

Research Article:**EFFICACY OF SINGLE SHOT GnRH VERSUS CIDR-PGF_{2α}-GnRH-FTAI
PROTOCOL FOR THE TREATMENT OF POSTPARTUM TRUE
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ABSTRACT

Postpartum true anestrus is a major cause of reduced fertility and production, particularly in high-yielding crossbred dairy cows. Hormonal interventions under proper nutrition and management can restore reproductive function. This study compared two hormonal protocols for treating postpartum true anestrus in dairy cows. Forty-five postpartum anestrus cows were examined by transrectal palpation and ultrasonography to classify them as true anestrus or silent estrus. Among these, 73.3% had true anestrus and were included in the treatment trial (n=33). In treatment 1 (GnRH, n=13), cows received a single intramuscular injection of buserelin acetate (20 µg). In treatment 2 (CIDR-PGF_{2α}-GnRH-FTAI, n=20), a CIDR device containing 1.9 g progesterone was inserted on day 0 and removed on day 7, with PGF_{2α} (cloprosterol 500 µg) injected at removal. GnRH (20 µg) was administered on day 9, followed by fixed-time artificial insemination on day 10. Estrus induction rate did not differ significantly between GnRH (53.9%) and CIDR-PGF_{2α}-GnRH-FTAI (60%) groups. The interval from treatment to estrus was shorter in the CIDR-based group (61.0±10.2 h) than in the GnRH group (163.7±36.0 h). Pregnancy rate tended to be higher in CIDR-PGF_{2α}-GnRH-FTAI (55.0%) than in GnRH (23.1%, P=0.07). Cows with good body condition score (BCS) had higher pregnancy rate than those with poor BCS (P=0.05). Other factors, including milk yield, endoparasitic infection and anemia, had no significant effect. Single-shot GnRH was as effective as CIDR-PGF_{2α}-GnRH-FTAI for estrus induction, while the CIDR-based protocol shortened the interval to estrus and tends to improve pregnancy outcomes.

Keywords: Estrus induction, fixed-time artificial insemination, hormonal protocols, infertility, pregnancy**INTRODUCTION**

Advances in genetics, nutrition, and breeding practices have markedly increased milk yield in dairy cows; however, intensive selection for high productivity and the associated physiological demands have also been linked to declining fertility worldwide (Cole et al., 2023). Infertility, particularly postpartum anestrus has emerged as a major reproductive challenge in crossbred and exotic cows (Gautam, 2023). Postpartum anestrus may manifest as silent estrus or true anestrus, the latter representing a complete absence of estrous behavior due to inactive ovaries (Ambrose, 2021; Roberts, 2004). True anestrus is recognized as a major cause of infertility in dairy cows (Gautam, 2023).

Various hormonal protocols using Gonadotropin-Releasing Hormone (GnRH), Prostaglandin F₂ alpha (PGF_{2α}) and progesterone-based regimens have been studied to treat true anestrus (Bhoraniya et al., 2012; Kumar et al., 2014). However, comparative effectiveness under diverse management systems and environmental conditions remains unclear, and many protocols are expensive and require frequent cow handling. For cows with adequate nutrition and a large dominant follicle, a single injection of GnRH may effectively induce ovulation, while the Controlled Internal Drug Release (CIDR[®])-based protocols such as CIDR-PGF_{2α}-GnRH followed by fixed-time artificial insemination (FTAI) are often more effective for true anestrus (Dhami et al., 2015). Identification of an effective yet low-cost and low-handling protocol is therefore critical for improving reproductive efficiency and reducing economic losses in dairy herds. This study, therefore, aimed to compare the effectiveness of single-shot GnRH versus CIDR-PGF_{2α}-GnRH-FTAI protocols for the treatment of true anestrus in crossbred dairy cows, while also evaluating the influence of factors such as breed, parity, stage of lactation, body condition score, milk yield, endoparasitic infection, and anemia on estrus induction and pregnancy outcomes.

