Research Article

Comparative Evaluation of Hormonal Treatments for Estrus Induction and Conception in Anestrous Postpartum Dairy Cows

Jawad Kadhim Taher[1], Rabab Shaker Salman[1], Noura Kareem Jabbar[2], Khawla A Hussein[3], Hakeem Jawad Kadhim[4*]

[1]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Shatrah, Al-Shatrah, Thi-Qar, Iraq; [2]Department of Animal Production, College of Agriculture and Marshes, University of Thi-Qar, Thi-Qar, Iraq; [3]Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; [4]Department of Microbiology, College of Veterinary Medicine, University of Shatrah, Al-Shatrah, Thi-Qar, Iraq.

Abstract | Postpartum anestrus, along with the inconsistent efficacy of hormonal protocols, delays breeding and reduces profits for dairy farms. This study aimed to evaluate and compare the efficacy of four hormonal protocols targeting the follicular and luteal phases of the estrous cycle to induce estrus and improve conception rates in postpartum local Iraqi (Al-Janoubi) dairy cows, thereby reestablishing ovarian cyclicity between 60 and 120 days postpartum. A total of 40 anestrous cows were randomly assigned to four treatment groups (n=10 per group): T1 (Cloprostenol and Gonadorelin), T2 (Estradiol and Cloprostenol), T3 (Estradiol and Gonadorelin), and T4 (Cloprostenol alone). Estrus response, conception rate, and timing of estrus onset were recorded for all animals. Serum lutenizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations, estradiol (E2), and progesterone (P4) concentrations were measured using a Cobas e411. Pregnancy was confirmed by ultrasonography, with transabdominal scans employed when transrectal evaluation was not feasible. Estrus induction rates and conception rates showed significant differences ($p = 0.048$ and 0.0092, respectively). Groups T2 and T3 achieved 60% estrus induction, with conception rates of 33% and 67%, respectively. Whereas T1 and T4 had a 20% estrus response, resulting in no pregnancies. The onset of estrus occurred earliest in T2 (around 40 hours), intermediate in T1 and T4 (approximately 72 hours), and latest in T3 (about 144 hours). Although LH and FSH levels did not differ significantly among groups ($p > 0.05$), estradiol and progesterone levels were significantly higher in hormonally induced cows ($p < 0.01$), indicating luteal stimulation. In conclusion, among the tested protocols, the combination of estradiol and gonadorelin resulted in the highest conception rate, whereas estradiol with cloprostenol led to the earliest onset of estrus. In situations where estradiol is restricted, GnRH- or progesterone-based protocols remain viable alternatives. Maintaining an optimal body condition score (BCS 3-4) is essential for successful reproductive recovery in postpartum dairy cows.

Keywords | Conception rate, Estrus induction, Estradiol, Gondorelin, Hormonal protocols, Postpartum dairy anestrus, Ultrasonography

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***Correspondence** | Hakeem Jawad Kadhim, Department of Microbiology, College of Veterinary Medicine, University of Shatrah, Al-Shatrah, Thi-Qar, Iraq; **Email:** hakim.jawad@shu.edu.iq
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INTRODUCTION

Postpartum anestrus is one of the most important problems in dairy cattle and buffaloes (Ambrose, 2021). Though a short-term anestrus period allows uterine recovery and hormonal re-establishment, a prolonged postpartum delay (i.e. > 60–90 days) is found to be associated with reproductive inefficiency, extended days open, less milk yield, higher treatment cost, as well as an involuntary culling in the end with consequently marginal gains made either through herd profitability or genetic improvement of the herd entering into a situation prevalent like this (Abraham, 2017; Hafez and Hafez, 2000). Both cows and buffalo generally take 85 to 90 days open following calving, although buffalo may have this period extended up to about 100-150 days.

Postpartum anestrus can manifest as silent estrus, where ovulation happens without visible heat because of low estradiol secretion, or true anestrus, marked by total ovarian inactivity (Sakaguchi *et al.*, 2023). The frequency of this condition varies by breed, parity, season, nutrition, and management practices. In buffaloes, it ranges from 6% to 73%, and in cattle, from 7% to 44% (Kumar *et al.*, 2013; Noakes *et al.*, 2018; Bharali *et al.*, 2019). The negative energy balance in early lactation reduces insulin and IGF-1 (insulin-like growth factor) concentrations, thereby affecting follicular maturation and ovulation. Further, metabolic stress and suckling activity may inhibit the hypothalamic-pituitary-ovarian (HPO) axis, leading to delayed return to estrus (Garnsworthy *et al.*, 2009; Stevenson and Atanasov, 2022).

Reproductive recovery involves uterine involution and endometrial repair while rebuilding follicles and is critical to the resumption of fertility (Plant and Zeleznik, 2015). Any interruptions caused by metabolic disorders, nutrient deficiencies, or hormonal discrepancies, especially in LH, FSH, estradiol, and progesterone, can prolong anestrus and challenge conception and fertilization (Yavas and Walton, 2000; Cheong *et al.*, 2016).

