STUDY ON THE EFFECT OF HUMAN CHORIONIC GONADOTROPIN ADMINISTRATION DURING LUTEAL PHASE ON FERTILITY IN DAIRY COWS

THESIS

By

TARUN THAKUR
(V-2008-30-14)

Submitted to

**CHAUDHARY SARWAN KUMAR
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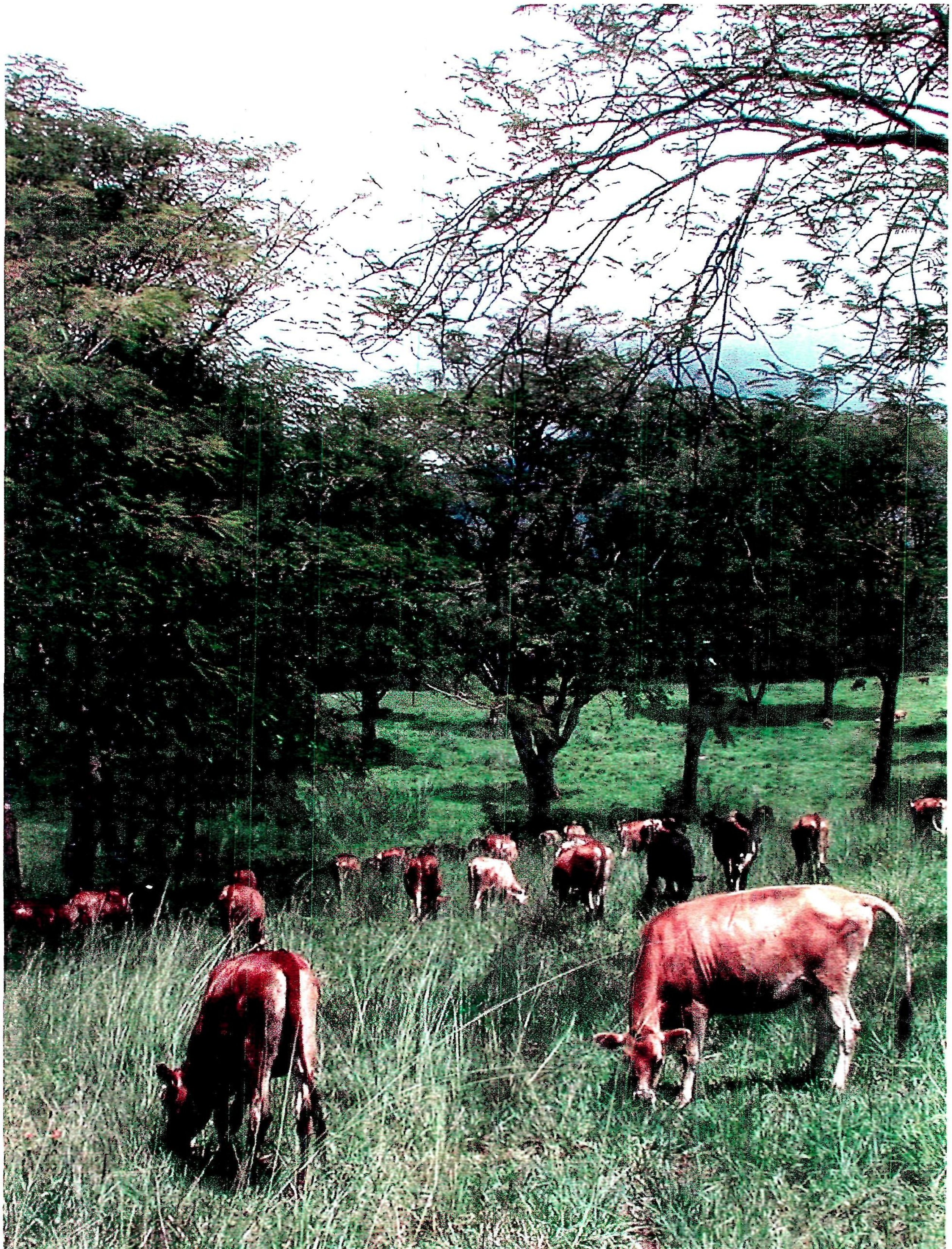

Dedicated to my

Parents

Who introduced me to the joy of reading from birth

and enabling such a study to take place today

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CERTIFICATE I

This is to certify that the thesis entitled **"Study on the effect of human chorionic gonadotropin administration during luteal phase on fertility in dairy cows"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the discipline of **Animal Reproduction, Gynaecology and Obstetrics** of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur is a *bonafide* research work carried out by **Dr. Tarun Thakur** (Admission No. V-2008-30-14) son of Sh. Roshan Lal Thakur under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

(Madhumeet Singh)
Major Advisor

Place: Palampur
Dated: 28.06.10

CERTIFICATE II

This is to certify that the thesis entitled **"Study on the effect of human chorionic gonadotropin administration during luteal phase on fertility in dairy cows"** submitted by **Dr. Tarun Thakur** (Admission No. V-2008-30-14) son of Sh. Roshan Lal Thakur to the CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science** in the subject of **Animal Reproduction, Gynaecology and Obstetrics** has been approved by the Advisory committee after an oral examination of the student in collaboration with an External Examiner.