RESEARCH METHODS

All research involving human participants, use of animals and field studies complied with institutional, national and international ethical standards.

Study area and duration

The study was conducted from June to November 2024, covering the summer and monsoon seasons. Research was carried out on 18 household and 5 commercial farms in Sainamaina Municipality and Kanchan Rural Municipality, Rupandehi district, Nepal. The district lies between 83°10'–83°30' E longitude and 27°10'–27°45' N latitude, at altitudes of 95–1219 m above sea level. The region has a tropical monsoon climate with four distinct seasons, a maximum summer temperature of 43.4°C, and a minimum winter temperature of 6°C.

Animal selection and data recording

Based on farm records, 45 cows more than 60 days postpartum with no history of estrus were selected. Reproductive organs were examined by transrectal palpation and ultrasonography. True anestrus was defined as absence of corpus luteum (CL) with small follicles, whereas silent estrus was characterized by the presence of CL and large follicles. Of the 45 cows, 33 (73.3%) had true anestrus and 12 (26.7%) had silent estrus. Only true anestrus cows were included in the trial. Body condition score (BCS; 1–5 scale, 0.25 increments) was recorded (Ferguson, 1998), and only cows with BCS >2.0 were used for the experiment. Among them, 32 were Holstein Friesian crosses and 13 Jersey crosses, reared under similar management with paddy straw, hay, grasses, and limited concentrates. Data regarding parity, stage of lactation, milk yield, date of calving and date of estrus were collected from farm records.

Pre-treatment before the start of hormonal treatment protocol

Prior to hormonal intervention, cows were dewormed with oxcyclozanide (10 mg/kg) and levamisole (7.5 mg/kg), followed by supplementation of a mineral-vitamin mixture (Agrimin Forte, 50 g/day/cow; Virbac, India) for 20 days.

Experimental design

After completion of the mineral-vitamin supplementation period, the selected true anestrus cows were divided into two treatment groups.

Cows in group 1 (n=13) were subjected to single shot GnRH group (Fig. 1). On day 0, GnRH analogue 20 µg buserelin acetate (Gynarich, Intas, India) was administered intramuscularly. Then those cows were observed for estrus three times daily until 3 weeks post treatment and artificial insemination (AI) was done as per AM-PM rule.

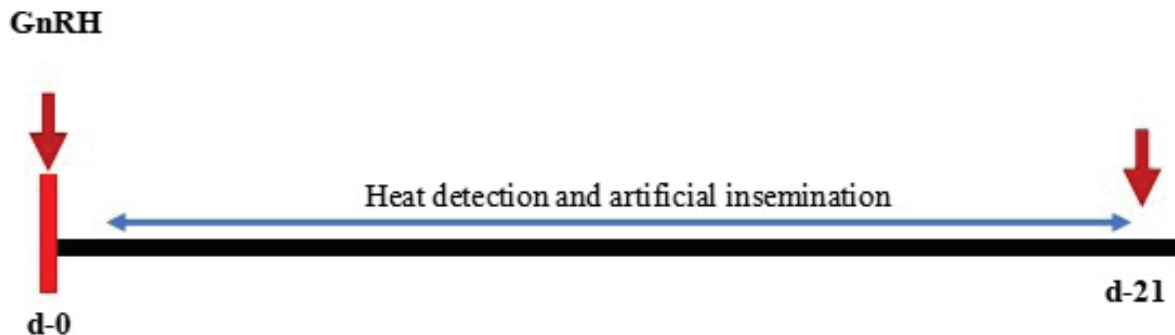


Fig. 1. Single shot GnRH protocol used in the experiment

Cows in group 2 (n=20) were treated with CIDR-synch protocol without GnRH on day 0 (CIDR-PGF_{2α}-GnRH-FTAI) (Fig. 2). Briefly, the CIDR (Zoetis, Australia) containing 1.90 grams progesterone was inserted intravaginally and kept for seven days. On day 7, CIDR was removed and 500 mcg cloprostenol (Pragma, Intas, India) was administered intramuscularly. Similarly, on day 9, GnRH analogue 20 µg buserelin acetate (Gynarich, Intas, India) was given I/M and fixed time artificial insemination (FTAI) was done on day 10 (16-20 hours after GnRH) regardless of the presence or absence of externally observed estrus signs.

