

EFFICACY OF HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION DURING LUTEAL PHASE IN DAIRY COWS ON PROGESTERONE CONCENTRATION AND CONCEPTION RATE

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Received: 06.06.2018; Accepted: 19.09.2018

ABSTRACT

The present study was conducted to study the efficacy of human chorionic gonadotropin (hCG) administration in cyclic dairy cows. The present research work was conducted at the Livestock Farm, Veterinary Clinical Complex of Palampur University and nearby field. One hundred and eighty seven cows were administered (i.m.) 1500 IU hCG at the time of AI (n=67), 5 days (n=65) and 12 days post AI (n=55). Pregnancy diagnosis was carried out on day 60 post AI. Conception rate achieved was 61.19, 63.07, 67.27 and 49.45 per cent in cows administered hCG simultaneous to AI, on day 5, day 12 post AI and control group, respectively. However, hCG administered 12th day post AI (67.27%) significantly ($p<0.05$) improved the conception as compared to control group (49.45%). Blood was collected at a weekly interval (day 0, 7, 14) for serum progesterone estimations and found numerically high ($P>0.05$) in hCG administered cows compared to control group cows. In brief, administration of hCG on day 12 post AI improves subsequent luteal profile with tendency for better conception rate in normal cyclic cattle.

Key words: Conception rate, Dairy cows, hCG, Progesterone.

Reproductive efficiency in dairy cows has declined over the last several years and is considerably lower than desired (Sharma *et al.*, 2018). Establishment and maintenance of pregnancy are complex processes that require precise communication and synchrony between the conceptus and the dam. The role of progesterone is critical in these processes. Maintenance of pregnancy is dependent on secretion of progesterone during early pregnancy (Gonzalez *et al.*, 2017).

Luteal deficiency during the first three weeks of gestation has been hypothesized as a cause of pregnancy failure (Santos *et al.*, 2004). To overcome these losses, the use of luteotropic hormones such as human chorionic gonadotropin (hCG) may directly or indirectly modify ovarian follicular dynamics that would be more conducive for the development and survival of embryo. Administration of hCG during the luteal phase induces ovulation of the dominant follicle from the first follicular wave and results in formation of an accessory CL. Similarly, numerous researchers (Gonzalez *et al.*, 2017; Kundu *et al.*, 2018) have reported that hCG administration during the luteal phase of cattle increases the size and weight of the already existing CL as well as serum concentrations of progesterone. The present experiment was conducted to evaluate the effect of post insemination administration of hCG during luteal phase on conception rate in dairy cows and comparing the effect of hCG administration in early or mid-luteal stages on progesterone concentration post insemination in dairy cows.

MATERIALS AND METHODS

The work was conducted at the University

Livestock Farm and Teaching Veterinary Clinical Complex of the university and in nearby field veterinary institutions from February 2015 to March 2016. In all, 278 normal cyclic cows were inseminated during this study. These cows were divided in three treatment and one control group. The animals included in treatment groups (n=187) with human chorionic gonadotrophin (Chorulon, MSD India Ltd.; 1200 I.U. i.m) were sub-divided into three groups *viz.* administration of hormone simultaneous to AI (day 0, group1; n=67), insemination and exogenous administration of hCG on 5th day post AI (group 2; n=65) and insemination and administration of hormone on 12th day post AI (group 3; n=55). Remaining 91 cows inseminated in standing heat without any treatment were kept as control (group 4).