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Place: Palampur
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Tarun Thakur

CONTENTS

LIST OF TABLES

LIST OF FIGURES

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ABSTRACT

The work was conducted at the university livestock farm and Veterinary clinical complex of College of Veterinary and Animal Sciences Palampur and in field veterinary institutions. In all, 278 normal cows were inseminated during this study. These cows were divided in three treatment and one control group. Depending upon different treatments groups, human chorionic gonadotrophin (Chorulon Intervet Schering Plough India Ltd.) was injected at the dose of 1500 IU. on different days (0, 5 or 12) of estrous cycle in these cows.

Blood was collected from 26 cows. This included 18 animals treated with hCG either on Day 0 (n=6) along with AI or on Day 5 (n=6) or Day 12 (n=6) post insemination and also from control group cows (n=8). In all the animals four blood samples were collected on the day 0 (day of insemination) and on days 7, 14 and 21 (post insemination) of estrous cycle.

hCG administration (along with AI or during luteal phase) improved conception in treated than control animals. Highest conception rates were recorded when hCG was administered on day 12 post AI. Consistently higher mean plasma progesterone concentration was recorded during luteal phase in hCG treated cows. The mean plasma progesterone values were highest in cows receiving hCG on day 12 post-AI.

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INTRODUCTION

CHAPTER 1

INTRODUCTION

Reproductive efficiency in dairy cows has declined over the last several years and is considerably lower than desired (Washburn *et al.* 2002; de Vries and Risco 2005). Decreased reproductive efficiency can be attributed to many factors including inefficiency and inaccuracy of estrus detection, improper timing of insemination, delayed ovulation and anovulation, negative energy balance and nutrition, selection for higher milk production and inbreeding (Hermas *et al.* 1987; Nebel and Jobst 1998; Butler 2000; Lucy 2001).

Another factor contributing to low pregnancy rates is embryonic loss (Lamming *et al.* 1989; Chebel *et al.* 2004; Santos *et al.* 2004; Diskin and Morris 2008). Although fertilization rates in cattle are reported to be greater than 90% (Diskin and Sreenam 1980), the majority of embryonic mortality (70-80% of total loss) occurs between days 8 and 16 after insemination (Dunne *et al.* 2000; Santos *et al.* 2004).

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. Regardless of the cause of low progesterone during early embryonic development, insufficient luteal activity has been associated with infertility in cattle as suboptimal progesterone level may contribute to low fertility in dairy cows by disturbing embryonic development and maternal recognition of pregnancy.

Luteal deficiency during the first three weeks of gestation has been hypothesized as a cause of pregnancy failure (Henricks *et al.* 1970; Butler *et al.* 1996; Mann and Lamming 2001; Santos *et al.* 2004). Many factors including nutrition (Gombe and Hansel 1973), heat stress (Wilson *et al.* 1998; Wolfensen *et al.* 2002), genetics (Lucy 2001), and rate of steroid metabolism in high producing cows (Wiltbank *et al.* 2000; Sartori *et al.* 2002;

Progesterone concentrations have been implicated in embryonic deaths before Day 6 post-mating, Day 4 through 9 post-mating, Day 14 through 17 during the maternal recognition of pregnancy and Day 28 through 42 while placentation and attachment are in progress (Inskeep 2004).

A wide array of the research aimed at reducing pregnancy losses has focused on providing a supplemental, exogenous source of progesterone or increasing endogenous concentrations of progesterone by inducing formation of accessory CL or enhancing the endogenous function of the existing CL. Several researchers (Lopez-Gatius *et al.* 2004; Stevenson *et al.* 2007) have attempted to reduce pregnancy loss by administering exogenous progesterone at various stages post-insemination. Others (Sterry *et al.* 2006; Bridges *et al.* 2000) have relied on induction of ancillary CL to increase endogenous concentrations of progesterone.

The increment in fertility can be achieved by increasing the fertilization rate or by reducing pregnancy losses after fertilization. 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 of embryo and conceptus survival. 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. Simultaneously, numerous researchers (Donaldson and Hansel 1965; Hansel and Seifart 1967; Moody and Hansel 1971) have reported that hCG administration during the luteal phase of cattle increased the size and weight of the already existing CL as well as serum concentrations of progesterone.

The present experiment was planned with the hypothesis that the treatment with hCG in early luteal phase induces ovulation of the dominant follicle of first follicular wave and helps formation of accessory CL, thereby increasing the concentration of progesterone during the luteal phase. The use of hCG injection at mid luteal stage after AI may enhance embryo survival rates by delaying the luteolytic mechanism that may occur due to failed maternal recognition of pregnancy.

