



# Effect of various hCG treatment protocols on luteal characteristics, plasma progesterone concentration, and pregnancy in normal cyclic Indian crossbred dairy cows

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## Abstract

Limited information exists on the application of human chorionic gonadotropin (hCG) post insemination in the Indian crossbred dairy cows. This study aimed to evaluate the effect of four hCG administration protocols on luteal characteristics and pregnancy outcomes following artificial insemination (AI). Using block randomization, 50 healthy lactating crossbred cows were allocated in equal numbers ( $n = 10$  cows per group) to receive either 5 mL normal saline (control) or 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14). All cows were scanned using sequential transrectal ultrasound examinations to evaluate primary luteal parameters, development of accessory corpora lutea, and pregnancy. Serial blood samples were collected to measure plasma progesterone concentrations. Data were analyzed using repeated measures ANOVA and Fisher's exact tests. The mean primary luteal area, total luteal area, and total luteal diameter values were significantly greater in the hCG-treated cows. Compared to the control, the hCG-14 group had a significantly higher percentage of cows with an accessory corpora luteum. However, there were no significant differences in the mean progesterone concentrations or the first service conception rates between any of the groups. Overall, the results of this study indicate that while hCG administration post AI in healthy Indian crossbred cows may enhance primary luteal dimensions or induce the formation of accessory corpora lutea, it does not appear to have any beneficial effect on luteal function or pregnancy.

**Keywords** Artificial insemination · Corpus luteum · hCG · Indian crossbred cattle · Pregnancy · Progesterone

## Introduction

The gradual reduction in fertility of dairy cows observed over the last several decades has been attributed to a

variety of reasons, including low progesterone concentrations in the first 3 weeks post insemination (Walsh et al. 2011). Elevated progesterone concentrations are associated with better conceptus elongation (Garrett et al. 1988; Carter et al. 2008), greater interferon-tau secretion (Mann and Lamming 1999, 2001), and increased pregnancy rates (Stronge et al. 2005; McNeill et al. 2006). Due to its luteotropic effect (Price and Webb 1989), human chorionic gonadotropin (hCG) has been used in multiple studies on purebred dairy cows to evaluate its effect on luteal function and pregnancy (Nascimento et al. 2013). Although the responses vary greatly between studies, hCG has been shown to promote primary luteal function, induce formation of accessory corpora lutea, and improve conception rates (Rajamahendran and Sianangama 1992; Sianangama and Rajamahendran 1992; Beltran and Vasconcelos 2008; Nascimento et al. 2013). The variability may be partly due to different hCG doses or days of administration used in different studies and partly due to

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breed differences and other factors including parity, days in milk, and total milk production.

Crossbred cattle constitute about 26.6% of the total cattle population in India, as mentioned in the latest census report published by the Department of Animal Husbandry and Dairying in 2019. Information on the application of hCG post insemination in Indian crossbred dairy cows is limited to a couple of studies in repeat breeding cows (Pandey et al. 2016; Kumar et al. 2020). However, the effects of hCG administration have not been investigated in healthy Indian crossbred cows. The objective of this study was to evaluate luteal characteristics, plasma progesterone, and pregnancy in normal cyclic crossbred cows administered 1500 IU hCG on single or multiple specific days post insemination.

## Materials and methods

All animal handling and experimental procedures used in this study were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Ethics Committee (IAEC/VGO/CVASC/181).

### Animals and management

This study was conducted at the Instructional Dairy Farm (IDF), Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India. Fifty healthy cyclic crossbred dairy cows [age 4 to 7 years, body condition score (BCS) between 3 and 4 on a scale of 1 to 5 in which 1 represents emaciated and 5 represents obese/extremely fat (Edmonson et al. 1989)] were enrolled in the study. The enrolled cows were at least 60 days in milk (DIM) and free of any apparent reproductive abnormalities detected through transrectal ultrasonography and the white side test (Bhat et al. 2014). All animals were kept under uniform housing and feeding management throughout the study.

