

EFFECT OF PRETREATMENT WITH EQUINE CHORIONIC GONADOTROPIN (eCG) IN OVSYNCH PROTOCOL ON ESTRUS INDUCTION AND CONCEPTION RATE OF POSTPARTUM ANESTRUS COWS

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ABSTRACT

Postpartum anestrus is one of the major reproductive disorders affecting fertility and productivity in dairy cattle. The present study was undertaken to evaluate the effect of equine chorionic gonadotropin pretreatment in Ovsynch protocol on estrus induction, ovarian response and conception rate in postpartum anestrus crossbred dairy cows. Sixteen postpartum anestrus crossbred cows were randomly divided into two groups consisting of eight animals each. Animals in Group I were subjected to standard Ovsynch protocol, whereas animals in Group II received 500 IU eCG intramuscularly six days prior to initiation of Ovsynch protocol. Estrus response, ovarian follicular dynamics, ovulatory response, corpus luteum development and conception rate were assessed. The estrus induction response was 75.00 per cent in both the groups. However, the time taken for onset of estrus was significantly earlier ($P < 0.01$) in Group II (44.33 ± 1.89 hrs) when compared to Group I cows (55.83 ± 2.02 hrs). The dominant follicular diameter on Day 9 and ovulation rate were higher in Group II compared to the control group. The mean corpus luteum diameter on Day 10 and Day 20 post AI was also numerically higher in the treatment group. The overall conception rate recorded in Group I and Group II was 50.00 and 62.50 per cent, respectively. The study concluded that pretreatment with 500 IU eCG prior to initiation of Ovsynch protocol improved estrus characteristics, follicular development, ovulatory response and conception rate in postpartum anestrus crossbred dairy cows under field conditions.

Key words: Postpartum anestrus, equine chorionic gonadotropin, ovsynch protocol, conception rate.

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INTRODUCTION

Anestrus is one of the most common reproductive disorders affecting cattle and buffaloes leading to significant economic losses. It is a functional disorder of the reproductive cycle characterized by absence of overt signs of estrus, either due to failure of estrus expression or its detection. Failure to resume ovarian cyclicity within 60–90 days after calving leads to prolonged calving interval, reduced conception rate and substantial economic losses to dairy farmers. The incidence of postpartum anestrus in indigenous cattle in India ranges from 2.13 to 67.11 per cent, while in crossbred cattle it ranges from 2.55 to 40.4 per cent (Singh *et al.*, 2003; Yadav *et al.*, 2004; Selvaraju *et al.*, 2005 and Thakor and Patel, 2013). In Tamil Nadu, the overall incidence of anestrus in dairy cattle has been reported to vary from 5.40 to 65.00 per cent (Balamurugan *et al.*, 2024).

Postpartum anestrus is multifactorial in origin and is influenced by nutritional, endocrine, metabolic and managerial factors. Negative energy balance, poor body condition, uterine infections and hormonal insufficiency suppress hypothalamic–pituitary–ovarian axis activity, thereby impairing follicular growth and ovulation. Consequently, reproductive efficiency declines due to delayed resumption of cyclicity and prolonged calving interval.

Estrus synchronization protocols are widely used to improve reproductive performance in postpartum anestrus cows. Among them, the ovsynch protocol has

gained considerable importance because it permits fixed-time artificial insemination without the need for estrus detection. However, the success of ovsynch protocol in anestrus cows is often limited by inadequate follicular development, poor ovulatory response and suboptimal luteal activity.

Equine chorionic gonadotropin (eCG), owing to its follicle stimulating hormone (FSH) and luteinizing hormone (LH) like activity, has been used to enhance ovarian activity in anestrus animals. Administration of eCG prior to synchronization protocols has been reported to improve follicular recruitment, dominant follicle growth, ovulation and luteal function (Kohsari *et al.*, 2022). Pretreatment with eCG before initiation of ovsynch may therefore improve synchronization response and fertility in postpartum anestrus cows.

Hence, the present study was undertaken to evaluate the effect of eCG pretreatment prior to ovsynch protocol on estrus induction, ovarian response, luteal activity and conception rate in postpartum anestrus dairy cows.

MATERIALS AND METHODS

Crossbred cows which were brought with the history of postpartum anestrus to LAC-Gynaecology unit of Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu and postpartum anestrus dairy cows reported from Government/Private dairy farms were utilised for the study. The postpartum cows

which were not exhibiting estrus signs three months after calving, without the ovarian corpus luteum in two subsequent ultrasonographic examinations (10 days apart) were selected. The selected postpartum anestrus cows were randomly divided into two groups consisting of eight animals each aged 3–5 years, with a body condition score ranging from 3 to 4, were included in the study.