The objectives of present study, therefore, were;

a. To evaluate the effect of post insemination administration of human chorionic gonadotropin during luteal phase on conception rate in dairy cows.
b. To compare the effect of hCG administration in early or mid luteal stages post insemination in dairy cows
c. To recommend a suitable day for hCG administration to enhance conception in cows.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Human chorionic gonadotropin (hCG) is synthesized by the developing embryo from 2 to 8 days after fertilization and is mainly a product of placental syncytiotrophoblast cells (de Medeiros and Norman 2009). hCG is a member of the glycoprotein hormone family (Stenman *et al.* 2006). Based on the high homology between hCG and LH b-subunits (80%), the applications of hCG were extended to induce ovulation. The half-life of hCG after treatment is biphasic, with a rapid phase of 5 to 9 hours and there after a slower phase lasting some 24 to 33 hours (Rizkallah *et al.* 1969; Stenman *et al.* 2006). Intramuscular administration of hCG results in a longer half-life than intravenous route (Rizkallah *et al.* 1969). hCG is used in domestic animals because its LH-like activity lasts longer than the activity of LH. 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.* 1996a). However, the repeated use of hCG in cattle induces an antibody response that can neutralize the hCG molecule and dramatically reduce binding to its receptor (Sundby and Torjesen 1978). In the cat, it has been observed that there is a large variability in immune response to repeated hCG administrations, and an interval of at least 4 months between two successive hCG treatments is recommended (Sanson *et al.* 1995).

The efficacy of hCG treatment has been studied in terms of it's role in inducing ovulation, its luteotropic effects, preventing embryonic mortality, its inclusion in estrus synchronization protocols and combating the effects of heat stress and cystic ovarian disease.

2.1 Administration of hCG along with insemination

It is suggested that in cattle, hCG is effective in increasing pregnancy rate when used at the time of AI. Significant improvement in conception rates had been reported when used along with AI (Brown *et al.* 1973; Wagner *et al.* 1973; Babler and Hoffman 1974; Breuel *et al.* 1989; Srivastava and Ahlawat 1998; Ingawale *et al.* 2002; Patel *et al.* 2005; Selvaraju *et al.* 2009). However, others (Hansel *et al.* 1960; Hansel *et al.* 1976; Echternkamp and Maurer

1983; Paksoy and Kalkan 2010) reported no significant improvement in conception rates following hCG administration.

Table R1: Effect of hCG administration along with AI on conception rate in cows.

Sr. No.	Author(s) and Year	Preparation / Dose	Conception Rate (%)	Remarks
1	Knoblauch 1975	500 IU of Gonabion (hCG)	-	19.6% higher conception rate than control
2	Hansel *et al.* 1976	hCG 1500 IU in 5% beeswax.	Dairy cows: Treatment (T) - 44.8 Control (C) - 52.8 Beef cows: T-54.4, C-54.5	-
3	Morris *et al.* 1976	1500 IU in 5% beewax and 95% sesame oil	-	Pregnancy percentage similar as control
4	Kudlac *et al.* 1982	1500 IU (Praedyn spofa) 3000 IU	T-54.83 C-51.54 T-66.00 C-53.98	Difference % is +3.29 +12.02
5	Sianangama and Rajamahendran 1992	hCG	T-47 C-40	Improved pregnancy rates
6	Lopez Gatius 2000	250 IU hCG	T-39 C-36 T-54 C-48	Synchronize with 500 mcg cloprostenol and 1mg estradiol benzoate 12 h later
7	Patel *et al.* 2005	1500 IU hCG (Chorulon)	T - 66.66 C - 33.33	Improved pregnancy rates
8	De Rensis *et al.* 2008	3300 IU hCG	T- 63 (hCG) C-36 (GnRH)	Cumulative pregnancy rate higher. Improved fertility in dairy cows in warmer season.
9	Selvaraju *et al.* 2009	1500 IU hCG	T-62.50 C-18.75	hCG in combination with PGF_2 alpha at AI improved conception rate.
10	Paksoy and Kalkan 2010	1500 IU hCG (Pregnyl)	T-46.7 C-40.0	-

The luteinizing activity of hCG in ovarian cells has long been recognized in several mammalian species. In cattle, treatment with hCG has a potent LH-like effect. It extends the life span of the corpus luteum (CL) increasing endogenous progesterone synthesis (Sianangama and Rajamahendran 1992), induces ovulation throughout the estrous cycle (Price and Webb 1989; Santos *et al.* 2001), promotes the formation of accessory corpora lutea when applied in the early luteal phase (Rajamahendran and Sianangama 1992) and modifies follicular wave dynamics, increasing the frequency of three-wave dominant follicular cycles (Diaz *et al.* 1998).