### Experimental design

Cows were observed twice a day (morning and evening) for behavioral signs of standing estrus. Cows suspected to be in estrus were examined using transrectal ultrasonography and those with a preovulatory follicle ( $\geq 10$ -mm diameter) were bred twice through AI at 12 and 24 h after standing estrus. Using block randomization, the inseminated cows were allocated in equal numbers ( $n = 10$  cows per group) to receive either 5 mL normal saline IM on days 0, 7, and 14 (control) or 1500 IU hCG (Chorulon®, MSD Animal Health, Pune, India) IM on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14).

A portable ultrasound scanner (DIGI-600 M, PROVET; S.S. Medical Systems (I) Private Limited, India) equipped with a 5.0-MHz linear-array transducer was used for transrectal ultrasonography. All cows were examined on days 7, 14, 20, 25, 30, 35, 40, and 60 to evaluate primary luteal parameters (primary luteal diameter and primary luteal area) and development of accessory corpora lutea. Diameter and area of the primary and accessory corpora lutea were measured using the inbuilt measurement functions in the ultrasound machine. Total luteal diameter and total luteal area were calculated by adding the dimensions of primary corpus luteum and any accessory corpora lutea. Pregnancy diagnosis was performed on day 60. All ultrasound examinations were performed by the same operator.

Blood samples were collected through jugular venipuncture in heparinized test tubes on days 0, 1, 2, 7, 14, 20, 25, 30, 35, 40, and 60. Plasma was harvested by centrifugation at 3000 rpm for 15 min and stored at  $-20$  °C. Progesterone concentration was estimated using C.T. RIA kits (M/s Beckman Coulter IM 1188) with a highly specific progesterone antibody and analytical sensitivity of 0.03 ng/mL.

### Statistical analysis

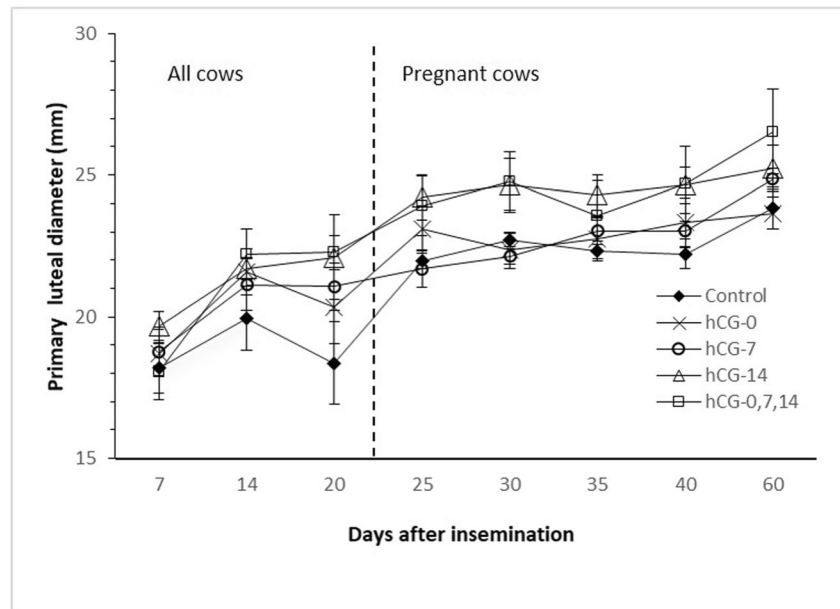
Statistical analysis of the data was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Data on continuous outcome variables (primary luteal diameter, primary luteal area, total luteal diameter, total luteal area, and plasma progesterone concentrations) were tested for normality through the Shapiro-Wilk test and plotted to identify any outliers before analyses. Differences in means of these variables between groups were evaluated for statistical significance through repeated measures ANOVA with least significant difference (LSD) post hoc tests. Differences in proportions of pregnant cows and cows with accessory corpus luteum between groups were tested for statistical significance using Fisher's exact test. A  $P$  value of less than 0.05 was considered statistically significant. Results are presented as means  $\pm$  SEM unless otherwise stated.