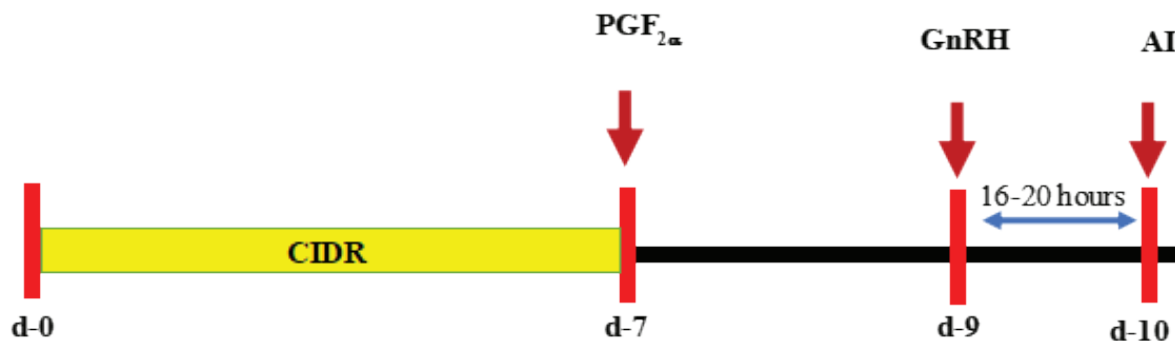


Fig. 2. CIDR-PGF_{2α}-GnRH-FTAI protocol used in the experiment

Determination of endoparasitic infection

The fecal samples were collected on day 0, directly from the rectum and examined using both sedimentation and floatation methods. After examination, data was recorded as absence or presence of gastrointestinal parasites.

Estrus detection

Estrus detection was performed three times daily until 21 days post-treatment. A small proportion of animals were managed under a free-range system, allowing observation of behavioural signs such as standing to be mounted and chin resting during natural interaction. In stall-fed animals, estrus detection was primarily based on physical and behavioural signs including mucus discharge, restlessness, frequent urination and reduced milk yield. Animals exhibiting one or more characteristic estrus signs were considered positive for estrus.

Pregnancy diagnosis

Pregnancy was diagnosed 75 days after insemination through transrectal palpation and ultrasonography using 7.5 MHz transducer (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China).

Statistical analysis

Data entry was done in Microsoft Excel- 16 and analyzed using IBM SPSS Statistics version 27. Chi-square test was used to compare the estrus induction rate and pregnancy rate between the treatment groups. If the expected frequency was less than five in more than 20% of cells, the Fisher's exact test was used. To analyze the effect of different factors on estrus induction and pregnancy outcomes, the true anestrous cows were classified as low BCS (<2.75) or good BCS (≥ 2.75 to ≤ 3.75); milk yield < average or \geq average; presence or absence of endoparasites; and moderate or severe clinical anemic score. Effect of these factors on estrus induction and pregnancy outcome was analyzed using binary logistic regression model. Likewise, a t-test was used to compare the interval from treatment to estrus expression (in hours) among the treatment groups. The values were expressed as Mean \pm SE. Differences with $P \leq 0.05$ were considered significant, while values between 0.05 and 0.1 were considered to indicate a tendency toward significance.

RESULTS

Proportion of different types of postpartum anestrus

The prevalence of different types of postpartum anestrus in crossbred dairy cows has been shown in Table 1. Out of total 45 anestrous cows, 33 (73.3%) had inactive ovaries and was classified as Type II anestrus or true anestrus. Remaining cows, i.e., 12 (26.7%) had palpable corpus luteum in the ovary, hence classified as Type I anestrus or silent estrus.

