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# Ultrasonography studies on effects of h CG administration at day 5 of the estrous cycle on pregnancy rate of repeat breeder cross-bred cow

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

## Abstract
The present study was designed to investigate the effects of hCG (Chorulon®, 1500 I.U.) administration on day 5 of the estrous cycle, inducing formation of accessory corpus luteum, thus preventing early embryonic mortality due to possible luteal insufficiency, monitoring the size of preovulatory follicle (day 0), dominant follicle (day 5), subsequent corpus luteum, formation of accessory corpus luteum vis-à-vis progesterone profile and pregnancy rate in repeat breeder cross-bred cows. Healthy and normal cyclic repeat breeder cross bred cows (n= 12), "White side negative", BCS between 3 and 4, 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, 5, 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 on various days of observation between and within control and treatment groups. Diameter and area of the corpus luteum increased in treatment group. There was no significant difference in diameter of corpus luteum, while area of corpus luteum differed significantly with control group. No accessory corpus luteum was formed in cows of control group. The formation and retention of 3 accessory corpora lutea were obtained in treatment group. The diameter and area of accessory corpus luteum was lesser than the diameter and area of corpus luteum present on the same day. Plasma progesterone concentration on day 14 was significantly high in comparison to day 0, 5 of the estrous cycle within treatment and control group. Progesterone concentration was significantly high on day 14 in treatment group in comparison to control group. The pregnancy rate was Nil in control group, while 50% in treatment group. It was concluded that there was no significant effect of hCG administration on diameter of pre-ovulatory or 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 5 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, ultrasonography, repeat breeding, accessory corpus luteum, progesterone

## Introduction
Repeat breeder is an important reproductive disorder which causes great economic loss to dairy farmers or industry. Repeat breeder animal is usually defined as a subfertile animal which has been served three or more times with a fertile bull or inseminated with fertile semen yet fails to conceive and continually returns to estrus in the absence of any obvious pathological disorder in the genital tract [1]. The repeat breeder cow continually returns to service in the absence of any obvious pathological diseases. Many factors are considered to intervene in this syndrome, such as impaired embryonic development, periovulatory abnormalities, damage to endometrium stress, and inadequate postovulatory progesterone concentrations. It was reported that ovarian cysts, mistimed AI, subluteal progesterone levels, luteal dysfunction, or ovulation defects are risk factors for repeat breeder syndrome. In repeat-breeder cows, 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 [2]. Also a slower than normal rise in progesterone concentration and a lower total progesterone concentration have been reported in low-fertility cows and repeat-breeder heifers in the first 6 days after estrus [2, 3]. Progesterone synthesis by the corpus luteum (CL) is necessary for maintenance of early pregnancy in the cow [4].

Premature regression of the CL, and the subsequent reduction in progesterone secretion, results in loss of pregnancy [5]. Various approaches have been made to improve embryonic survival in repeat breeder cows like direct application of progesterone, progesterone releasing intravaginal device (PRID), Controlled Internal Drug Release (CIDR) and Synchromate ear implants are which are commercially available [6]. Another recent approach for endogenous production of progesterone is administration of hCG during the early luteal phase to induce ovulation of the dominant follicle from the first follicular wave and results in the formation of an accessory corpus luteum [7]. The most dramatic effects of hCG administration on conception rates have been observed in dairy herds of low fertility [8]. Human chorionic gonadotropin has activity similar to LH, and after binding to LH receptors, causes small luteal cells to increase progesterone synthesis [9]. The GnRH and hCG treatments were equally effective in inducing an accessory CL, but the subsequent increase in progesterone concentrations was greater in hCG-treated heifers [7]. Such treatments however had different results on pregnancy rates in lactating cows [7, 10-13]. Therefore keeping above facts in view the aim of the present investigation is to study the effect of human chorionic gonadotropin (hCG) on conception in repeat breeder crossbred cows that are occurring due to luteal deficiency thus preventing early embryonic mortality (EEM) by administration on 5$^{th}$ day of AI, resulting in accessory corpus luteum (by luteinizing dominant follicle of Ist follicular wave) thus producing more progesterone, necessary for better embryo survival in luteal deficient repeat breeder cross-bred 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 [14] 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 [15]. 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 5/ did not received any treatment and treatment group: inj. hCG (Chorulon®) 1500 IU i.m. at day 5 of AI.