Blood was collected in vials with anticoagulant from only 26 cows for plasma progesterone estimation which included six animals each from three treatment groups and eight animals from control group (on day 0, 7 and 14 post insemination) Plasma was separated by centrifugation of blood sample @ 3000 rpm for 10 min. and frozen at -20°C in 2 ml capacity micro-centrifuge tubes till further analysis. Plasma progesterone was estimated

Table 1
Effect of administration of hCG on different days of estrous cycle on conception in normal dairy cows

Dose	Day of Administration post-AI	Cows inseminated	Pregnant	CR (%)
hCG	0	67	41	61.19 ^{ab}
1500	5	65	41	63.07 ^{ab}
units	12	55	37	67.27 ^b
(n=187)				
Control (n=91)		91	45	49.45 ^a

Figures with different superscripts differ significantly ($p<0.05$)

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Table 2
Plasma progesterone concentration (ng/ml) (Mean±SE) in dairy cows administered with human chorionic gonadotropin at estrus or during luteal phase

Treatment	Administration	Progesterone concentration (ng/ml)		
		day 0	day 7	day 14
hCG 1500 units (n=18)	Simultaneous to AI (n=6)	0.26±0.06 (0.10-0.46)	2.15±0.51 (0.98-4.20)	2.77±0.41 (1.60-4.60)
	5 th day post AI (n=6)	0.39±0.09 (0.10-0.68)	1.91±0.37 (1.20-3.70)	3.47±0.42 (1.80-4.80)
	12 th day post AI (n=6)	0.30±0.07 (0.01-0.60)	2.02±0.16 (1.70-2.70)	3.55±0.43 (2.20-5.20)
Control group (n=8)		0.47±0.34 (0.10-1.10)	1.91±0.61 (0.80-2.60)	2.61±0.90 (1.70-4.40)

using Radioimmunoassay (RIA). Pregnancy diagnosis was carried out on day 60 post AI in cows not returning to estrus within this duration by per rectal palpation method.

The data was statistically analyzed using Chi-square and Student's t-test with SPSS® 20 level version for windows.

RESULTS AND DISCUSSION

Perusal of the Table 1 indicates that conception rate (CR) achieved was 61.19, 63.07 and 67.27 per cent in group 1, 2 and 3, respectively. However, in control group cows, CR was 49.45 per cent. Clinically, hCG administered 12th day post AI (67.27%) significantly (P<0.05) improved the conception as compared to control group (49.45%).

Perusal of Table 2 shows increase in plasma progesterone concentration on day 7 following treatment with hCG on day 0 was non-significantly (p>0.05) higher than other treatment groups. Similarly, an increase in plasma progesterone concentration on day 14 following treatment with 1500 IU hCG on day 5 or/and 12 was non-significantly (p>0.05) higher than other groups.

A number of studies have been conducted to examine the effect of hCG on reproductive performance of dairy cows when administered on the day of AI (Selvaraju *et al.*, 2009) or in early (Gonzalez *et al.*, 2017; Kundu *et al.*, 2018) or mid luteal phase (Paksoy and Kalkan, 2010).

Our results regarding the conception rate were also in agreement with the findings that use of hCG during the (early or mid) luteal phase enhances the conception rate in the dairy cows (Gonzalez *et al.*, 2017; Kundu *et al.*, 2018). Contrary to our findings it has been reported that the use of hCG has no effect or did not improve conception or reproductive performance in dairy cows (Schmitt *et al.*, 1996). Our results are in agreement with the findings of Selvaraju *et al.* (2009) who used hCG at the time of AI and found increased conception rate in dairy cows. Contrary to these findings it has also been suggested that hCG administered at the time of AI has no effect on conception rate (Paksoy and Kalkan, 2010).

The improvement in fertility due to hCG

treatment during estrus in the present study might be influenced by a series of events *viz.* the time of ovulation, fertilization, corpus luteum development, progesterone secretion and embryonic survival. Human chorionic gonadotropin injection, if administered at the time of insemination, increases the chances of pregnancy by inducing ovulation (Gonzalez *et al.*, 2017). A dose of hCG at AI can increase subsequent plasma progesterone concentration (Machado *et al.*, 2008), which is related to hypertrophy and hyperplasia of corpus luteum cells and differentiation of theca and granulosa cells into small and large luteal cells and which is probably due to increase in LH pulses (Rajamahendran and Sianangama, 1992). hCG injection at the time of estrus causes LH surge and thus, ovulation (Gonzalez *et al.*, 2017). In dairy cows, plasma LH concentrations are markedly increased for 30 hours after hCG administration and do not return to baseline concentrations for 66 hours (Schmitt *et al.*, 1996). The treatment of hCG also decreases the time interval between estrus to LH surge, which in turn leads to decreased production of mitotic inhibitor factor in granulosa cells (Thatcher *et al.*, 1993). Following hCG injection, changes start and maturation and ovulation occur under the influence of LH (Kudlac *et al.*, 1982). The greater incidence of ovulation after hCG administration is probably a function of hCG's greater half-life compared to GnRH-induced LH release in response to hCG's four sites of O-linked glycosylation (Jameson and Hollenberg, 1993).