## Results

### Primary luteal diameter and area

Analysis of the data on primary luteal diameter revealed neither a group effect nor a group-by-day interaction (Fig. 1). In contrast, there was a group-by-day interaction ( $P < 0.05$ ) for the primary luteal area. Compared to the control, a significantly higher mean primary luteal area was observed on day 20 post AI in the hCG-0 group; days 20, 40, and 60 post AI in the hCG-7 group; days 20 and 30 post AI in the hCG-14 group; and days 40 and 60 post AI in the hCG-0,7,14 group (Fig. 2).

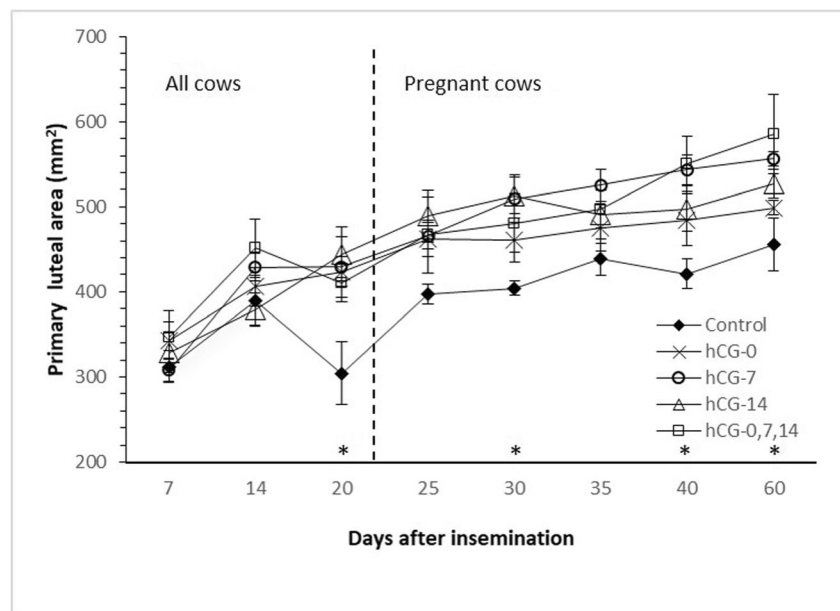
**Fig. 1** Mean ( $\pm$  SEM) primary luteal diameter of control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14)



**Total luteal diameter and area**

A group-by-day interaction ( $P < 0.05$ ) was observed for both total luteal diameter (Fig. 3) and total luteal area (Fig. 4). Compared to the control, a significantly higher mean total luteal diameter was observed on days 14 and 20 post AI in the hCG-7 group, days 20 and 25 post AI in the hCG-14 group, and days 20, 25, 30, 35, 40, and 60 post AI in the hCG-0,7,14 group (Fig. 3). Similarly, compared to the control, a significantly higher mean total luteal diameter was observed on days 14, 20, 40, and 60 post AI in the hCG-7 group; days 20, 25, 30, and 60 post AI in the hCG-14 group; and days 20, 25, 30, 35, 40, and 60 post AI in the hCG-0,7,14 group (Fig. 4).

**Fig. 2** Mean ( $\pm$  SEM) primary luteal area of control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14). Asterisks indicate the days on which a significant difference was observed in the mean values between groups