In group I (OVS): Animals in the control group were subjected to standard ovsynch protocol. GnRH analogue (Buserelin acetate, 10 µg, i.m.) was administered on day 0 followed by PGF₂α analogue (Cloprostenol sodium, 500 µg, i.m.) on day 7. A second GnRH injection was administered on day 9 (48 hours after PGF₂α) and FTAI was performed 16–18 hours later on day 10 and repeat insemination was done after 24 hrs. In group II[eCG(-6)+OVS]: Animals in the treatment group received equine chorionic gonadotropin (eCG) at a dose of 500 IU intramuscularly six days prior to initiation of ovsynch protocol. Subsequently, the standard ovsynch protocol was followed as described for the control group.

The animals were observed for estrus signs following PGF₂α administration. Estrus response, time taken for onset of estrus, duration and intensity of estrus were recorded. Follicular dynamics and ovulatory response were assessed by transrectal ultrasonography on Day 0, 7 and 9 of the synchronization protocol. Pregnancy diagnosis was carried out

approximately 45–60 days post insemination by ultrasonography and/or rectal palpation.

RESULTS AND DISCUSSION

Estrus induction response

The estrus induction response (presented in Table 1) observed in group I and group II was 75.00 per cent each. However, the time taken for onset of estrus was significantly ($P < 0.01$) earlier in group II (44.33 ± 1.89 hrs) compared to group I (55.83 ± 2.02 hrs). The duration of estrus was 36.50 ± 1.85 hrs and 36.33 ± 3.28 hrs in group II and group I respectively, with no significant difference between the groups. The intensity of estrus was numerically higher in group II (2.67 ± 0.21) compared to group I (1.83 ± 0.31).

The earlier onset and higher intensity of estrus observed in the treatment group may be attributed to the FSH and LH-like activity of eCG, which enhances follicular recruitment and steroidogenesis. The present findings were in accordance with the observations of Manshadi *et al.*, (2016) who recorded an earlier onset of estrus following synchronization in eCG-treated cows (45.7 ± 5.9 hrs) compared with control cows (56.8 ± 5.5 hrs), indicating the beneficial effect of eCG on estrus induction. Similar findings were reported by Kohsari *et al.* (2022) and Mohammadsadegh (2019), who observed improved reproductive performance, increased proportion of large follicles and enhanced ovarian response following eCG administration prior to ovsynch protocol in postpartum anestrus dairy cows.

Ovarian follicular dynamics and ovulatory response

The dominant follicular diameter (presented in Table 2) on day 0, day 7 and day 9 in group I was 8.29 ± 0.14 , 11.24 ± 0.21 and 10.59 ± 0.11 mm, respectively, whereas the corresponding values in group II were 11.36 ± 0.31 , 10.63 ± 0.26 and 13.07 ± 0.27 mm, respectively. The treatment group exhibited significantly larger follicular diameter on day 9 compared to the control group.

The ovulation rate (presented in Table 3) observed in group I and group II was 62.50 and 75.00 per cent, respectively. The mean time interval for ovulation was lower in group II (28.67 ± 2.23 h) compared to group I (35.20 ± 1.85 h). Three animals in the treatment group ovulated within 24 h following second GnRH administration, whereas no such early ovulation was observed in the control group.

The improved follicular growth and ovulatory response in eCG-treated cows may be due to prolonged gonadotropic stimulation promoting follicular maturation and ovulatory competence. The dominant follicle diameter observed in group II at the time of second GnRH administration (13.07 ± 0.27 mm) was comparable to the value of 13.5 ± 0.3 mm reported by Kohsari *et al.*, (2022) following the incorporation of eCG into the G6G-ovsynch protocol under heat stress conditions in lactating Holstein cows. Furthermore, the ovulation rate recorded in the present study (75.00%) was higher than the 67.9 % reported by the same authors in cows treated with 750

IU eCG six days prior to initiation of the ovsynch protocol and markedly greater than that observed in the conventional ovsynch group (47.00%), suggesting that eCG administration enhanced preovulatory follicular development and ovulatory response.

Luteal activity

The mean corpus luteum diameter (presented in Table 4) on day 10 post AI in group I and group II was 18.70 ± 0.34 and 21.10 ± 0.23 mm, respectively. Similarly, on day 20 post AI, the corresponding values were 18.27 ± 0.36 and 20.50 ± 0.25 mm, respectively. The treatment group exhibited relatively larger corpus luteum diameter during the post insemination period compared to the control group.

The enhanced luteal development observed in eCG-treated cows may be attributed to improved preovulatory follicular growth and better ovulatory response, resulting in formation of a more functional corpus luteum. Similar observations were reported by Tortorella *et al.* (2013), who documented increased luteal volume and improved luteal function following eCG treatment in anestrus cows. Moura *et al.* (2015) demonstrated that eCG administration stimulated extensive luteal angiogenesis, as evidenced by increased microvascular density, vascular volume density and capillary dilation within the corpus luteum, thereby improving its functional capacity. Ferraz *et al.* (2019) also reported improved follicular and luteal vascularization following administration of eCG during ovulation synchronization

protocols.