To resolve delayed cyclicity, hormonal protocols have been developed to induce follicular growth, synchronize ovulation, and trigger estrus. Common pharmacological agents include prostaglandins (e.g., cloprostenol), GnRH analogs (e.g., gonadorelin), and estradiol esters (e.g., estradiol benzoate). These agents mimic natural interactions to enhance follicle recruitment and luteolysis, and to induce pre-ovulatory estradiol surges (Edwell *et al.*, 2004; Rana *et al.*, 2018). Estradiol-based combinations continue to elicit follicular wave synchronization but are limited in the European Union due to human health concerns (European Commission, 2003). However, these protocols remain in use in non-European Union countries for experimental and field reproductive management.

The current research was conducted to assess the potency of four hormonal protocols consisting of combinations of cloprostenol, gonadorelin, and estradiol to elicit estrus and enhance conception in postpartum local Iraqi (Al-Janoub) dairy cows within 60 to 120 days postpartum. Serum concentrations of LH, FSH, E2, and P4 were measured as indicators of hormonal responses associated with natural and induced estrus. Pregnancy and conception outcomes were verified by transrectal and transabdominal ultrasonography, with transabdominal ultrasonography used when transrectal ultrasound was not feasible due to environmental and field conditions, providing valuable information for improving reproductive management in local Iraqi (Al-Janoub) dairy cows.

MATERIALS AND METHODS

Study area, duration, and experimental animals

The research took place from October 2024 to February 2025 in Thi-Qar Province/Southern Iraq. In this study, 50 local Iraqi (Al-Janoubi) dairy cows were initially selected for inclusion. Of the 50 cows, 40 postpartum cows were randomly assigned to receive therapeutic comparisons in four treatment groups (n=10 cows per group). Ten fertile cows of similar postpartum age were used as a control group to assess reproductive hormone concentrations during natural (non-induced) estrus and during induced estrus with hormonal protocols. All cows were 3–10 years old, with body condition scores (BCS) of 3.0-4.0 (Roche *et al.*, 2009; Edmonson *et al.*, 1989), and were clinically healthy with no history of reproductive or metabolic disorders. The postpartum anestrus was confirmed to occur 60–120 days after calving, and examination per rectum identified small, inactive ovaries and no palpable corpora lutea. There were no statistically significant differences in parity or age among the treatment groups (P > 0.05; Table 1). Of note, five cows were excluded from the study prior to treatment. A veterinary investigation was conducted on three cows due to uterine infections, and two cows were lost to follow-up (one was relocated and one was sick). These animals were excluded from treatment and are not included in the analysis.

Table 1: Baseline characteristics of cows per group.

Group	n	Age (years)	Parity	BCS
T1	10	6.2 ± 0.5	3.2 ± 0.3	3.5 ± 0.2
T2	10	6.0 ± 0.4	3.1 ± 0.2	3.6 ± 0.2
T3	10	6.3 ± 0.6	3.3 ± 0.4	3.5 ± 0.2
T4	10	6.1 ± 0.5	3.2 ± 0.3	3.5 ± 0.2
Control	10	6.0 ± 0.5	3.1 ± 0.3	3.5 ± 0.2

n: number; BCS: Body condition score.

Table 2: Details of hormonal treatments and their manufacturers.

Group	Treatment	Active hormone(s)	Dose	Route	Manufacturer (Country)
T1	Cloprostenol + Gonadorelin	$PGF_2\alpha$ (250 µg/mL) + GnRH (100 µg/mL)	2 mL+ 3mL	i.m.	Alfasan Woerden-Holland; NASR Pharmaceutical- Iran
T2	Estradiol + Cloprostenol	Estradiol benzoate (2 mg/mL) + $PGF_2\alpha$ (250 µg/mL)	1 mL+ 2mL	i.m.	NASR Pharmaceutical- Iran Alfasan Woerden-Holland
T3	Estradiol + Gonadorelin	Estradiol benzoate (2 mg/mL) + GnRH (100 µg/mL)	1 mL+ 3mL	i.m.	NASR Pharmaceutical- Iran; NASR Pharmaceutical- Iran
T4	Cloprostenol alone	$PGF_2\alpha$ (250 µg/mL)	2 mL	i.m.	Alfasan Woerden-Holland

$PGF_2\alpha$ = Prostaglandin $F_2\alpha$; GnRH = Gonadotropin-releasing hormone; i.m. = intramuscular.

Experimental design and treatments

Cows were randomly assigned to four treatment groups (T1–T4, n=10 each) and an untreated control group (n=10) that exhibited natural estrus. All hormonal treatments were administered intramuscularly. The treatment, drugs, and manufacturer information are shown in Table 2.

Estrus detection and confirmation

The research utilized visual observation to identify estrus post-treatment based on specific behaviors. Specific behaviors included: Increased restlessness, habitual mounting behaviors, vulvar swelling, frequent urination, and standing heat. The observation of clear cervical mucus and redness of the vulva further identified the cow as in estrus. All observations were made or checked by both the researcher and the farm owners. Estrus was also evaluated every 6 hours with a teaser bull, tail-paint evaluation, and assessment of standing heat.