Table 1. Prevalence of different types of postpartum anestrus in crossbred dairy cows

| Parameters | Number of cows (%) |
|--|--------------------|
| Total number of postpartum anestrous cows examined | 45 |
| True anestrus | 33 (73.3) |
| Silent estrus | 12 (26.7) |

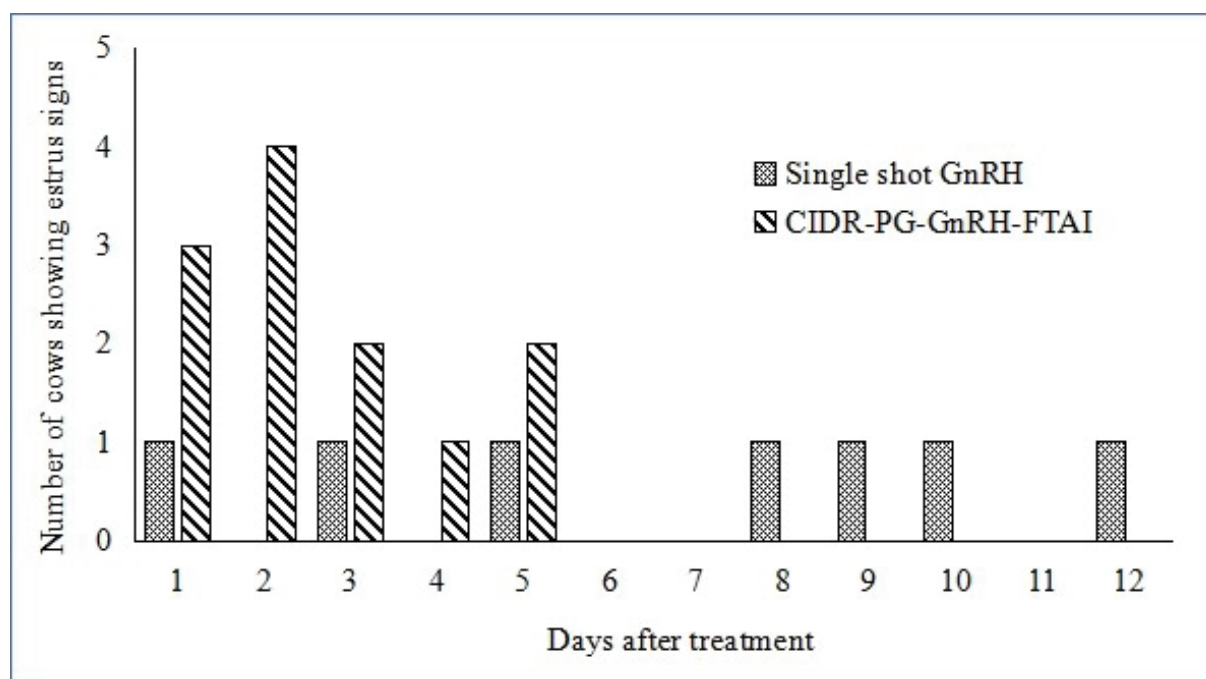
Estrus induction rate

The estrus induction rate was not significantly different between two treatment groups (Table 2). The overall estrus induction rate among the treated true anestrous cows was 57.6% (19/33). However, the interval from treatment to estrus expression was significantly shorter in cows treated with CIDR- PGF_{2 α} -GnRH-FTAI protocol (61.0 \pm 10.2 hours) as compared to the cows treated with single shot GnRH (163.7 \pm 36.0 hours).

Table 2. Estrus induction in postpartum true anestrous cows treated with two different hormonal protocols

| Parameters | Treatment protocol | | p-value |
|---|--------------------|---|---------|
| | Single shot GnRH | CIDR-PGF _{2α} -GnRH-FTAI | |
| No. of cows treated | 13 | 20 | |
| No. of estrus induced cows | 7 | 12 | |
| Estrus induction rate (%) | 53.9 | 60.0 | 0.83 |
| Interval from treatment to estrus expression; hours (Mean \pm SE) | 163.7 \pm 36.0 | 61.0 \pm 10.2 | 0.03 |

The numbers of cows showing estrus at different days after the end of the protocol has been shown in Fig. 3. The cows treated with CIDR-PGF_{2 α} -GnRH-FTAI protocol came to estrus within 5 days of the treatment, whereas, the cows treated with single shot GnRH protocol showed estrus till 12 days after treatment.

