

DISCUSSION

In this study, the overall estrus and pregnancy rates across all treatments for anestrus cattle were 52.4%, and 34.5%, respectively, while the overall pregnancy rate per estrus was 65.9%. Comparatively higher estrus rates and varying pregnancy rates per estrus have been reported in previous studies; for instance, Akter *et al.* (2022) recorded 73.8% and 100%, respectively, Srilatha and Phaneendra (2024) observed 81% and 73%, respectively, and Shrestha *et al.* (2025) reported 97.5% and 45%, respectively. This variation may be attributed to differences in selection criteria for anestrus cattle, management systems, treatment protocols, and institutional farm settings. In contrast, the present study was conducted under field conditions on smallholder farms characterized by diverse management practices.

The expression of estrus signs and subsequent pregnancy following per-rectum massage of the reproductive organs

were non-significantly higher than in the control group. Manual massage of the reproductive organs might induce a bio-stimulatory effect, which stimulates the hypothalamic-pituitary-gonadal axis to initiate hormonal activity. Furthermore, strategic administration of vitamin-minerals also significantly increased both estrus rate and pregnancy rates. Because vitamin-minerals deficiencies suppress gonadotropin secretion and inhibit reproduction (Chen *et al.*, 2023). Therefore, supplements (especially with vitamin A, D, and E, and selenium) helps maintain immune function and enhances adaptation to stress conditions, such as nutritional and heat stress. Similarly, utilizing a nutritional management approach, Akter *et al.* (2022) reported a 71.4% estrus rate, while Srilatha and Phaneendra (2024) reported estrus and conception rates of 85.7% and 83.3%, respectively. Nutritional deficiencies and mineral imbalances are key contributors to anestrus. Correcting these deficiencies improves the hypothalamic-pituitary-ovarian axis, enhances follicular development, and increases ovulation rates (Song *et al.*, 2026). Furthermore, the energy status of a female animal regulates the hormones essential for follicular development, ovulation, and fertilization (Hao *et al.*, 2026). During the transition period, feed shortages cause a negative energy balance (NEB) that disrupts reproductive cycles. Additionally, deficiencies in minerals can lead to anestrus by hindering cellular-level hormone and enzyme activity (Sammad *et al.*, 2022).

This study revealed that two injections of GnRH at 11-day intervals significantly increased the estrus and pregnancy rates (65.2% and 43.5%, respectively) of anestrus cattle [estrus: OR =9.75; pregnancy: OR =11.15; $p < 0.05$]. In comparison, Akter *et al.* (2022) reported a higher estrus rate (75%), while Srilatha and Phaneendra (2024) observed estrus and pregnancy rates 57.1% and 50%, respectively. Exogenous GnRH injection stimulates the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH is responsible for follicular development, which increases the estrogen levels to initiate estrus behavior, whereas LH mediates oocyte maturation and ovulation. Furthermore, GnRH administration increases circulating LH concentrations and the resulting estrous intensity appears to modulate the effect of GnRH on post-estrus corpus luteum development and progesterone concentrations (Burnett *et al.*, 2022).

In this study, combination treatment protocols significantly increased the estrus and pregnancy rates compared to a single treatment regimen in anestrus cows. These combined treatment regimens might exert a synergistic effect that improves the reproductive performance of anestrus cattle. Specifically, the administration of GnRH in combination with per-rectum massage of the reproductive organs, vitamin-mineral supplementation, or both, significantly

increased estrus induction rates and pregnancy outcomes. Synergizing massage or nutritional therapy with GnRH interventions, particularly in cows with ovarian inactivity, helps maintain an optimal body condition score and regulates hormonal action on fertility (Hao *et al.*, 2026).

Regarding age, although animals aged 2.6-3.5 years exhibited a numerically higher estrus rate (63.3%), those aged 3.6-4.5 years had a higher pregnancy rate (50%) than the other age categories. Animals in the 3.6-4.5 years cohort had 2.9-fold higher odds of becoming pregnant than younger. This finding aligns with the study by Biswas *et al.* (2022), who reported a 77.5% pregnancy rate in 3.6-4.5-year-old cattle, representing a 5.3-fold greater probability of becoming pregnant than other age groups. Furthermore, Fahim *et al.* (2025) demonstrated that cows aged 4-6 years are more productive than other age groups because mature cows possess better metabolic reserves and endocrine stability, enabling improved responsiveness to estrus induction protocols.