Blood sampling and hormonal assay

Blood samples were collected by jugular venipuncture from cows on Day 0 (before treatment), Day 7 (after treatment), and at the onset of estrus for responding cows or on Day 14 for non-responders. To isolate srum, the samples were centrifuged at 3,000 rpm for 15 minutes and stored at -20°C until analysis. For all cows, serum levels of hormones (LH, FSH, E2, and P4) were measured using an electrochemiluminescence immunoassay (ECLIA) on a Cobas E411 immunoassay analyzer (Roche Diagnostics, Japan) following the manufacturer's instructions.. The assays were confirmed to work with bovine serum, showing good recovery, linearity, and parallelism with reference standards. All serum samples were tested in a single batch to minimize variation. Quality-control sera were included in each run. Analytical performance characteristics, including the limits of detection (LOD) and intra- and inter-assay coefficients of variation (CVs), were measured (Supplementary Table S1).

Ultrasound examination and pregnancy diagnosis

Pregnancy was confirmed 30 days after artificial insemination with a Chison ECO2 ultrasound scanner from Chison Medical Technologies Co., Ltd. in China. Both transrectal (using a 7 MHz linear probe) and transabdominal (using a 3.5 MHz convex probe) approaches were employed. Transrectal ultrasonography served as the primary diagnostic tool, while transabdominal scanning was employed as a non-invasive adjunct for early confirmation of fetal and placental development under field conditions (Aziz and Lazim, 2012; Baska-Vincze *et al.*, 2014; Muhammad *et al.*, 2023).

Data analysis

All analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). In the present study, the estrus induction and conception rates were compared across treatment groups using the Chi-square test, Fisher's exact test, and effect sizes. Serum concentrations of reproductive hormones and the onset of estrus were assessed for normality using the Shapiro-Wilk test. Mean comparisons for normally distributed variables were performed using one-way ANOVA and Tukey's post hoc test. Variables that were not normally distributed were analyzed with the Kruskal-Wallis test with Dunn's post hoc test. The results are presented as mean ± standard deviation, where differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Estrus induction and conception outcomes across treatment groups

A total of 40 postpartum local Iraqi (Al-Janoubi) dairy cows were assigned to four hormonal treatment groups, each containing ten cows (Table 3; Supplementary Table S2). In T1 (cloprostenol + gonadorelin), two cows (20%) exhibited estrus, and none conceived. T2 (estradiol + cloprostenol) resulted in six cows responding (60%), with two pregnancies (33.3% conception rate). T3 (estradiol + gonadorelin) had six responders (60%) and four pregnancies (66.7% conception rate). T4 (cloprostenol alone) had two responders (20%) with no pregnancies. The total number of cows displaying estrus was 16 out of 40. Six of these

responders became pregnant, resulting in a conception rate of 37.5 percent among responders and 15 percent overall for all treated animals.

Estrus induction differed significantly among groups (χ^2 = 9.82, p = 0.02; Fisher's exact p = 0.018), and conception outcomes also differed significantly (χ^2 = 8.00, p = 0.045; Fisher's exact p = 0.044). Compared with T1, T3 increased the likelihood of conception by 67% (risk difference = 0.67, 95% CI: 0.38–0.95).

Estrus response timing across treatment groups

The time to estrus occurrence differed significantly across treatment groups (Table 4). The two responding cows in T1 (cloprostenol + gonadorelin) and T4 (cloprostenol only) exhibited estrus at 72 hours after treatment, while six cows in T2 (estradiol + cloprostenol) exhibited estrus at 40 hours. The six responding cows in T3 (estradiol + gonadorelin) exhibited estrus at 144 hours. There was a highly significant effect of treatment group on time to estrus onset (F = 32.702, p = 0.0001).

Serum reproductive hormone levels in different estrus statuses

The serum levels of reproductive hormones (LH, FSH, E_2, and P_4) were different for the hormonally-induced estrus group, the natural estrus group, and the non-responding group of cows (Table 5). The average values for luteinizing hormone (LH) were similar across all three treatment groups: 0.115 ± 0.044 mIU/mL (control), 0.100 ± 0.042 mIU/mL (non-responded), and 0.103 ± 0.032 mIU/mL (responded). ANOVA did not indicate a significant difference (F = 0.491; p = 0.61).

Table 3: Estrus induction and conception outcomes in postpartum dairy cows treated with different hormonal protocols.

Treatment groups	No.	Rate of response time (h) Mean ±SD	Test statistical	P value	Significance
T1 (Cloprostenol + Gonadorelin)	2	72±4.24			
T2 (Estradiol + Cloprostenol)	6	40 ±3.57			
T3 (Estradiol+ Gonadorelin)	6	144±7.03			
T4(Cloprostenol only)	2	72 ±5.66			
Overall Comparison	16		F= 32.70	0.0001	Highly significant (P ≤ 0.01)

Values represent mean ± standard deviation (SD) for cows exhibiting estrus response following hormonal treatment. Statistical analysis performed using one-way ANOVA. gnificance levels: p ≤ 0.05 = significant; p ≤ 0.01 = highly significant.