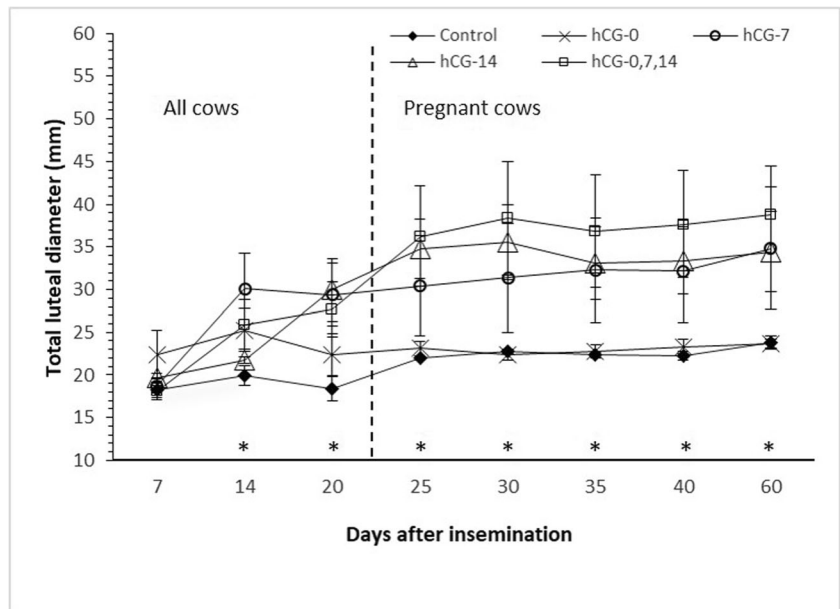
**Plasma progesterone concentration**

There was neither a group effect nor a group-by-day interaction for plasma progesterone concentration (Fig. 5).

**Accessory corpus luteum**

The proportions of cows with an accessory corpus luteum in each of the five groups are presented in Fig. 6. Compared to the control group, the proportion of cows with an accessory corpus luteum was significantly higher in the hCG-14 group.

**Fig. 3** Mean ( $\pm$  SEM) total luteal diameter of control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14). Asterisks indicate the days on which a significant difference was observed in the mean values between groups



**Pregnancy**

As shown in Fig. 7, there were numerical differences in the proportions of pregnant cows between the groups but these differences did not attain statistical significance.

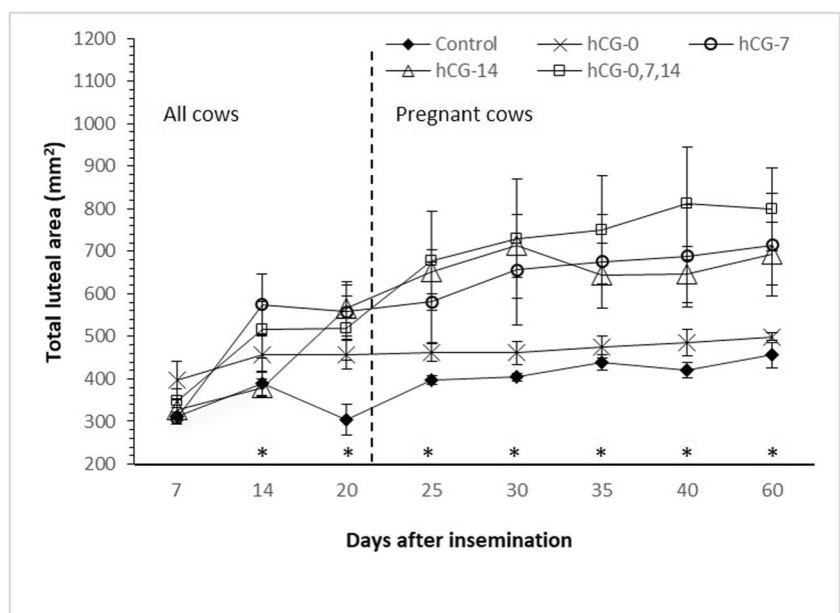
**Discussion**

The results of this study suggest that hCG administration post insemination is effective in enhancing the development of primary corpus luteum and inducing accessory corpora lutea in healthy Indian crossbred cows. These