In the present study, observations for progesterone concentration are in agreement with the findings of Shahneh *et al.* (2008) that hCG administration at the time of AI caused a non-significant increase in the concentration of plasma progesterone on day 5 post AI and are contrary to the findings of significant increase in progesterone concentration (Machado *et al.*, 2008) or no effect on progesterone concentration by other researchers (Paksoy and Kalkan, 2010).

The explanation and rationale for the increased conception rate with the use of hCG during luteal phase has been attributed to the fact that hCG on day 5 (early

luteal phase) induces ovulation of the first wave dominant follicle thus forming an accessory CL and enhancing progesterone production early in the cycle, as an increase in progesterone secretion may facilitate embryonic development (Gonzalez *et al.*, 2017). Treatment with hCG increases serum concentrations of progesterone in at least two ways: first by induction of accessory CL (Stevenson *et al.*, 2007) and second by an increase in number of progesterone-secreting luteal cells in existing CL (Niswender *et al.*, 1995) or both. Beltran and Vasconcelos (2008) also reported that hCG administered 5th day post AI caused a proportional increase in serum progesterone concentration between days 5 and 7 after AI. This increase in CL number and thus total CL tissue area is most likely responsible for the increased serum concentration of progesterone observed with hCG treatment. Mann *et al.* (2006) also noted that augmented progesterone concentration in blood on day 5 to 9 post-insemination increased trophoblast size and interferon- τ concentrations.

The administration of hCG on day 12 probably enhanced progesterone production which may have provided luteotrophic stimulation to CL. The administration of 1000 IU hCG in postpartum beef cows was sufficient to induce luteinization irrespective of follicle size or dominance at the time of treatment (Cooper *et al.*, 1991). The effectiveness of hCG to luteinize follicles can vary during the estrous cycle. Thus, the formation of accessory luteal structures was found to be greater when hCG was given during the early luteal (Days 4 to 7) than during the follicular (Days 0 to 3) or mid luteal (Days 8 to 12) stage of the estrous cycle (Price and Webb, 1989).

The non-significant ($p>0.05$) increase in progesterone concentration following hCG administration on day 12th post AI is in agreement with the findings of Paksoy and Kalkan (2010). Progesterone is a vital hormone during early pregnancy that promotes embryo development and controls the luteolytic mechanism (Kundu *et al.*, 2018). Exogenous administration of hCG could initiate the endogenous increase in progesterone probably due to combined effect on the original and induced CL formation (Esfandabadi *et al.*, 2007). hCG has been reported to stimulate blastocyst expansion and larger blastocysts secrete more IFN- τ (Nephew *et al.*, 1994) which by down-regulation of oestradiol and oxytocin receptors suppresses PGF_{2 α} release more effectively. Consequently, the luteolytic mechanism is either blocked or delayed which gives blastocyst more time to establish and help in maternal recognition of pregnancy. However, it is yet to be investigated whether the increased blastocyst expansion following hCG treatment leads to increased

conceptus growth and improved placentation (Santos *et al.*, 2001).

Thus, it can be deduced that hCG administration on day 12 post AI significantly improved pregnancy rates in dairy cows. Also, progesterone concentrations in hCG administered dairy cows was also higher and it could have favored embryonic development which increased embryonic competence to establish the maternal pregnancy recognition.

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