By inducing ovulation of the dominant follicle, hCG stimulates the differentiation of theca and granulosa cells into small and large luteal cells and the transformation of small into large luteal cells (Farin *et al.* 1988; Sianangama and Rajamahendran 1992). This in turn leads to an increase in the steroidogenic capacity of the primary CL (Veenhuizen *et al.* 1972; Breuel *et al.* 1990; Schmitt *et al.* 1996c; Diaz *et al.* 1998). Follicles can also become accessory corpora lutea after hCG treatment. Luteinization of follicles by hCG was recorded in 70% beef cows in which the primary CL was removed on day 28 of gestation (Bridges *et al.* 2000) and in more than 70% of dairy cows receiving hCG between day 4 and 9 after artificial insemination (Santos *et al.* 2001; Stevenson *et al.* 2007). 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). In effect, even follicles smaller than 10 mm in diameter were able to form luteal tissue in response to hCG (Sheffel *et al.* 1982). 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 (Helmer and Britt 1986; Price and Webb 1989).

hCG induced three-wave follicular cycles could result in a higher conception rate as some authors have reported a higher conception rate in inseminated cows with three follicular waves compared to cows with two follicular waves (Ahmad *et al.* 1997). The occurrence of three dominant follicular waves could also explain the slightly lengthened estrous cycles after hCG treatment (Rajamahendran and Sianangama 1992). However, cycle length was not affected by treatment in other studies (Veenhuize *et al.* 1972; Seguin *et al.* 1977; Sianangama and Rajamahendran 1996; De Rensis *et al.* 2008) and there have even been descriptions of a high incidence (23%) of short estrous cycles in cows (Geary *et al.* 2001) and heifers (16%) after hCG treatment (Schmitt *et al.* 1996c). These differences between studies could be mainly due to the stage of the estrous cycle at which hCG was administered. In effect, there was an increased estrous cycle length after hCG administration on days 10 or 15, but not on day 17 of the estrous cycle (Seguin *et al.* 1977).

The effects of hCG at breeding on conception rates are variable. hCG administration at breeding or immediately around breeding increases conception rates in beef heifers (Brown *et al.* 1973; Wagner *et al.* 1973) and in dairy cows (Rajamahendran and Sianangama 1992). However, in another study in lactating cows, this effect was not observed (Hansel *et al.* 1976). These differences could be related to the period of the year in which the treatment was performed. In fact, De Rensis *et al.* (2008) observed a positive effect of hCG after synchronization in dairy cows only during the warm but not the cold period of the year.

One of the more utilized protocols to induce ovulation for timed AI is the sequential administration of GnRH, prostaglandin $F_2\alpha$ ($PGF_2\alpha$), and GnRH (GPG or Ovsynch protocol: GnRH on day 0, PGF on day 7, GnRH on day 9) followed by timed AI (Pursley *et al.* 1995; Nebel *et al.* 1998). In this protocol, the second dose of GnRH is designed to synchronize and induce ovulation but does not always achieve sufficient gonadotrophic stimulation of the preprogrammed follicle, and the effectiveness of the protocol in some animals is compromised (Vasconcelos *et al.* 1999; Moreira *et al.* 2000). Applying hCG instead of GnRH as the second gonadotrophic dose in the GPG protocol (GPH: GnRH on day 0, PGF on day 7, hCG on day 9) led to elevated plasma progesterone levels on days 3, 6, and 9 post-AI, reduced the calving-conception interval, and gave rise to similar conception rates as those recorded in cows receiving GPG (De Rensis *et al.*1999; De Rensis *et al.* 2002; De Rensis *et al.* 2008).

The first reports of decreasing fertility in the past century started approximately 60 to 70 years ago (Erb *et al.* 1940; Seath *et al.* 1941). Heat stress can disrupt both the physiology and productive performance of dairy cows. The most significant consequence of heat stress in cows is reduced fertility (Wolfenson *et al.* 1995; De la Sota *et al.* 1998) due to detrimental effects on follicle development and hormone profiles viz. suppressed progesterone production (Hansen *et al.* 1999).

A few research studies have investigated the actions of hCG in heat stressed dairy cows. De Rensis *et al.* (2008) demonstrated that cows treated with hCG within a GPG protocol (GPH) had a higher cumulative pregnancy rate than cows treated with GPG during the warm period, suggesting that hCG can to some extent counteract the negative effects of heat stress on fertility. The effectiveness of hCG administration at AI may be related to better synchronization between ovulation and AI in cows under heat stress. A delayed interval between ovulation and insemination is a main factor affecting fertility during the warm season (De la Sota *et al.* 1998). In another study, hCG applied during the warm season did not improve pregnancy rates to first service but reduced the calving to conception interval (Moreira *et al.* 2000). hCG tested 5 days post AI during the warm season also did not affect the pregnancy rate (Schmitt *et al.* 1996b; Hansen *et al.* 1999). Probably, as noted above, different forms of stress after conception can mask the positive effects of hCG treatments subsequent to AI.