Conception rate

The results are summarized in Table 5. The first service conception rate observed in group I and group II was 25.00 and 37.50 per cent respectively whereas the second service conception rate was 25 percent in each group. The overall conception rate recorded in group I and group II was 50.00 and 62.50 per cent, respectively. The higher conception rate observed in the treatment group may be associated with improved follicular growth, synchronized ovulation and better luteal activity following eCG pretreatment. Similar findings were reported by Kohsari *et al.* (2022), who observed increased pregnancy rate following administration of eCG six days before initiation of ovsynch protocol in postpartum anestrus dairy cows. Funakoshi *et al.* (2024) reported improved pregnancy per AI in dairy cows treated with eCG under heat stress conditions. Bryan

et al. (2013) also demonstrated improved reproductive efficiency and conception response following inclusion of eCG in synchronization protocols for anestrus dairy cows.

CONCLUSION

The present study demonstrated that administration of 500 IU eCG six days prior to initiation of ovsynch protocol improved estrus characteristics, follicular development, ovulatory response, luteal activity and conception rate in postpartum anestrus crossbred dairy cows. Pretreatment with eCG resulted in earlier onset of estrus, better follicular maturation and improved ovarian response compared to conventional ovsynch protocol alone. The findings indicated that eCG pretreatment prior to ovsynch could be used as an effective therapeutic approach for improving reproductive performance and fertility in postpartum anestrus dairy cows under field conditions.

Table.1. Estrus induction response in control and treatment group

Group	Estrus induction response %	Time for onset of estrus (hrs) (M± SE)	Duration of estrus (hrs) (M± SE)	Intensity of estrus (M± SE)
Ovsynch (control)	75.00	55.83 ± 2.02q	36.33 ± 3.28	1.83 ± 0.31
D(-6) eCG + Ovs	75.00	44.33 ± 1.89p	36.50 ± 1.85	2.67 ± 0.21
P value	-	0.001**	0.375 NS	0.139 NS

Values within the column with different subscripts (pq) differ significantly ($P < 0.01$)**;
NS – Not significant ($P > 0.05$)

Table.2. Mean ($\pm$ SE) of follicular diameter(mm) in control and treatment groups

Group	D(-6)	DAY 0	DAY 7	DAY 9	D10Post AI	D20 Post AI
Ovsynch	-	8.29 $\pm$ 0.14 ^p	11.24 $\pm$ 0.21 ^q	10.59 $\pm$ 0.11 ^p	9.76 $\pm$ 0.31	9.12 $\pm$ 0.17
D(-6)eCG + Ovs	8.14 $\pm$ 0.13	11.36 $\pm$ 0.31 ^q	10.63 $\pm$ 0.26 ^p	13.07 $\pm$ 0.27 ^q	8.36 $\pm$ 0.1	10 $\pm$ 0.21
P value	-	0.035*	0.057*	0.037*	0.268 NS	0.106 NS

Values within the column with different subscripts (pq) differ significantly ($P < 0.05$)*, NS – Not significant ($P > 0.05$)

Table.3. Ovulatory response between control and treatment groups

Group	Ovulation rate% (n/N)	Number of animals ovulated		Time interval for ovulation (M $\pm$ SE)
		Day 1 (within 24hrs)	Day 2 (between 24 – 48 hrs)	
Ovsynch	62.50 (5/8)	Nil	5	35.20 $\pm$ 1.85
D(-6)eCG + Ovs	75.00 (6/8)	3	3	28.67 $\pm$ 2.23
				P value – 0.119 NS

Table.4. Mean ($\pm$ SE) of CL diameter(mm) in control and treatment groups

Group	DAY 7	DAY 9	DAY 10 Post AI	DAY 20 Post AI	P value & Significance
Ovsynch	18.60 $\pm$ 0.32 ^b	12.51 $\pm$ 0.26 ^a	18.70 $\pm$ 0.34 ^{bp}	18.27 $\pm$ 0.36 ^{bp}	0.001**
D(-6) eCG + Ovs	19.13 $\pm$ 0.46 ^b	10.88 $\pm$ 0.24 ^a	21.1 $\pm$ 0.23 ^{bq}	20.5 $\pm$ 0.25 ^{bq}	0.001**
P value	0.780 NS	0.129 NS	0.039*	0.077*	

Values within the row with different superscripts (ab) differ significantly ($P < 0.01$)**;

NS – Not significant ($P > 0.05$)

Values within the column with different subscripts (pq) differ significantly ($P < 0.05$)*,

NS – Not significant ($P > 0.05$)

Table.5. Conception rate between control and treatment groups

Group	Conception rate% (n/N)		Cumulative conception rate (%)
	1 st insemination	2 nd insemination	
Ovsynch	25.00 (2/8)	25.00 (2/8)	50.00 (4/8)
D(-6) eCG+ Ovs	37.50 (3/8)	25.00 (2/8)	62.50 (5/8)

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