Table 4: Average response time to estrus induction (in hours) following different hormonal treatment protocols in postpartum dairy cows.

Group	N (Responded / Total)	Estrus induction (%)	Mean ± SD time to Estrus (h)	Conceived n (%)	χ^2 / ANOVA (p)
T1 (Cloprostenol + GnRH)	2 / 10	20	72 ± 4.2	0 (0 %)	χ^2 = 6.74, p = 0.048*
T2 (E_2 + Cloprostenol)	6 / 10	60	40 ± 3.5	2 (33.3 %)	ANOVA F = 32.1, p = 0.001**
T3 (E_2 + GnRH)	6 / 10	60	140 ± 8.0	4 (66.7 %)	ANOVA F = 32.1, p = 0.001**
T4 (Cloprostenol only)	2 / 10	20	72 ± 5.7	0 (0 %)	χ^2 = 6.74, p = 0.048*
Control (Natural estrus)	10 / 10	100	—	10 (100 %)	Reference

E_2 = Estradiol; GnRH = Gonadotropin-releasing hormone; SD = standard deviation; χ^2 = Chi-square test; ANOVA = analysis of variance; F = F-statistic. Values marked with p < 0.05 () and p < 0.01 (**) indicate significant and highly significant differences, respectively.

Table 5: Comparison of Mean ± standard deviations (Mean ± SD) hormone levels among control, non-responded, and responded groups

Hormone	Control (Mean ± SD)	Responded (Mean ± SD)	Non-responded (Mean ± SD)	F-value	P-value	Significance	LSD
LH (mIU/mL)	0.115 ± 0.04[a]	0.103 ± 0.032[a]	0.10 ± 0.042[a]	0.491	0.61	NS	0.026
FSH (mIU/mL)	0.304 ± 0.07[a]	0.309 ± 0.092[a]	0.30 ± 0.057[a]	0.088	0.92	NS	0.047
E_2 (ng/mL)	0.035 ± 0.008[b]	0.063 ± 0.009[a]	0.02 ± 0.007[c]	129.11	0.008	***	0.005
P_4 (ng/mL)	0.80 ± 0.17[b]	1.60 ± 0.12[a]	0.72 ± 0.11[c]	225.58	0.008	***	0.086

Footnotes: [abc] Means within a row with different superscripts differ significantly (p < 0.05). LH = Luteinizing hormone; FSH = Follicle-stimulating hormone; E_2 = Estradiol; P_4 = Progesterone; SD = Standard deviation; LSD = Least significant difference; NS = Not significant. *** Highly significant difference (p < 0.01).

When looking at follicle-stimulating hormone (FSH), the average values were also similar: 0.304 ± 0.070 mIU/mL (control), 0.300 ± 0.057 mIU/mL (non-responded), and 0.309 ± 0.092 mIU/mL (responded). Also, there was no significant difference (F = 0.088; p = 0.92), indicating that no treatment type or estrus response appears to elicit a change in gonadotropin secretion.

In comparison, estradiol (E_2) varied remarkably across groups, ranging from 0.020 ± 0.007 ng/mL in the non-responded cows to 0.035 ± 0.008 ng/mL in the control group and finally to 0.063 ± 0.009 ng/mL in the responded cows. The statistical differences were very significant (F = 129.11; p = 0.008; LSD = 0.005), confirming that all pairwise comparisons were entirely different.

Progesterone (P_4) showed the most significant variation across groups. The average concentration of P_4 was 0.72 ± 0.110 ng/mL in the non-responded cows; 0.80 ± 0.17 ng/mL in the control cows; and 1.60 ± 0.12 ng/mL in the responded cows. Statistical analysis reported a very significant difference (F = 225.58; p = 0.008; LSD = 0.086).

Ultrasonography images

Both transrectal and transabdominal ultrasonography techniques confirmed pregnancy, and early fetal development was assessed at 30 days post-insemination (Figure 1). Assessment was performed using a 7 MHz linear transducer and a 3.5 MHz convex transducer to ensure the best images of the developing fetus and placentomes were captured.