2.2 Administration of hCG post insemination in luteal phase

In cyclic cows, several studies have achieved improved conception rates using hCG on days 5 to 7 post-AI (Breuel *et al.* 1989; Sianangama and Rajamahendran 1992; Rajamahendran and Sianangama 1992; Santos *et al.* 2001; Ideta *et al.* 2003). Conversely, although hCG treatment induced the development of accessory corpora lutea and increased plasma progesterone levels for 4 weeks, it did not reduce embryonic losses compared to GnRH-treated or control cows (Stevenson *et al.* 2008). The latter observation is consistent with previous reports where no difference in conception rates were recorded between control and treated cows receiving hCG either during the luteal phase of the cycle (Pratt *et al.* 1982; Eduvei and Seguin 1982; Helmer and Britt 1986; Schmitt *et al.* 1996b) or at AI (Hansel *et al.* 1976).

Table R2: Effect of hCG administration in luteal phase on conception rate in cows.

Sr. No.	Author(s) and Year	Preparation /Dose	Day of Administration Post AI	Conception Rate (%)
1	Eduvie and Seguin 1982	10000 IU hCG	Day 10,11,12	T-59, C-63 (Inter-estrous interval prolonged approximately five days in non pregnant cows)
2	Helmer and Britt 1986	1000hCG , 5000hCG	Day 2,3,4	11% increase in first service conception rate.
3	Price and Webb 1989	1500 IU hCG (Chorulon)	Between day 0 to day 16	Large, luteal phase follicle of cow is capable of ovulating in response to hCG and no significant difference between left-right orientation of existing and hCG induced CL.
4	Walton *et al.* 1990	1500 IU hCG (Progon)	Day 5	Normal cows: T-57.1, C-57.1 Repeat breeder T-47.2, C-39.5
5	Lewis *et al.* 1990	3500 IU hCG (Lyphomed)	Day 15	First service C-41.1, T-50 2nd service C-47.2, T-46.0 3rd service T-46.2 C-44.8 Overall T-48.5, C-45
6	Terma *et al.* 1990	3000 IU hCG	Day 4	Did not affect the first service pregnancy rate
7	Sianangama and Rajamahendran 1992	hCG	Day 7	T-62, C-40
			Day 14	T-55, C-40
8	Nephew *et al.* 1994	100 IU hCG	Day 11	T-94%, C-83.5 (ewes)
9	Sianangama and Rajamahendran 1996	1000 IU hCG	Day 7	Alteration in follicular dynamics, but this did not affect cycle length
10	Ishida *et al.*1999	500 IU hCG	Day 6, 9	Pregnancy and lambing rates and prolificacy were not significantly different in groups
11	Shabannkareh *et al.* 2009	3000 IU hCG	Day 5	T-37.9, C-23.6 Pregnancy rates are higher in warmer season

12	Santos *et al.* 2001	3300 IU hCG	Day 5	T-45.8, C-38.7 (28 day) T-40.4, C-36.3 (45 day) T-38.4, C-31.9 (90 day)
13	Funston *et al.* 2003	3300 IU hCG (Chorulon)	Day 5	T-61, C-50
14	Hanlon *et al.* 2005	1500 IU hCG (Chorulon)	Day 5	T-43.6, C-46.3
15	Walker *et al.* 2005	1500 IU hCG (Chorulon)	Day 5	T-67, C-65 (first service CR)
16	Kalaimani *et al.* 2007	1500 IU hCG (Chorulon)	Day 7	T-30, C-30 (Faliure to improve conception rate)
17	Esfandabadi *et al.* 2007	3000 IU hCG (Chorulon)	Day 5	T-27.14, C-35.22
18	Khan *et al.* 2007	200 IU hCG (Chorulon)	Day 5	Increased ovarian function, conceptus growth and improved implantation in ewes, prolonged luteotropic and embryotrophic effect
19	Larson *et al.* 2007	1000 IU hCG (Chorulon)	14 days prior to synchronization	hCG did not alter conception rates.
20	Stevenson *et al.* 2007	3300 IU hCG (Chorulon)	Between day 4 to day 9	T-37.8 C-26.9
21	Stevenson *et al.* 2008	1000 IU hCG (Gonadostimulon)	Between day 26 to 71 of pregnancy	No effect on CR
22	Beltran and Vasconcelos 2008	2500 IU hCG (Vetecor)	Day 5	T-32.8, C-10.1 (temp.below 39.7 degree) T-24.4, C-15.2 (temp.above 39.7 degree)
23	Machado *et al.* 2008	3000 IU hCG (Vetecor)	Day 5	Enhanced luteal function and reprogrammed follicular development
24	Tenhagen *et al.* 2010	2500 IU hCG	Day 4	T-35.4, C-35 hCG in combination with PGF_2 alpha improved conception rate.
25	Selvaraju *et al.* 2009	1500 IU hCG	Day 4	T-37.5, C-18.75
26	Buttery *et al.* 2010	1000 IU hCG (Chorulon)	Between day 22 to day 35	T-6.6%, C-3.6% (pregnancy losses higher in hCG group)
27	Paksoy and Kalkan 2010	1500 IU hCG (Pregnyl)	Day 12	T-46.7, C-40
28	Kaim *et al.* 2010	2500 IU hCG (Pregnyl)	Day 6	T-43.6 C-36.6(N.S)

On the other hand, several studies have shown that cows with lower progesterone concentrations during diestrus subsequent to AI had lower conception rates (Lukaszewska and Hansel 1980; Mann and Lamming 1999).