### Collection of blood

Blood samples were collected at each ultrasound session by jugular venipuncture in heparinized test tube on day 0, 5 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.

### Ultrasonography studies

Portable Ultrasound Diagnostic Imaging System, CHISON Model: ECO 1 VET equipped with a 5.0 MHz linear-array transducer was used for trans-rectal scanning of ovaries, follicular study (Figure 1), luteal study and early pregnancy diagnosis (Figure 2). Ovarian maps were drawn to record size and position of the follicles and corpus luteum as per [16] and pregnancy diagnosis on day 45 post AI with inbuilt calipers.

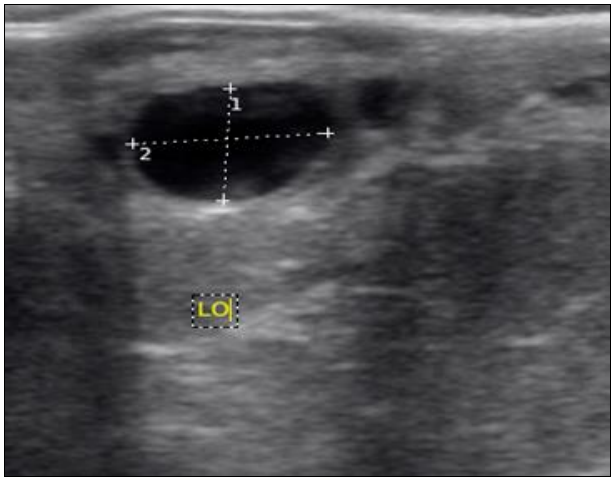


**Fig 1:** Ultrasonographic image of ovary showing measurement of follicle

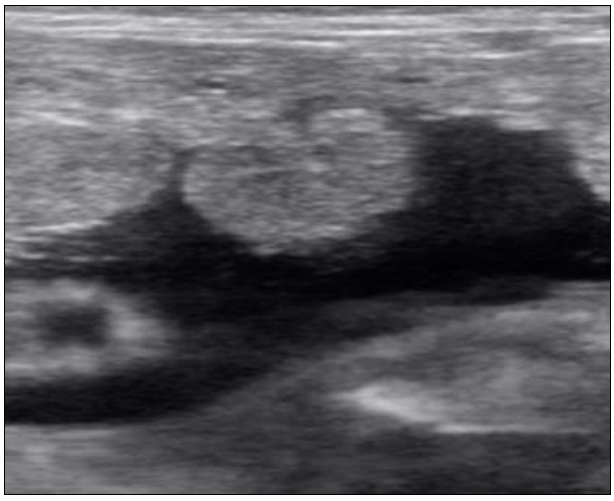

**Fig 2:** Ultrasonography image of pregnant uterus showing Place tome

### Statistical analysis

The data were analyzed statistically using Analysis of Variance (ANOVA) [17].

## Results and Discussion

### Follicular development

The effect of hCG administration on day 5 on follicular development at day 0, 5 and 14 are given in table 1. The follicle diameter (mm) in control and treatment group on day

0, 5 and 14 were 12.27 ± 1.30, 9.67 ± 1.39, 10.70 ± 0.49 and 11.73 ± 1.1, 11.13 ± 1.1, 10.99 ± 0.92, respectively.

There was no significant difference in follicular diameter (mm) between and within the control and treatment group on various days of observation i.e. day 0, day 5 and day 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. This finding was in agreement with finding of [18] Sianangama and Rajamahendran (1996) who reported that the preovulatory follicle (day 0) or dominant follicle (day 7 and day 14) are of greater than 11 mm, and findings of [19] Ginther *et al.* (2003) who defined dominant follicle that has reached diameter > 10 mm.

The larger diameter in treatment group on day 5 might be due to change in the number of follicular wave in few animals of treatment group. [16]Savio *et al.* (1988) reported that in cows mainly there are two follicular waves (15%) or three follicular waves (81%) and rarely one or four follicular waves per cycle in cattle.