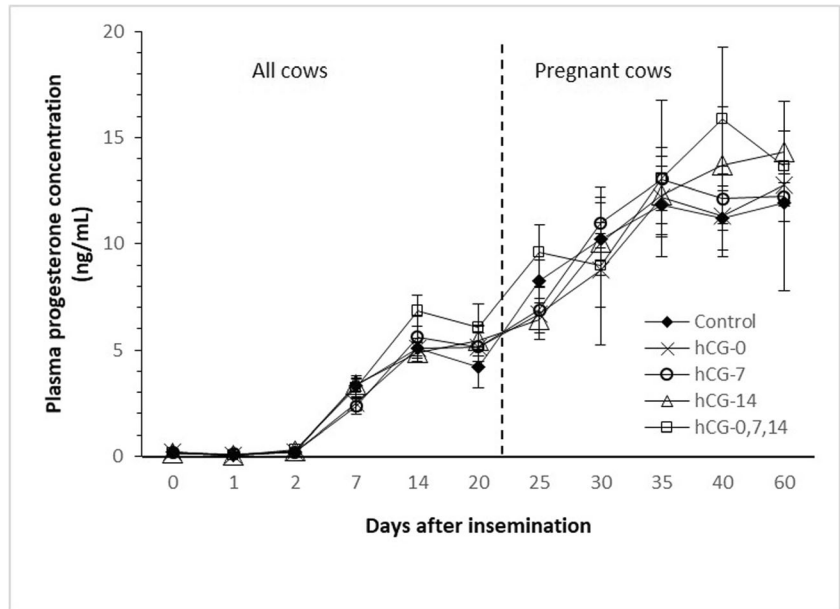
findings are consistent with the reported luteinizing effects of hCG treatment in dairy cows, as reviewed previously (De Rensis et al. 2010). An increase in the primary and total luteal dimensions of cows has been reported after hCG administration on various days post AI (Rajamahendran and Sianangama 1992; Santos et al. 2001; Maillo et al. 2014). The stimulatory effect of hCG administration on the primary luteal structural characteristics could be attributed to its effects on the growth and transformation of small luteal cells into large luteal cells (Farin et al. 1988).

The higher mean total luteal diameter and area in the hCG-treated groups were partly due to the luteotropic

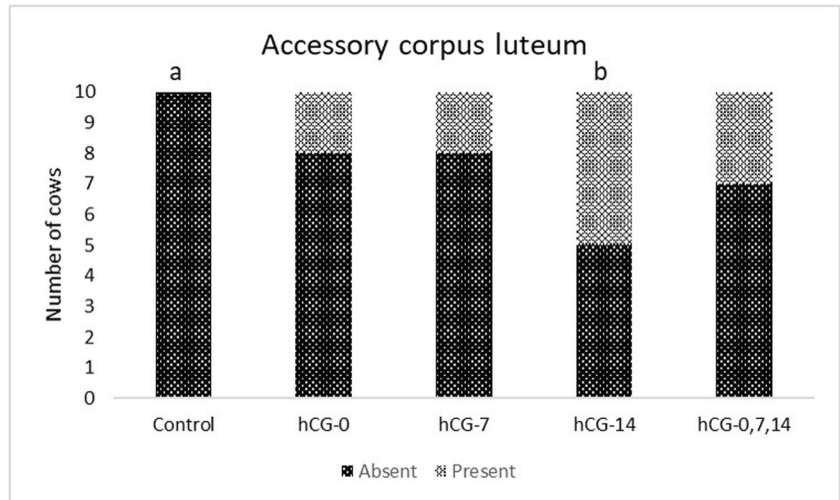
**Fig. 4** Mean ( $\pm$  SEM) total luteal area of control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14). Asterisks indicate the days on which a significant difference was observed in the mean values between groups



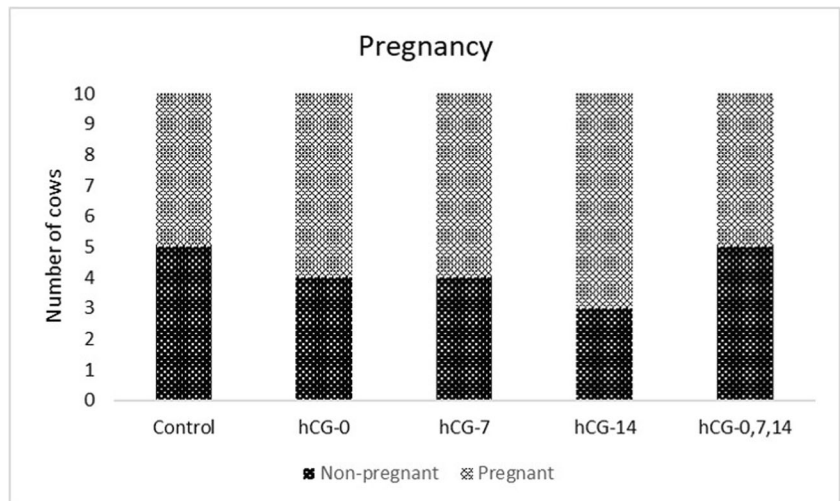
**Fig. 5** Mean ( $\pm$  SEM) plasma progesterone concentration of control cows and cows treated with control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14)