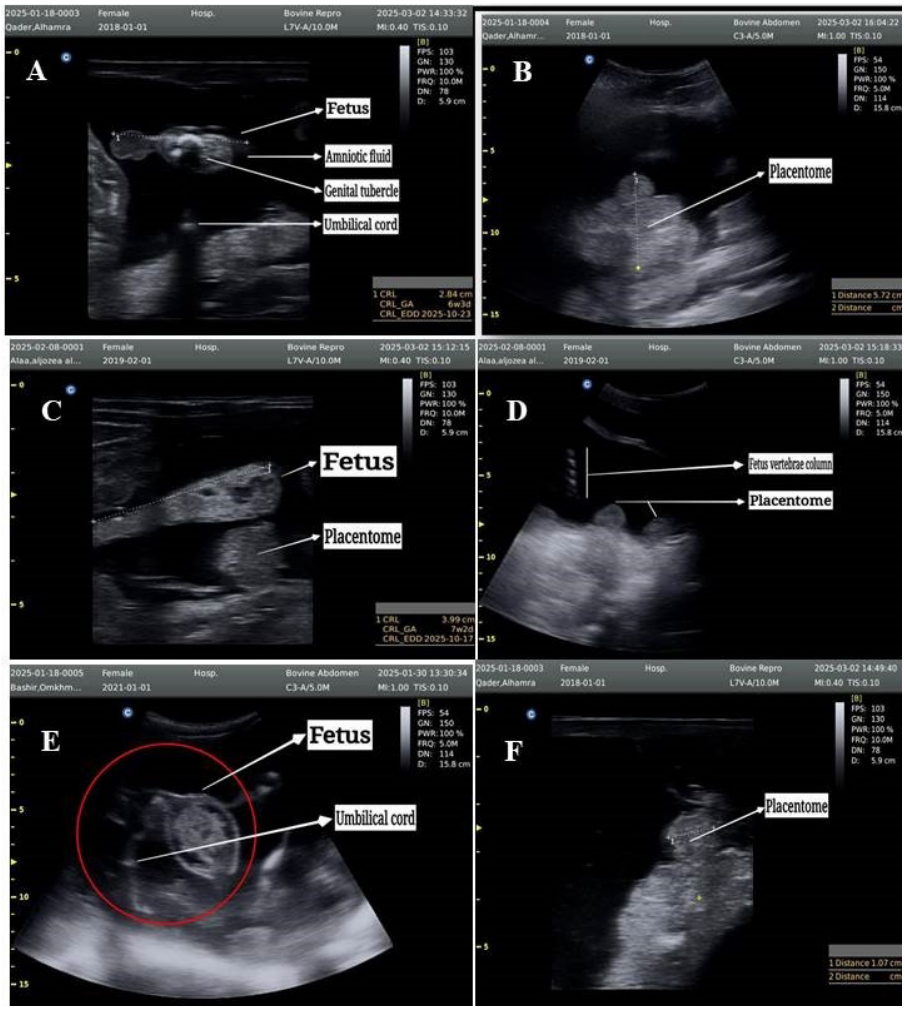

Figure 1: Ultrasonographic assessment of bovine pregnancy during early gestation (Panels A-F). (A) Transrectal ultrasound at 6 weeks 3 days (CRL: 2.84 cm) showing the fetus with amniotic fluid, genital tubercle, and umbilical cord. Linear probe, 7 MHz, transverse plane, gain 60%, scale bar = 1 cm. (B) Transabdominal scan of placentome (5.72 cm in diameter). Convex probe, 5 MHz, longitudinal plane, gain 65%, scale bar = 1 cm. (C) Longitudinal view of the fetus at 7 weeks and 2 days (CRL: 3.99 cm) near a placentome. Linear probe, 7 MHz, longitudinal plane, gain 60%, scale bar = 1 cm. (D) Sagittal view highlighting the fetal vertebral column and placentome. Linear probe, 7 MHz, sagittal plane, gain 60%, scale bar = 1 cm. (E) A cross-sectional transabdominal image displaying the fetus and umbilical cord within the uterus. Convex probe, 5 MHz, transverse plane, gain 65%, scale bar = 1 cm. (F) Early-stage placentome (1.07 cm) captured with a 7 MHz transrectal linear transducer, transverse plane, gain 60%, scale bar = 0.5 cm.

Based on crown-rump length (CRL) and internal anatomy, elements indicated that fetuses were 6-7 weeks of age. The fetus was seen on ultrasound as a bright echogenic structure floating within the well-defined anechoic amniotic cavity. The head, vertebral column, and curved body shape could be distinguished. Placentomes were also seen quite clearly in a relatively even distribution along the uterine wall. The dimensions of the placentomes were recorded to be generally circular and varied in size from 1.07 to 5.72 cm in diameter, with the CRL of the fetuses appearing equivalent to the dimensions of the placentomes. The higher-frequency probe allowed detection of slightly smaller placentomes, while the lower-frequency probe provided deeper information about the same placentomes, albeit with better development indicated.

There was no apparent placental separation, calcification, or fluid collection, an echotextured placental surface displayed blood flow in the umbilical cord, indicating normal fetal and placental development. These observations confirmed viable early pregnancies in the cows that had successfully conceived. Also, transabdominal sonography was identified as a helpful, non-invasive, supplementary diagnostic procedure in the field.