**Corpus luteum development**

The effect of hCG administration on day 5 on corpus luteum development on day 14 is given in table 2. The corpus luteum diameter (mm) in control and treatment group on day 14 was 17.19 ± 0.70 and 22.74 ± 2.48, respectively and the corpus luteum area ($mm^2$) in control and treatment group on day 14 was 317.02 ± 8.27 and 396.8 ± 1.94, respectively. There was no significant difference in corpus diameter (mm) between the control and treatment group, whereas there was a significant ($P$<0.05) increase in the corpus luteum area ($mm^2$) of the treatment group in comparison to control group. This finding of increase in corpus luteum might be due to luteotropic effect of hCG. This luteotropic effect of hCG on corpus luteum is also reported by various scientists [7-9, 20-23].

**Progesterone concentration**

The progesterone concentration (ng/ml) in control and treatment group on day 0, 5, 14 were 0.13 ± 0.06, 0.54 ± 0.26, 5.11 ± 0.58 and 0.15 ± 0.06, 0.50 ± 0.08, 17.96 ± 5.41, respectively (Table 1). It was observed that there was no significant difference in the progesterone concentration (ng/ml) on day 0 and 5 between control and treatment group, whereas, the progesterone concentration was significantly higher ($P$<0.05) on day 14 of treated animal in comparison to control group. It was also 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 day 5 within the same group.

It was revealed that progesterone concentration (ng/ml) on day 0 and 5 in both the groups were below 1 ng/ml (Table 1). Whereas, on day 14 the concentrations increased more than 1 ng/ml in both groups. This finding was in agreement with [24] Hansel *et al.*(1973) who reported that corpus luteum takes approximately 4-5 days from ovulation to gain progesterone producing capacity in excess of 1 ng/ml.

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 5 within the same group. This finding was in agreement with [25] Patel *et al.* (2006) who reported that the level of plasma progesterone steadily increase from day 0 of estrous cycle and attains peak on day 14.

It was also observed that progesterone concentration was significantly higher ($P$<0.05) on day 14 in treatment group in comparison to control group this finding of increase in progesterone concentration in treatment group is in agreement with [26] Donaldson *et al.*,1964; [27] Hansel and Seifart, 1967; [28] Moody and Hansel, 1971 and [29] Machado *et al.*, 2008, who reported that hCG administration during luteal phase of cattle increased the size and weight of already existing corpus luteum, as well as serum progesterone concentration.

**Accessory corpus luteum (ACL)**

Total number of ACL produced in control and treatment group were 0 and 3, respectively (Table 3). The formation and retention of ACL as a result of hCG inj. on day 5 in treatment group was 50 per cent. The diameter (mm) and area ($mm^2$) of ACL on day 14 was 15.99 ± 0.94 and 263.46 ± 16.12, 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 corpus luteum is formed in control group, while 3 ACL (50%) were produced in treatment group, which was less than the findings [30] Thatcher *et al.*(2002), who obtained 86.2% ACL with inj. of hCG on day 5 in cows.

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 [18] Sianangama and Rajamahendran, 1996 and [31] 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 5 the pregnancy rate was 50 percent in the treatment group (Table 3) in comparison to 0 in the control group.

It was observed that the pregnancy rate was 50 percent, while no conception was obtained in control group. The higher Ist service conception rate (50%) might also be due to larger number of 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 5) on day 0, 5 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 | $12.27 \pm 1.30^{Aa}$ | $11.73 \pm 1.1^{Aa}$ | $0.13 \pm 0.06^{Aa}$ | $0.15 \pm 0.06^{Aa}$ |
| 5 | $9.67 \pm 1.39^{Aa}$ | $11.13 \pm 1.1^{Aa}$ | $0.54 \pm 0.26^{Aa}$ | $0.50 \pm 0.08^{Aa}$ |
| 14 | $10.70 \pm 0.49^{Aa}$ | $10.99 \pm 0.92^{Aa}$ | $5.11 \pm 0.58^{Ba}$ | $17.96 \pm 5.41^{Bb}$ |

- 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 5) 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 | $17.19 \pm 0.70^a$ | $22.74 \pm 2.48^a$ | $317.02 \pm 8.27^a$ | $396.8 \pm 1.94^b$ |

- 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 5) 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 | 3 | 15.99 ± 0.94 (n=3) | 263.46 ± 16.12(n=3) | 3 | 50 |

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

## Conclusion

It was concluded that there was no significant effect of hCG administration on diameter of pre-ovulatory or 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 5 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. Further, more studies with larger populations and complete endocrine profile should be done in future studies for its validation and recommendation.

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