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**Harjyote Singh**
Divison of Veterinary Gynaecology & Obstetrics, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Sanjay Agarwal**
Divison of Veterinary Gynaecology & Obstetrics, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Padamveer Singh**
Divison of Veterinary Gynaecology & Obstetrics, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Sharad Kumar**
Division of Teaching Veterinary Clinical Complex, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Pawan Kumar Verma**
Division of Veterinary Pharmacology and Toxicology, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Sudhir Kumar**
Divison of Veterinary Gynaecology & Obstetrics, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

**Corresponding Author:**
**Sanjay Agarwal**
Division of Teaching Veterinary Clinical Complex, F.V.Sc. & A.H., S.K.U.A.S.T.-J., R. S. Pura, Jammu, India

# Role of hCG administration at day 4 of the estrous cycle in repeat breeder cross-bred cow

**Harjyote Singh, Sanjay Agarwal, Padamveer Singh, Sharad Kumar, Pawan Kumar Verma and Sudhir Kumar**

## Abstract
The aim of this study was to investigate the effects of hCG (Chorulon®, 1500 I.U.) administration on day 4 of the estrous cycle. Healthy and normal cyclic repeat breeder cross bred cows (n= 12) aging 4-7 years were used in this study. Animals with pre-ovulatory follicle of ≥ 10 mm were bred artificially. The plasma progesterone was estimated using RIA kit. Ultrasonography and blood collection was done on day 0, 4, 14 of estrous cycle. Pregnancy diagnosis was confirmed at day 45 post breeding. Preovulatory follicle had the largest diameter among all the days of estrous cycle. There was no significant difference in diameter of follicle between control and treatment group and within the control group. Diameter and area of the corpus luteum increased in treatment group. There was no significant difference in diameter and area of corpus luteum between control and the treatment group. No accessory corpus luteum was formed in cows of control group, whereas, two accessory corpora lutea were induced in the treatment group. Plasma progesterone concentration on day 14 was significantly high in comparison to day 0, 4 of the estrous cycle within treatment and control group. The pregnancy rate was Nil in control group, while 16.66% in treatment group. It was concluded that there was no significant effect of hCG administration on diameter of dominant follicle. The diameter and area of corpus luteum increased post hCG administration. The accessory corpus luteum could be produced by hCG administration on day 4 of the estrous cycle, which is lesser in size. After hCG administration there was significant rise in progesterone concentration, may be due to accessory corpus luteum formation or stimulatory effect on existing corpus luteum.

**Keywords:** hCG, day 4, Repeat breeding, Accessory corpus luteum, Progesterone

## Introduction
Reproductive efficiency of dairy cows has declined worldwide and pregnancy rates after artificial insemination (AI) are usually between 30 and 40% [1, 2]. This reduced fertility leads to great economic losses to the farmers and dairy producers due to more AI services, increased calving interval and increased culling rates [3, 4]. The reduced fertility though may be due to various reasons, but without any obvious pathological signs, the majorities of animals repeatedly comes in estrus at regular interval even after being inseminated with fertile semen.

Repeat breeding is an important reproductive disorder in which an animal which has been served three or more times with a fertile bull or inseminated with fertile semen doesn't conceive and continually returns to estrus in the absence of any obvious pathological disorder in the genital tract [5]. Though many factors are considered to play an important role in this syndrome e.g. nutritional, managemental practices, genetic, diseases, physiological disturbances, anatomical defects, estrus detection errors, embryonic mortalities and summer heat stress [6-8], thereby decreasing overall pregnancy rates [9, 10], but major factor is inadequate postovulatory progesterone concentrations. The time when the embryo enters the uterus and undergoes blastocoels formation (Day 6–8) has been suggested to be a critical period during which embryonic death occurs [11]. Thus slower than normal rise in progesterone concentration and a lower total progesterone concentration in cows and heifers during first 6 days after estrus may result low-fertility and repeat-breeder syndrome [11, 12]. Increasing concentrations of circulating P4 in the immediate post-conception phase can hasten elongation of the embryo and increase embryonic interferon-τ production [13-15], while lower circulating P4 is associated with reduced fertility in lactating dairy cows [16-18]. The elevation in progesterone (P4) concentration during the first week of pregnancy reduce embryonic mortalities [19, 20] through increasing interferon-tau (INF-τ) [21] which extends the lifespan of the corpus luteum (CL) by

suppressing estradiol and oxytocin receptor genes [22] and by attenuating the endometrial secretion of PGF2α [23]. Thus progesterone synthesis by the corpus luteum (CL) is necessary for maintenance of early pregnancy in the cow [23]. Premature regression of the CL, and the subsequent reduction in progesterone secretion, results in loss of pregnancy [8].