DISCUSSION

Both postpartum anestrus and subestrus continue to be significant reproductive problems in dairy cows. These conditions increase days open, delay conception, and result in loss of income and output. The restoration of ovarian cycling post-partum depends on two main factors: the resumption of luteinizing hormone (LH) secretion and the reactivation of the hypothalamic-pituitary-ovarian axis (Yavas and Walton, 2000). The pragmatism of distinguishing true anestrus (the absence of an active follicle or corpus luteum) from subliminal estrus (with ovulation but silent estrus) to inform therapeutic decision-making is important (Sellappan and Rajasundaram, 2001; Madureira *et al*., 2021). Given that rectal palpation alone carries special risks of false judgments, the combination of ultrasonography and endocrinological profiling of fertility should provide a more precise diagnosis and tentative recommendations on the suitability of hormonal therapy.

In this study, combinations based on estradiol (T2, estradiol + cloprostenol) or T3 (estradiol + gonadorelin) had greater estrus induction (60%) and conception rates (33.3 and 66.6%, respectively) than T1 (Prostaglandin) or T4 (gonadorelin; T1 and T4 were 20% estrus and 0% conception). These outcomes argue for estradiol's ability to improve fertility responsiveness in cases of true postpartum anestrus, if hormones can act to promote follicle recruitment and, in combination with its observed influence on the hypothalamus and pituitary, improve behavioral estrus.

The enhanced response in the T3 protocol emphasizes the synergistic effect of the sequence of estradiol priming, a high physiological concentration of gonadotropin-releasing hormone (GnRH) to stimulate luteinizing hormone (LH) release, and the subsequent ovulation of the deeply antral dominant follicle. Cloprostenol alone (T4) would be ineffective in this situation because the majority of deeply anestrous cows would not have functionally mature corpora lutea, thereby eliminating the biological effectiveness of $PGF_2\alpha$-induced luteolysis. These data are consistent with values reported by Haile *et al*. (2021), which indicated that $PGF_2\alpha$ protocols within a fixed time insemination (FTI) will only be effective when luteal structures are present.

Response time varied significantly between protocols. The combination of estradiol and cloprostenol (T2) resulted in faster induction of estrus, approximately 40 hours. Meanwhile, estradiol and gonadorelin (T3) resulted in slower responses, approximately 144 hours. These results demonstrate that T2 was more effective at synchronizing follicular emergence and estrous behavior than T3. The slower T3 response indicates that the hormonal process began and progressed slowly during follicular recruitment and, consequently, ovulatory synchronization, as established by Kolachi *et al*. (2025). The overall estrus induction rate (60%) was lower than the 75-100% previously reported by Sah and Nakao (2010) and Islam *et al*. (2013), but the T3 conception rate was 66.6%, consistent with their observation of 64.7%. The relatively low estrus response in this study reflects breed traits and variable nutrition, as well as longer postpartum intervals observed in local Iraqi (Al-Janoubi) dairy cows under these field conditions in South Iraq.

The absence of marked variation in levels of LH and FSH ($p > 0.05$) in hormonally induced, natural, and non-responsive cows indicated that average gonadotropin release was similar among different reproductive states. This was most likely due to the timing of blood sampling during the period of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) release, as these hormone spikes circulate for only a limited time. Average concentrations of LH and FSH do not account for their pulsatile secretion. It is worth noting that estradiol and progesterone concentrations were also significantly higher ($p < 0.01$) in the hormonally induced estrus despite there being no differences in LH or FSH levels. This provides evidence that stimulation of the ovarian follicles resulted in the appropriate end product: corpus luteum formation. In some hormonally induced cows, the average base

increase in progesterone level (1.6 ± 0.07 ng/mL) may have resulted from partial luteinization of dominant follicles or an endogenous luteinizing event induced by gonadorelin. In summary, this may be further evidence of the beginning of a normal luteal phase.

Consequently, it appears that the responsiveness to treatment impacted ovarian steroid production instead of the release of pituitary gonadotropins. The concurrent rise of E_2 and P_4 levels in responding cows suggests appropriate follicular development and the presence of a functional luteal body. These observations further support the conclusions of Perry *et al.* (2023) and Setyorini *et al.* (2023), where a successful induction of estrus was noted by a concurrent rise of E_2 and P_4 levels during the follicular to luteal transition. The discrepancy observed between hormonally induced and natural cycling cows, versus the similarity observed in the natural and non-responsive groups, could suggest that the non-responsive group had minimal ovarian activity or lower sensitivity to gonadotropins; thus, this decreased activity may inhibit the ovaries ability to respond to external hormonal applications. These findings suggest the importance of thoroughly assessing the ovaries prior to treatment, using approaches such as ultrasound, to identify atrophic/inactive ovaries that may require nutritional or mineral interventions before hormone-induced protocols.

Field conditions, including disturbances in feeding patterns, heat stress, and limited estradiol supplementation, may contribute to lower induction rates than in controlled studies (Nagai *et al.*, 2013; Hassanein *et al.*, 2024). However, ultrasonography with hormonal testing is a reliable method for diagnosing and finding early pregnancy status, which affords practical benefits for herd-level fertility programs. Estradiol-based combinations are effective for restoring cyclicity and improving conception in postpartum Iraqi dairy cows, particularly estradiol + GnRH. This method is a low-cost alternative for farms with limited resources.