**Fig. 3. The distribution of cows showing estrus at different days after the end of the treatment protocols**

Pregnancy rate

The overall pregnancy rate among the treated true anestrous cows was 42.4%. There was a tendency that the pregnancy rate was higher in cows treated with CIDR- PGF_{2 α} -GnRH-FTAI protocol compared to the single shot GnRH group (55.0% vs 23.1%; P=0.07) (Table 3). Although behavioral estrus signs were expressed at varying times after treatment, all cows in the CIDR-PGF_{2 α} -GnRH-FTAI group, irrespective of estrus, received insemination according to the fixed-time schedule. Therefore, pregnancy outcomes reflected the response to the synchronization protocol rather than insemination based on observed estrus alone.

Table 3. Pregnancy rate in postpartum true anestrous cows treated with two different hormonal protocols

| Parameters | Treatment protocol | | p-value |
|--------------------------------|--------------------|------------------------------------|---------|
| | Single shot GnRH | CIDR- PGF _{2α} -GnRH-FTAI | |
| No. of cows treated | 13 | 20 | |
| No. of pregnant cows diagnosed | 3 | 11 | 0.07 |
| Pregnancy rate (%) | 23.1 | 55.0 | |

Effect of various factors on estrus induction

None of the factors considered in the study had significant effects on estrus induction in cows treated with two different protocols (Table 4).

Table 4. Effect of various factors on estrus induction in true anestrous cows treated with single shot GnRH versus CIDR- PGF_{2α}-GnRH-FTAI protocols

| Factors | Level | No. of cows | Estrus induction (%) | Odds ratio | 95% CI | | p-value |
|--------------------------|----------|-------------|----------------------|------------|--------|-------|---------|
| | | | | | Lower | Upper | |
| BCS | Poor | 20 | 55.0 | Ref. | | | 0.96 |
| | Good | 13 | 61.5 | 1.054 | 0.167 | 6.661 | |
| Milk yield | ≥average | 17 | 53.0 | Ref. | | | 0.61 |
| | <average | 16 | 62.5 | 1.568 | 0.281 | 8.766 | |
| Endo-parasitic infection | Negative | 23 | 56.5 | Ref. | | | 0.65 |
| | Positive | 10 | 60.0 | 1.493 | 0.271 | 8.214 | |
| Clinical anemic score | Severe | 19 | 52.6 | Ref. | | | 0.50 |
| | Moderate | 14 | 64.3 | 1.787 | 0.328 | 9.728 | |

Effect of various factors on pregnancy rate

Table 5 shows the effect of various factors on pregnancy outcome in true anestrous cows treated with single shot GnRH and CIDR-PGF_{2α}-GnRH-FTAI protocols. Among the various factors considered in the study, the pregnancy outcome among the treated cows was affected only by the BCS. Cows with poor BCS had significantly lower pregnancy rate as compared to the cows with good BCS (35.0% vs. 53.9%; OR = 12.35, 95% CI = 1.01–150.76; P = 0.05).