As direct administration of P4 though exogenous source has many drawbacks thus now-a-days considerable attention has been drawn towards enhancing the level of P4 endogenously. One common method for supplementing P4 after AI is induction of the dominant follicle of the first follicular wave or early luteal phase using human chorionic gonadotropin (hCG) treatment to produce an accessory corpus luteum [24] subsequently increased circulating P4 concentrations [25-27]. The hCG has activity similar to LH, and after binding to LH receptors, causes small luteal cells to increase progesterone synthesis [27]. Such treatments however had different results on pregnancy rates in lactating cows [24, 28-31]. In continuation to our previous reported findings on effects of hCG administration at day 5 [32] and day 6 [33] of the estrous cycle on pregnancy rate of repeat breeder cross-bred cow. The aim of the present investigation is to study the effect of human chorionic gonadotropin (hCG) administration at day 4 of A.I. on follicular development, luteal development, progesterone secretion, accessory corpus luteum induction and pregnancy in repeat breeder crossbred cows.

## Materials and Methods

### Animal and management

This study was conducted at Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu (SKUAST-J), Jammu. The experimental animals comprised of 12 healthy and cyclic crossbred repeat breeder cattle, aging 4-7 years, kept at ILFC, SKUAST-J, Jammu, organized dairy farms, commercial dairy farms and local nearby area, with a body condition score (BCS) between 3 and 4 (Scale 1 to 5; BCS 1 = emaciated; BCS 5 = obese/ extremely fat [34] at initiation of the study were selected for experimentation.

### Experimental design

Animal at standing estrous, confirmed on the basis of records for regular estrous cycle, signs of estrus followed by trans-rectal ultrasonography when ovary contains pre ovulatory follicle of ≥ 10 mm diameter. All the animals at standing estrus negative to 'White Side Test' (a test of endometritis [35] were selected for experimentation. All selected animals were bred twice by artificial insemination at 12 and 24 hrs after onset of estrus with fertile frozen semen and were randomly divided into 2 groups (each group containing 6 animals) as control group: inj. 5ml Normal Saline on day 4/ did not received any treatment and treatment group: inj. hCG (Chorulon ®) 1500 IU i.m. at day 4 of AI.

### Collection of blood

Blood samples were collected at each ultrasound session by jugular venipuncture in heparinized test tube on day 0, 4 and 14 and plasma was harvested by centrifuging at 3000 rpm for 15 minutes within 30 minutes of collection and stored at -20°C pending progesterone assay.

### Progesterone estimation

The progesterone estimation from blood plasma was done using progesterone RIA Kits. Analytical sensitivity: 0.03 ng/ml (0.10 nmol/L). Specificity: the antibody used in the immunoassay is highly specific for progesterone.

### Ultrasonographic studies

Portable Ultrasound Diagnostic Imaging System, CHISON Model: ECO 1 VET (Figure: 1.0) equipped with a 5.0 MHz linear-array transducer was used for trans-rectal scanning of ovaries, follicular study (Figure: 2.0), luteal study and early pregnancy diagnosis. Ovarian maps were drawn to record size and position of the follicles and corpus luteum as per [36] Savio *et al*. pregnancy diagnosis on day 45 post AI with inbuilt calipers.

### Statistical analysis

The data were analyzed statistically using Analysis of Variance (ANOVA) [37].

## Results and Discussion

### Follicular development

The effect of hCG administration on day 4 on follicular development at day 0, 4 and 14 are given in table 1.0. The follicle diameter (mm) in control and treatment group on day 0, 4 and 14 were 12.27 ± 1.30, 9.06 ± 1.12, 10.70 ± 0.49 and 11.10 ± 0.75, 8.27 ± 0.94, 10.10 ± 0.79, respectively.