**Fig. 6** Accessory corpus luteum formation in control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14). Different lowercase letters indicate a significant difference



**Fig. 7** First service conception rate in control cows and cows treated with 1500 IU hCG on the day of AI (hCG-0), day 7 post AI (hCG-7), day 14 post AI (hCG-14), or days 0, 7, and 14 post AI (hCG-0,7,14)



action of hCG on the primary CL and partly due to the formation of accessory corpora lutea. Our findings are in agreement with the previous studies in dairy cows that reported accessory CL formation after hCG treatment (Price and Webb 1989; Rajamahendran and Sianangama 1992; Santos et al. 2001; Stevenson et al. 2007). The rate of accessory CL formation has been shown to be greater with hCG administration during the early luteal phase compared to the follicular and midluteal phases (Price and Webb 1989; Rajamahendran and Sianangama 1992). In the present study, the best response for accessory CL formation was observed in cows treated with hCG on day 14 post AI. This variation may be due to subtle differences in the follicular dynamics of Indian crossbred cows. Future studies in hCG-treated normal cyclic Indian crossbred cows are warranted to investigate the underlying follicular dynamics that might explain the variation in response.

In contrast to the stimulatory effect of hCG on primary CL and formation of accessory corpora lutea, none of the hCG treatments had any effect on the luteal function as assessed by measuring plasma concentrations. While a lack of hCG treatment effect on plasma progesterone concentration has been reported previously in dairy cows (Sianangama and Rajamahendran 1996), a vast majority of studies showed an increase in concentration after hCG treatment (Rajamahendran and Sianangama 1992; Sianangama and Rajamahendran 1992; Santos et al. 2001; Beltran and Vasconcelos 2008; Nascimento et al. 2013). Higher progesterone concentrations have also been reported in hCG-treated repeat breeding Indian crossbred cows (Pandey et al. 2016). Although the lack of higher progesterone concentrations in hCG-treated cows despite the increase in luteal dimensions is surprising, the finding may be explained by previously reported inconsistent relationships between luteal structural dimensions and plasma progesterone concentrations (Assey et al. 1993; Yung et al. 1996; Sartori et al. 2002).

Similar to the findings of two previous studies (Helmer and Britt 1986; Rajamahendran and Sianangama 1992), hCG treatment did not have a significant effect on pregnancy. In contrast, other studies have shown an increase in conception rate after hCG treatment (Sianangama and Rajamahendran 1992; Santos et al. 2001; Stevenson et al. 2007; Nascimento et al. 2013; Pandey et al. 2016). The absence of a statistically significant difference in first service conception rates between groups in the present study could be attributed to smaller sample sizes or a lack of requirement for additional luteotropic support in healthy animals.

In conclusion, the results of this study suggest that while hCG administration post AI in healthy Indian crossbred cows may enhance primary luteal dimensions or induce the formation of accessory corpora lutea, it does not appear to have any beneficial effect on luteal function or pregnancy. Further studies involving larger sample sizes are required to determine conclusively if hCG administration post AI has any beneficial

effect on the reproductive performance of healthy Indian crossbred cows.

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**Author's contribution** Sanjay Agarwal: conceptualization, methodology, investigation, writing—original draft preparation. Harihar Prasad Gupta: funding acquisition, project administration, supervision. Shiv Prasad: project administration, resources, supervision. Pawan Kumar Verma: data curation, formal analysis. Afroza Khanam: data curation, formal analysis, visualization. Firdous Ahmad Khan: conceptualization, formal analysis, software, writing—review and editing.

**Data availability statement** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interests** The authors declare no competing interests.

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