Poorly developed embryos (16 days old) and embryonic losses are associated with a delayed increase in systemic progesterone concentration as well as with lower progesterone concentrations during days 7 to 16 post-AI (Mann *et al.* 1996). Administration of hCG between day 5 and 14 post-AI increases conception and embryo survival rates (Breuel *et al.* 1990; Sianangama and Rajamahendran 1992; Kerbler *et al.* 1997; Santos *et al.* 2001).

A further study on embryo transfer recipients reported a higher pregnancy rate in recipients treated with hCG on day 6 (67.5%) than in control cows (45.0%) or cows receiving hCG on day 1 (42.5%) after estrus (Nishigai *et al.* 2002). This reinforces the idea that induction of an accessory CL and increased progesterone concentrations reduces early embryonic mortality in cattle. Differences among studies concerning the utility of hCG to improve conception and embryo survival rates could be related to the fact that hCG is effective at reducing embryo loss only during early pregnancy. Thus, in a study in which conception rates on days 28, 42, and 90 post-AI were increased by hCG administration, the authors noted that late embryo losses were not improved (Thatcher *et al.* 2006).

2.3. Effect of hCG administration on progesterone concentration in cows

Progesterone is essential for the maintenance of pregnancy and embryo development (McDonald *et al.* 1952). Progesterone is produced from the corpus luteum and is indicative of its function. Its levels are high in progesterone dominance phase of estrous cycle and also in pregnant animals in comparison to the non-pregnant animals. The progesterone levels are significantly higher at mid-luteal phase (Kimura *et al.* 1987). The maintenance of pregnancy in ruminants depends upon the continued secretion of luteal progesterone resulting from the prevention of luteolysis.

In cyclic ruminants luteolysis is caused by the release of luteolytic episodes of $PGF_2\alpha$ in response to the binding of ovarian oxytocin to newly developed oxytocin receptors on the endometrium, where as in pregnant animals the development of these receptors is inhibited by the production of trophoblastic interferon by the embryo (Flint *et al.* 1992). Any factor that influences the development of the luteolytic mechanism in the mother or interferon production by the embryo may affect the outcome of pregnancy. Ovarian hormones, progesterone and oestradiol, influence the development of luteolytic mechanism (Lau *et al.* 1992; Beard *et al.* 1994) and the production of endometrial secretions necessary for embryo development (Geisert *et al.* 1992).

In inseminated cows progesterone levels were lower in animals subsequently found not to be pregnant than in those animals in which pregnancy was successfully maintained (Lukaszewska and Hansel 1980; Lamming *et al.* 1989). However, some workers have suggested higher progesterone concentrations in non-pregnant than pregnant cows due to incomplete luteolysis or premature ovulation (Selvaraj and Kumar 2001).

Cows with elevated concentrations of progesterone earlier in the estrous cycle had embryos that were more advanced developmentally. These embryos had greater capacity to produce interferon-τ which is capable of inhibiting the $PGF_2\alpha$ release 16 days after breeding. Cows that had a delayed rise in progesterone had less developed embryos, less capacity to produce interferon-τ, and were not capable of inhibiting the $PGF_2\alpha$ release on day 16 (Kerbler *et al.* 1997; Mann *et al.* 1999; Mann and Lamming 2001).

Therefore, several strategies have been used to increase progesterone concentration after breeding (Binelli *et al.* 2001). Treatment with hCG during the periovulatory period enhances the subsequent luteal activity of the primary CL. Given at the time of AI, hCG increases plasma progesterone levels from day 5 to 12 of the estrous cycle in cows with no accessory CL (Helmer and Britt 1986; Geary *et al.* 2001; Machado *et al.* 2008; De Rensis *et al.* 2008). Extended binding of hCG to the luteal cell membrane could explain the enhanced stimulatory effects of hCG on plasma progesterone concentrations during the luteal phase.

Table R3: Effect of hCG administration in luteal phase on progesterone concentration in cows.