Table 5. Effect of various factors on pregnancy outcome in true anestrous cows treated with single shot GnRH versus CIDR- PGF_{2α}-GnRH-FTAI protocols

| Factors | Level | No. of cows | Pregnancy (%) | Odds ratio | 95% CI | | p-value |
|--------------------------|----------|-------------|---------------|------------|--------|---------|-------------|
| | | | | | Lower | Upper | |
| BCS | Poor | 20 | 35.0 | Ref. | | | 0.05 |
| | Good | 13 | 53.9 | 12.350 | 1.011 | 150.764 | |
| Milk yield | ≥average | 17 | 35.3 | Ref. | | | 0.17 |
| | <average | 16 | 50.0 | 4.998 | 0.513 | 48.706 | |
| Endo parasitic infection | Positive | 10 | 40.0 | Ref. | | | 0.60 |
| | Negative | 23 | 43.5 | 1.778 | 0.203 | 15.549 | |
| Clinical anemic score | Moderate | 14 | 35.7 | Ref. | | | 0.84 |
| | Severe | 19 | 47.4 | 1.248 | 0.148 | 10.496 | |
| Estrus expression | Yes | 19 | 42.1 | Ref. | | | 0.82 |
| | No | 14 | 42.9 | 1.235 | 0.200 | 7.623 | |

DISCUSSION

In this study, among anestrous cows, 73.3% had true anestrus and the remaining 26.7% had silent estrus. The incidence of true anestrus in the present study was higher than that (64.3% and 61.4%, respectively) reported by previous studies while, the prevalence of silent estrus was lower than that (35.7% and 33.3%, respectively) reported by the same studies (Rana et al., 2018; Sah & Nakao, 2010). Different hormonal protocols have been practiced over many years for the treatment of postpartum anestrus in cattle. Despite the differences in treatment strategies, the common goal should be to minimize the cost of treatment and reduce the frequency of cow handling as much as possible, at the same time maintaining the optimal pregnancy rate. Therefore, this study was carried out to compare the effectiveness of two different hormonal protocols for estrus induction and pregnancy outcome in postpartum true anestrous dairy cows. For this, true anestrous cows were divided into two groups and subjected to single shot GnRH and CIDR- PGF_{2α}-GnRH-FTAI protocols.

The estrus induction rate with single shot GnRH in the present study (53.9%) was lower than the finding (75%) of a previous study that also used single shot of GnRH in anestrous cows (Islam et al., 2014). The pregnancy rate observed in the present study (23.1%) was also much lower compared to the pregnancy rate observed (64.7%) in the previous study (Campbell et al., 2009). Likewise, another study observed 10% and 90% estrus induction rate within a week and a month of administration of single shot GnRH in anestrous dairy cows respectively with 40% pregnancy rate after first artificial insemination (Rana et al., 2018). GnRH and its analogues indirectly affect ovarian follicular development and corpus luteum (CL) formation by triggering the release of pituitary luteinizing hormone (LH) and Follicle stimulating hormone (FSH) (Campbell et al., 2009). GnRH therapy in cattle results in ovulation and the formation of a new follicular wave within 3-4 days, regardless of the estrous cycle stage (Twagiramungu et al., 1995).

The estrus induction rate while using CIDR-PGF_{2α}-GnRH-FTAI protocol in the present study (60%) was lower than the finding (100%) of a previous study that also used CIDR based treatment in cows of unknown estrous cyclicity (Bhoraniya et al., 2012; Ramakrishnan et al., 2012). On the other hand, compared to the present finding, a similar estrus expression rate ranging from 43%-83% was observed in other studies (Cevik et al., 2010; Tauck et al., 2007). Similarly, conception

rate observed in the present study (55.0%) was in accordance with the previous studies that reported 60%, 53.3% and 69% conception rates in cows treated with Ovsynch CIDR protocol (Cevik et al., 2010; Martinez et al., 1998; Tauck et al., 2007), and higher than that (33%) reported by a previous study (Ramakrishnan et al., 2012). Among the treatment groups, cows treated with CIDR-PGF_{2α}-GnRH-FTAI protocol had a tendency of higher pregnancy outcome compared to cows treated with single shot GnRH protocol similar to that reported in previous study (Rana et al., 2018). Insufficient plasma progesterone concentrations during the follicular development are a major concern in lactating dairy cows. Lower progesterone concentrations lead to an increased pulse frequency of LH and premature meiotic resumption, ultimately decreasing oocyte quality. Exogenous progesterone, either alone or in combination with other hormones, is widely used to induce estrus in non-cyclic cows and synchronize estrous cycles in cyclic cows. Progesterone based protocols sensitize the hypothalamus-pituitary-gonadal axis and regardless of presence or absence of functional CL, combination of progesterone for 7 to 9 days and PGF_{2α} upon P4 withdrawal, was found to have induced estrus in true anestrus cows (Madhuri et al., 2017). By keeping progesterone at basal levels, it prolongs follicular growth and allows the dominant follicle to mature via endogenous GnRH and LH pulses (Yavas & Walton, 2000). Such a progesterone therapy followed by PGF_{2α} efficiently synchronizes estrous cycles, regardless of whether a functional CL is present (Patterson et al., 1989).