Regarding genotypes, the L×SL breed was observed comparatively higher estrus and pregnancy rates (61.4%, and 42.9%, respectively) than the others genotypes. This genotypes also demonstrated 1.3-fold and 1.5-fold higher odds for estrus and pregnancy, respectively, compared to local breed. In contrast, the L×HF breed showed a significantly lower estrus rate (35.5%) than the local breed ($p < 0.05$). These findings are consistent with those of Roy *et al.* (2025), who observed higher pregnancy rates in SL crossbred cows and comparatively lower pregnancy rates in HF crossbred cows than in the local breed. In the agro-ecological context of Bangladesh, local and L×SL genotypes are well-adapted to the environment, whereas the L×HF breed exhibits discomfort behaviors specially in summer season (Islam *et al.*, 2021).

Although parity had no significant effect on the estrus and pregnancy rates, parity 2 cows were exhibited comparatively higher estrus and pregnancy rates (58.3%, and 41.7%, respectively) than others parity cohorts. Similarly, Roy *et al.* (2025) and Biswas *et al.* (2022) observed pregnancy rates of 71.5%, and 65%, respectively, in second-parity cows. Conversely, Shrestha *et al.* (2025) reported a higher estrus expression rate in primiparous (P1) cows than in multiparous. Such variations occur because physiological activity and pituitary responses heavily depend on rapid post-partum recovery from negative energy balance.

Regarding BCS, although no significant differences were observed across the categories, the anestrus animals with a BCS ≥ 3.5 were found more responsive to the treatment protocols, yielding higher estrus induction and pregnancy rates. This observation is supported by Biswas *et al.* (2022) and Roy *et al.* (2025). Poor body condition reflects a

prolonged negative energy balance and associated vitamin-minerals deficiencies, which suppress gonadotropin secretion and follicular growth. Concurrently, the estrus response has been shown to increase from 52% to 88% as BCS improves, confirming that nutritional therapy is a key of anestrus management (Taher *et al.*, 2026).

LIMITATIONS

The study was conducted across diverse management systems. Ultrasonography was not utilized for the pregnancy diagnosis. Neither biochemical analyses nor hormonal assays were performed in this study.

CONCLUSION

This study demonstrates that combination treatment regimens are highly effective in resolving anestrus and optimizing reproductive performance in dairy cattle under smallholder field conditions. The integration of exogenous GnRH administration with per-rectum reproductive massage and strategic vitamin-mineral supplementation exerts a powerful synergistic effect, yielding significantly higher estrus induction and pregnancy rates than single-treatment interventions. Furthermore, Optimal treatment outcomes are achieved in mature cows (aged 3.6–4.5 years), second-parity animals, well-adapted local or L×SL crossbred genotypes, and cattle maintaining an adequate nutritional status (BCS ≥ 3.5). Future research should incorporate with comprehensive biochemical analysis to better understand the metabolic mechanisms involved with anestrus in cattle.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Research and Training Centre, Patuakhali Science and Technology University for financial assistance (Project code: ANSVM-87, FY 2024-2025).

NOVELTY STATEMENT

This study is novel in evaluating both pre- and post-partum anestrus in cattle with field level applicable protocols.

AUTHOS'S CONTRIBUTION

AKP: Conceptualization, experimental design, statistical analysis and final manuscript writing, supervision, and final review. HM, AR and SAK: Conducted experiment, data collection and laboratory analyses, writing draft manuscript. DB: Statistical analysis, validation, interpretation of results and critical revision of the manuscript. All authors read and approved the final version of manuscript.

ABBREVIATIONS

AI: Artificial insemination, BCS: Body condition score, CI: Confidence interval, GnRH: Gonadotropin-releasing hormone, HF: Holstein-Friesian, L: Local, MRO: Massage of the reproductive organs, NEB: Negative energy balance, OR: Odd ratio, P: Parity, PGF2 α : Prostaglandin F2 α , SL: Sahiwal, VM: Vitamin-minerals

ETHICAL APPROVAL

The methodology of this study was evaluated and approved by the Institutional Ethical Committee of Patuakhali Science and Technology University, considering internally acceptable animals ethics and welfare criteria (Approval No. PSTU/IEC/2025/11/i).

DATA AVAILABILITY

All data supporting the findings of this study are available within the manuscript. Additional data are available from the corresponding author upon request.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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