Sr. No.	Authors and Year	GnRH used / Dose	Days/Time of administration	Days of Blood collection	Mean(±SE) Progesterone Concentration (ng/ml)
1	Morris *et al.*1976	1500 IU in 5% beewax and 95% sesame oil	Day 0, simultaneous to AI	Day 9 & day 16	Serum progesterone level higher in hCG group than control heifers.
2	Helmer and Britt 1986	1000 IU hCG, 5000 IU hCG.	Day 2, 3 and 4 post AI	-	Progesterone concentration during mid-cycle higher in hCG treated heifers than control
3	Price and Webb 1989	1500 IU hCG (Chorulon)	-	-	Large and luteal phase follicle is capable of ovulating in response to hCG. No significant difference between left-right orientation of existing CL and hCG induced CL.
4	Walton *et al.* 1990	1500 IU hCG (Progon)	Day 5 post AI	Between day 8 and day 20	Sustained increase in plasma progesterone concentration.
				Day 14	T-4.3±0.46 C-3.2±0.30 (Milk progesterone)
				Day 20	T-4.1±0.60 C-2.9±0.30 (Milk progesterone)
5	Lewis *et al.* 1990	3500 IU hCG	Day 15 post AI	-	Increased concentration of progesterone
6	Sianangama and Rajamahendran 1992	hCG	Day 0 simultaneous to AI and Days 7 and14 post AI	-	Significant increase in progesterone concentration observed in day 7 & day 14 treated cows from days 18 to 42 after breeding compared with the day 0 or control cows
7	Nephew *et al.* 1994	100 IU hCG	Day 11 post AI	Day 11	T-2.3,C-2.2
				Day 12	T-3.1,C-2.1
				Day 13	T-3.6,C-2.4

8	Sianangama and Rajamahendran 1996	1000 IU hCG	Day 7 post AI	-	Cows with 6 accessory C.L. and altered follicular dynamics in treatment group. Mean conc. of progesterone was similar in both groups until day 16, but thereafter a rapid decline in progesterone in hCG treated group.
9	Kesler and Favero 1996	hCG	Day 0(12 hour after estrus)	-	Sustained-release formulation doubled the ovulation rate, increased corpora lutea size, increased the incidence of cystic corpora lutea, and tended to increase progesterone concentrations
10	Schmitt *et al.* 1996a	2000 IU hCG	Day 5 post AI	-	Higher level of progesterone from day 6 to day 13 of estrous cycle. It is due to greater response of hCG induced C.L.
11	Ishida *et al.* 1999	500 IU hCG	Mid luteal phase(day 6 or day 9)	-	On day 12 and 15 plasma p4 level higher in hCG treated group than control.
12	Santos *et al.* 2001	3300 IU hCG	Day 5 post AI	-	Plasma progesterone concentration were increased by 5.0 ng/ml.
13	Funston *et al.* 2003	3300 IU hCG (Chorulon)	Day 5 post AI	Day 14	T-8.6, C-4.6
				Day 17	T-11.2, C-5.2
14	Hanlon *et al.* 2005	1500 IU hCG (Chorulon)	Day 5 post AI	Day 12	T-6.2±2.7, C-4.9±1.3
15	Patel *et al.* 2005	1500 IU hCG (Chorulon)	Day 0	0 to 7th week post AI	T-5.19±0.52 C-4.00±0.23
16	Walker *et al.* 2005	1500 IU hCG (Chorulon)	Day 5 post AI	Day 4	T-0.5, C-0.3
				Day 5	T-1.1, C-0.9
				Day 6	T-1.5, C-1.0
				Day 7	T-2.4, C-1.4
				Day8	T-2.6, C-1.4

17	Kalaimani *et al.* 2007	1500 IU hCG (Chorulon)	Day 7 post AI	Day 0	T-0.57±0.02, C-0.43±0.04
				Day 7	T-4.51±0.39 C-3.16±0.11
				Day 14	T-9.91±0.82 C-4.73±0.64
18	Esfandabadi *et al.* 2007	3000 IU hCG (Chorulon)	Day 5 post AI	Day 5	T-2.94±0.65 C-2.98±0.36
				Day 12	T-16.07±3.64 C-8.71±0.86
19	Khan *et al.* 2007	200 IU hCG (Chorulon)	Day 12 post AI	-	hCG prolonged luteotrophic effect and increased plasma progesterone concentration in ewes.
20	Stevenson *et al.* 2007	3300 IU hCG (Chorulon)	Between day 4 to 9 post AI	-	More induced CL. Serum progesterone increased in response to hCG.
21	Larson *et al.* 2007	1000 IU hCG (Chorulon)	Pre-synchronized the estrous cycle with hCG.	-	Concentration of progesterone greater on day 0 and 2 for heifers treated with hCG and those heifers had more CL on ovaries on day 0.
22	Kharche *et al.* 2008	250 IU hCG (Chorulon)	Day 9 post AI	-	Increased number of CL and significant increase in plasma progesterone concentration
23	Stevenson *et al.* 2008	1000 IU hCG (Gonadostim ulon)	Between day 26 to 71.	-	Serum progesterone did not differ among treatments, but among females forming new luteal structures, progesterone was greater at 1 (7.2±0.3 vs. 6.3 ±0.2 ng/mL) and 2 wk (7.0 ±0.3 vs. 6.1 ±0.2 ng/mL) after treatment.
24	Beltran and Vasconcelos 2008	2500 IU hCG (Vetecor)	Day 5 post AI	Day 5	T-3.2±0.5, C-2.7±0.4
				Day 7	T-5.7±0.5, C-4.8±0.4
				Day 12	T-8.5±0.5, C-5.2±0.4
25	Machado *et al.* 2008	3000 IU hCG (Vetecor)	Day 5 post AI	-	Enhanced luteal function by inducing accessory CL, increased CL volume and plasma progesterone conc.