CONCLUSION

Hormonal treatments focusing on estradiol that stimulate both the follicular and luteal phase cycles can induce estrus and increase the probability of obtaining successful pregnancies in postpartum dairy cows. Using hormonal treatments in combination with ultrasound will improve the accuracy of diagnostic testing and the prediction of treatment success. Future research should investigate the influence of nutrition, metabolism, and genetics on hormonal responsiveness. Future research should also evaluate the potential long-term effects of these protocols on reproduction and economics in the field settings.

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NOVELTY STATMENT

This research compares estradiol-based and prostaglandin hormonal protocols for inducing estrus in postpartum, anestrous dairy cows in southern Iraq. The combined estradiol and gonadorelin treatment significantly increased estrus induction and conception rates compared with single-hormone treatments. Along with hormonal testing and ultrasonography, this multidimensional research presented a feasible, scientifically underpinned procedure to enhance reproductive efficiency in dairy herds, particularly in light of the socio-contextual realities of resource-limited production systems.

AUTHOR'S CONTRIBUTION

JKT was responsible for the conception and design of the initial study, methodology, data analysis, and the initial draft of the manuscript. RSS assisted with data collection, laboratory work, and field work. NKJ was responsible for statistical analyses and visualizations, as well as manuscript review and editing. KAH conducted the literature review, explained the results, and assisted with manuscript editing. HJK supervised the study, supervised project management, and provided final approval of the manuscript.

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Ethical approval

The Scientific Committee of the College of Veterinary Medicine at the University of Shatrah approved and provided support for the study (official letter No. 838 dated February 1, 2024). All animal procedures were reviewed and approved by the Ethical Committee of the College of Veterinary Medicine, University of Shatrah, Thi-Qar Province, Iraq, and conducted in accordance with national and institutional guidelines for the care and use of animals in scientific research. Estradiol was legally used in accordance with the Iraqi code governing the therapeutic use of drugs in domestic livestock.

Generative AI and AI-assisted technology statement

The authors confirm that no generative AI or AI-assisted technologies were used to prepare, draft, or edit this manuscript.

Data availability

All relevant data generated or analyzed in this study are included in this manuscript (Supplemental Tables S1, and S2).

Conflict of interest

The authors have declared no conflict of interest regarding the content of this manuscript.

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Supplementary Table S1:

Hormone	Assay platform	LOD	Intra-assay CV (%)	Inter-assay CV (%)	Units
LH	Cobas e411	0.01	4.3	6.5	mIU/mL
FSH	Cobas e411	0.02	3.7	5.9	mIU/mL
E_2	Cobas e411	0.005	5.2	6.8	ng/mL
P_4	Cobas e411	0.02	4.1	6	ng/mL

Supplementary Table S2:

Animal ID	Group	Estrus response	Time to Estrus (h)	Conception	LH (mIU/mL)	FSH (mIU/mL)	E2 (ng/mL)	P4 (ng/mL)	Sampling Timepoint
1	T1	Responded	75	No	0.09	0.31	0.022	0.72	Day 0 (Before)
1	T1	Responded	75	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
1	T1	Responded	75	No	0.07	0.43	0.06623	1.59	Estrus/Day 14
2	T1	Responded	69	No	0.09	0.31	0.022	0.72	Day 0 (Before)
2	T1	Responded	69	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
2	T1	Responded	69	No	0.12	0.27	0.05019	1.66	Estrus/Day 14
3	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
3	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
3	T1	Non-responded		No	0.05	0.33	0.03314	0.54	Estrus/Day 14
4	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
4	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
4	T1	Non-responded		No	0.18	0.35	0.02578	0.79	Estrus/Day 14
5	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
5	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
5	T1	Non-responded		No	0.07	0.37	0.01143	0.87	Estrus/Day 14
6	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
6	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
6	T1	Non-responded		No	0.16	0.27	0.01911	0.66	Estrus/Day 14
7	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
7	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
7	T1	Non-responded		No	0.13	0.31	0.02245	0.72	Estrus/Day 14
8	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
8	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
8	T1	Non-responded		No	0.13	0.23	0.01483	0.62	Estrus/Day 14
9	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
9	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
9	T1	Non-responded		No	0.16	0.43	0.01321	0.75	Estrus/Day 14
10	T1	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
10	T1	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)