The mean (\pm SE) from the end of protocol to estrus induction interval of CIDR-PGF_{2α}-GnRH-FTAI protocol in this study was 61.0 \pm 10.2 hours which was in accordance to estrus induction interval after treatment (58.27 \pm 1.93, 64.13 \pm 1.33, and 54.00 \pm 1.17 hours) in cows under CIDR, Ovsynch and Norgestomet ear implant protocols respectively, observed in a previous study (Dhami et al., 2015). When GnRH is injected during the follicular phase, it causes a rise in luteinizing hormone (LH), and the estrus may be induced within 2 to 3 days (48 to 72 hours) following injection (Hassanein et al., 2024). The continuous release of progesterone from the CIDR facilitates a more progressive and regulated stimulation of the reproductive system, hence increasing the probability of estrus expression compared to the abrupt GnRH injection (Smith et al., 2015; Twagiramungu et al., 1995). This might be the reason for a longer mean estrus induction interval of 163.7 \pm 36.0 hours in anestrus cows treated with single shot GnRH as compared to the cows treated with CIDR-PGF_{2α}-GnRH-FTAI protocol in this study.

The association of various factors with estrus induction rate and pregnancy rate in postpartum true anestrus cows was analyzed. The effects of BCS, milk yield, endoparasitic infection and clinical anemic score were evaluated. It was observed that the cows with good BCS had a significantly higher pregnancy outcome compared with the cows with poor BCS. Previous study also reported significant effect of BCS on postpartum estrous cyclicity and conception in crossbred dairy cows (Pokharel & Gautam, 2025). The BCS influences the development and maintenance of pregnancy (López-Gatius et al., 2002). Postpartum BCS alterations were significant predictors of first AI pregnancy in dairy cows. Cows with significant BCS loss during early postpartum displayed increased impairments of embryo development during the first week after AI. Cows that lost BCS during the first 30 days after calving were at greater risk of losing their pregnancy from 35 to 60 d after first AI. Cows with high BCS loss in early lactation had longer intervals between the first AI and were less likely to conceive (Domecq et al., 1997). Apart from BCS, none of the other factors had significant influence on estrus induction and pregnancy outcome. Similar findings have been reported by different studies conducted previously (Gautam et al., 2024; Kandel et al., 2024). Such a non-significant association of these factors with the estrus induction rate and pregnancy outcome may be due to an irregular number of animals across different factors, poor nutrition, and low breed variability (as both were exotic crossbreeds).

CONCLUSION

In conclusion, the single shot GnRH protocol was as effective as CIDR-PGF_{2α}-GnRH-FTAI protocol in terms of estrus induction in true anestrous postpartum dairy cows. However, CIDR-PGF_{2α}-GnRH-FTAI protocol resulted in shorter interval from treatment to estrus expression and showed a tendency towards a higher pregnancy rate. Furthermore, the pregnancy outcome among the treated cows was affected by the body condition score of the cow.

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AUTHOR CONTRIBUTIONS

JM: Methodology, Data curation, Investigation, Funding acquisition, Resources, Project administration, Writing – original draft; **GG:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Supervision, Funding acquisition, Resources, Project administration, Writing – review & editing; **AP:** Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing; **SS:** Formal analysis, Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL APPROVAL AND PERMITS

Not applicable.

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