There was no significant difference in follicular diameter (mm) between the control and treatment group and within control group, while in treatment group it was significantly higher ($P<0.05$) on day 0 in comparison to day 4 and 14 of the estrous cycle.

The diameter (mm) of follicle present on day 0, 14 in both the groups i.e. control group and treatment group was ≥ 10 mm which was in agreement with finding of [38] Sianangama and Rajamahendran (1996) and [39] Ginther *et al*.

### Corpus luteum development

The effect of hCG administration at day 4 on corpus luteum development on day 14 is given in table 2.0. The corpus luteum diameter (mm) in control and treatment group on day 14 was 17.19 ± 0.70 and 21.00 ± 1.85, respectively and the corpus luteum area ($mm^2$) in control and treatment group on day 14 was 317.02 ± 8.27 and 334.65 ± 3.15, respectively. There was no significant difference in corpus diameter (mm) and corpus luteum area ($mm^2$) between the control and treatment group. This finding of increase in corpus luteum might be due to luteotropic effect of hCG. Increase in CL size was observed on day 9 and day 10 [40] (Maillo *et al*., 2013). This luteotropic effect of hCG on corpus luteum is also reported by various researchers [24-27, 40-43].

### Progesterone concentration

The progesterone concentration (ng/ml) in control and treatment group on day 0, 4, 14 were 0.13 ± 0.06, 0.23 ± 0.12, 5.11 ± 0.58 and 0.08 ± 0.05, 0.36 ± 0.17, 15.17 ± 6.89, respectively (Table 1.0). It was observed that there was no significant difference in the progesterone concentration (ng/ml) between control and treatment group, whereas, within control and treatment group the progesterone concentration was significantly higher ($P< 0.05$) on day 14 in comparison with the levels at day 0 and day 4 within the same group.

The progesterone concentration (ng/ml) on day 0 and 4 in both the groups were below 1 ng/ml, whereas, on day 14 the concentrations increased more than 1 ng/ml in both groups. This finding was in agreement with [44] Hansel *et al.* (1973). It was observed that within control and treatment group the

progesterone concentration (ng/ml) at day 14 was significantly high ($P<0.05$) in comparison with the levels at day 0 and 4 within the same group. This finding was in agreement with [45] Patel *et al.* (2006). It was also observed that progesterone concentration was higher on day 14 in treatment group in comparison to control group this finding of increase in progesterone concentration in treatment group was supported with the findings of [46] Donaldson *et al.*,1964; [47] Hansel and Seifart, 1967; [48] Moody and Hansel, 1971 and [49] Machado *et al.*, 2008.

**Accessory corpus luteum (ACL)**

Total number of ACL produced in control and treatment group were 0 and 2, respectively (Table 3.0). The formation and retention of ACL as a result of inj. hCG on day 4 in treatment group was 33.33 per cent. The diameter (mm) and area ($mm^2$) of ACL on day 14 was 14.28 ± 0.92 and 271.95 ± 21.25, respectively. The size of ACL both diameter and area were least when compared with the size of primary CL on day 14 of the estrous cycle.

It was observed that no accessory corpus luteum was formed in control group, while 2 ACL were induced in the treatment group i.e. about 33.33% which was less than the findings [40] Maillo *et al.*, 2013, who obtained 90% ACL with inj. of hCG on day 4 in heifers.

It was observed that average diameter and area of ACL was less than the primary CL in same group on the same day of estrous cycle. This finding was in agreement with the findings of [38] Sianangama and Rajamahendran, 1996 and [50] Stevenson *et al.*, 2008, who reported that induced accessory corpus luteum are smaller in size compared with primary corpus luteum.

**Pregnancy rate**

As a result of hCG inj. on day 4 the pregnancy rate was 16.66 percent in the treatment group (Table 3.0) in comparison to 0 in the control group.

It was observed that the pregnancy rate was 16.66 percent, while no conception was obtained in control group. This findings was in agreement with the findings of [41] Breuel *et al.*, 1989. In contrast, other studies reported no effects or reduced pregnancy rates (Tefera *et al.*, 2001; Fischer-Tenhagen *et al.*, 2010) [51, 52]. In our study the increased pregnancy rate (16.66 %) might also be due to ACL production.