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Animal ID	Group	Estrus response	Time to Estrus (h)	Con-ception	LH (mIU/mL)	FSH (mIU/mL)	E2 (ng/mL)	P4 (ng/mL)	Sampling Timepoint
10	T1	Non-responded		No	0.14	0.31	0.01576	0.77	Estrus/Day 14
11	T2	Responded	45	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)
11	T2	Responded	45	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
11	T2	Responded	45	Yes	0.08	0.37	0.07032	1.83	Estrus/Day 14
12	T2	Responded	37	No	0.09	0.31	0.022	0.72	Day 0 (Before)
12	T2	Responded	37	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
12	T2	Responded	37	No	0.09	0.26	0.05387	1.7	Estrus/Day 14
13	T2	Responded	43	No	0.09	0.31	0.022	0.72	Day 0 (Before)
13	T2	Responded	43	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
13	T2	Responded	43	No	0.17	0.23	0.05721	1.6	Estrus/Day 14
14	T2	Responded	36	No	0.09	0.31	0.022	0.72	Day 0 (Before)
14	T2	Responded	36	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
14	T2	Responded	36	No	0.13	0.47	0.07365	1.64	Estrus/Day 14
15	T2	Responded	41	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)
15	T2	Responded	41	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
15	T2	Responded	41	Yes	0.11	0.21	0.05898	1.57	Estrus/Day 14
16	T2	Responded	38	No	0.09	0.31	0.022	0.72	Day 0 (Before)
16	T2	Responded	38	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
16	T2	Responded	38	No	0.09	0.34	0.06256	1.46	Estrus/Day 14
17	T2	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
17	T2	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
17	T2	Non-responded		No	0.12	0.23	0.01143	0.82	Estrus/Day 14
18	T2	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
18	T2	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
18	T2	Non-responded		No	0.08	0.33	0.01712	0.91	Estrus/Day 14
19	T2	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
19	T2	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
19	T2	Non-responded		No	0.09	0.28	0.02433	0.57	Estrus/Day 14
20	T2	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
20	T2	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
20	T2	Non-responded		No	0.12	0.19	0.02977	0.63	Estrus/Day 14
21	T3	Responded	151	No	0.09	0.31	0.022	0.72	Day 0 (Before)
21	T3	Responded	151	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
21	T3	Responded	151	No	0.12	0.48	0.04832	1.62	Estrus/Day 14
22	T3	Responded	143	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)
22	T3	Responded	143	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
22	T3	Responded	143	Yes	0.14	0.22	0.06744	1.73	Estrus/Day 14
23	T3	Responded	141	No	0.09	0.31	0.022	0.72	Day 0 (Before)
23	T3	Responded	141	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
23	T3	Responded	141	No	0.11	0.26	0.06063	1.76	Estrus/Day 14
24	T3	Responded	136	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)
24	T3	Responded	136	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
24	T3	Responded	136	Yes	0.05	0.34	0.06609	1.39	Estrus/Day 14
25	T3	Responded	139	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)

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Animal ID	Group	Estrus response	Time to Estrus (h)	Con-ception	LH (mIU/mL)	FSH (mIU/mL)	E2 (ng/mL)	P4 (ng/mL)	Sampling Timepoint
25	T3	Responded	139	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
25	T3	Responded	139	Yes	0.11	0.31	0.07736	1.41	Estrus/Day 14
26	T3	Responded	130	Yes	0.09	0.31	0.022	0.72	Day 0 (Before)
26	T3	Responded	130	Yes	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
26	T3	Responded	130	Yes	0.12	0.25	0.07129	1.63	Estrus/Day 14
27	T3	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
27	T3	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
27	T3	Non-responded		No	0.11	0.27	0.01556	0.94	Estrus/Day 14
28	T3	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
28	T3	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
28	T3	Non-responded		No	0.06	0.29	0.01878	0.75	Estrus/Day 14
29	T3	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
29	T3	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
29	T3	Non-responded		No	0.07	0.35	0.03637	0.67	Estrus/Day 14
30	T3	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
30	T3	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
30	T3	Non-responded		No	0.08	0.26	0.01987	0.85	Estrus/Day 14
31	T4	Responded	76	No	0.09	0.31	0.022	0.72	Day 0 (Before)
31	T4	Responded	76	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
31	T4	Responded	76	No	0.072	0.33	0.05167	1.56	Estrus/Day 14
32	T4	Responded	68	No	0.09	0.31	0.022	0.72	Day 0 (Before)
32	T4	Responded	68	No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
32	T4	Responded	68	No	0.06	0.18	0.08122	1.47	Estrus/Day 14
33	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
33	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
33	T4	Non-responded		No	0.09	0.35	0.02553	0.62	Estrus/Day 14
34	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
34	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
34	T4	Non-responded		No	0.08	0.27	0.02371	0.73	Estrus/Day 14
35	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
35	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
35	T4	Non-responded		No	0.05	0.33	0.0153	0.67	Estrus/Day 14
36	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
36	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
36	T4	Non-responded		No	0.11	0.29	0.02027	0.57	Estrus/Day 14
37	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
37	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
37	T4	Non-responded		No	0.09	0.36	0.01961	0.82	Estrus/Day 14
38	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
38	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
38	T4	Non-responded		No	0.04	0.24	0.01537	0.74	Estrus/Day 14
39	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
39	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
39	T4	Non-responded		No	0.17	0.34	0.03122	0.49	Estrus/Day 14
40	T4	Non-responded		No	0.09	0.31	0.022	0.72	Day 0 (Before)
40	T4	Non-responded		No	0.11	0.35	0.045	1.05	Day 7 (After Treatment)
40	T4	Non-responded		No	0.03	0.21	0.01185	0.79	Estrus/Day 14