**Table 1:** Diameter (mm) of the largest follicle and plasma progesterone concentration (ng/ml) of control group versus treatment group (inj. hCG day 4) on day 0, 4 and 14 of the estrous cycle.

| Days of estrous cycle | Diameter of the largest follicle (mm) | | Progesterone concentration (ng/ml) | |
|---|---|---|---|---|
| | Control group | Treatment group | Control group | Treatment group |
| 0 | $12.27 \pm 1.30^{Aa}$ | $11.10 \pm 0.75^{Ba}$ | $0.13 \pm 0.06^{Aa}$ | $0.08 \pm 0.05^{Aa}$ |
| 4 | $9.06 \pm 1.12^{Aa}$ | $8.27 \pm 0.94^{Aa}$ | $0.23 \pm 0.12^{Aa}$ | $0.36 \pm 0.17^{Aa}$ |
| 14 | $10.70 \pm 0.49^{Aa}$ | $10.10 \pm 0.79^{Aa}$ | $5.11 \pm 0.58^{Ba}$ | $15.17 \pm 6.89^{Ba}$ |

Values are given as Mean ± S.E.M. of 6 animals.
Values with different superscript (smaller alphabets) within row differ significantly ($P<0.05$)
Values with different superscript (capital alphabets) within column differ significantly ($P<0.05$)

**Table 2:** Diameter (mm) and area ($mm^2$) of the corpus luteum of control group versus treatment group (inj. hCG day 4) on day 14 of the estrous cycle.

| Days of estrous cycle | Diameter of the corpus luteum (mm) | | Area ($mm^2$) of the corpus luteum | |
|---|---|---|---|---|
| | Control group | Treatment group | Control group | Treatment group |
| 14 | $17.19 \pm 0.70^{a}$ | $21.00 \pm 1.85^{a}$ | $317.02 \pm 8.27^{a}$ | $334.65 \pm 3.15^{a}$ |

Values are given as Mean ± S.E.M. of 6 animals.
Values with different superscript (smaller alphabets) within row differ significantly ($P<0.05$)

**Table 3:** Induction of accessory corpus luteum (ACL) and pregnancy rate in control group versus treatment group (inj. hCG day 4) on day 14 of the estrous cycle.

| Groups | Total no. of Accessory Corpus Luteum | Diameter (mm) of ACL | Area ($mm^2$) of ACL | Pregnancy | Pregnancy rate |
|---|---|---|---|---|---|
| **Control** | 0 | - | - | - | - |
| **Treatment** | 2 | 14.28 ± 0.92 (n=2) | 271.95 ± 21.25(n=2) | 1 | 16.66 |

Values of ACL are given as Mean ± S.E.M. of number of ACL (n)

**Fig 1:** Portable Ultrasound machine used, CHISON Model: ECO 1 VET

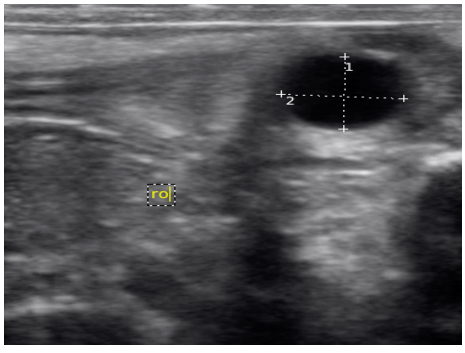

**Fig 2:** Ultrasonographic image of a dominant follicle

## Conclusion

It was concluded that there was no significant effect of hCG administration on diameter of dominant follicle. The diameter and area of corpus luteum increased post hCG administration. The accessory corpus luteum could be produced by hCG administration on day 4 of the estrous cycle, which is lesser in size. After hCG administration there was rise in progesterone concentration, may be due to accessory corpus luteum formation or stimulatory effect on existing corpus luteum. Further, more studies with larger populations, complete endocrine profile of animals pre and post treatment along uniform feeding schedule should be done in future studies for its validation and final recommendation.

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