26	De Rensis *et al.* 2008	3300 IU hCG	Day 0 post AI	-	Elevated plasma progesterone levels were observed on day 3, 6 and 9 after time fixed AI.
27	Selvaraju *et al.* 2009	1500 IU hCG	Day 0	-	Higher conc. of progesterone observed on day of estrus induction in pregnant (7.19±0.46) compared to non pregnant cows (5.24±0.43 ng/ml).
28	Buttery *et al.* 2010	1000 IU hCG (Chorulon)	Between day22 to 35 post AI	-	T-6.1±0.4, C-5.4±0.4
29	Paksoy and Kalkan 2010	1500 IU hCG (Pregnyl)	Day 0 and day 15	Day 0	T-0.55±0.12 C-0.42±0.16
				Day 5	T-5.56±0.88 C-6.68±2.72
				Day 10	T-7.27±1.70 C-11.50±3.27
				Day 15	T-9.76±2.40 C-15.12±4.05
				Day 21	T-10.53±4.52 C-22.48±4.94
30	Kaim *et al.* 2010	2500 IU hCG (Pregnyl)	Day 6 post AI	-	T-4.8, C-3.7 (mid-luteal phase)

On day 5, the post-ovulatory CL is capable of responding to the steroidogenic stimulus provided by hCG (Bennett *et al.*1989; Fricke *et al.* 1993; Schmitt *et al.* 1996a). Thus, the luteotropic effects of hCG treatment on primary CL have been attributed to an increased size of luteal cells (Schmitt *et al.* 1996a), an increased number of large luteal cells concomitant with a reduction in the number of small luteal cells (Helmer and Britt 1987; Breuel *et al.* 1989; Sianangama and Rajamahendran 1992; Fricke *et al.* 1993; Schmitt *et al.* 1996b; Diaz *et al.* 1998) and to an increased surface area, volume, and diameter of the CL (Rajamahendran and Sianangama 1992; Sianangama and Rajamahendran 1992; Santos *et al.* 2001; Stevenson *et al.* 2007). The luteotropic activity of hCG has also been attributed to the newly formed luteal tissue (Bennett *et al.* 1989; Fricke *et al.* 1993; Schmitt *et al.* 1996a; Kerbler *et al.* 1997).

A significant increase in progesterone concentration following hCG treatment was also reported in other studies (Rajamahendran and Calder

1993; Kharche *et al.* 2001; Hanlon *et al.* 2005). The increased progesterone concentration might have resulted from hypertrophy of luteal cell in spontaneous CL (Helmer and Britt 1986). Luteinization of granulosa cells of unovulated follicles may also contribute towards increase in progesterone concentration after hCG treatment (Booth *et al.* 1975; Opavsky and Armstrong 1989). The progesterone concentration increases after superovulatory estrus until the day of flushing due to the formation of multiple CL (Booth *et al.* 1975; Saumande 1980; Rajamahendran and Calder 1993; Kharche *et al.* 2001).

The increase in plasma progesterone concentrations after GnRH or hCG treatment on day 12 of pregnancy suggests that GnRH through LH release and hCG by its LH like activity may provide luteotrophic stimulation to CL, which could explain the increase in CL weight observed in the ewes given these treatments. This luteotrophic stimulation may either be in the form of conversion of small luteal cells to large luteal cells which then secrete higher concentrations of progesterone (Farin *et al.* 1988) or may even be due to an increase in the size of large luteal cells (Fitz *et al.* 1982). Whatever the mechanism of higher progesterone secretion from the CL after hCG or GnRH treatment, this additional luteotrophic support at this stage of pregnancy seems to be beneficial for improving fertility by decreasing embryo mortality.

In conclusion, hCG can increase plasma progesterone levels through two possible mechanisms; a direct effect on the primary CL and/or by developing accessory corpora lutea. Treatment with hCG at AI increases plasma progesterone levels (Helmer and Britt 1986; Breuel *et al.* 1989; Schmitt *et al.* 1996a; Diaz *et al.* 1998; Santos *et al.* 2001; De Rensis *et al.* 2008) with a greater area under the progesterone curve recorded 2 to 3 weeks after treatment when compared with that of control cows (Santos *et al.* 2001; Stevenson *et al.* 2007; Machado *et al.* 2008).

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The work was conducted at the university livestock farm, CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur, Veterinary clinical complex of Dr.G.C.Negi college of Veterinary and Animal Sciences Palampur and in field veterinary institutions from February 2009 to March 2010. Animals were maintained under loose housing system and standard farm conditions at livestock farm where as in field conditions the animals were maintained under tied/conventional systems. Animals considered for clinical trials were in good health, normal cyclic with normal genitalia and no apparent clinical reproductive abnormalities based upon their history records and/or by rectal examinations. Inseminations were carried out in both heifers as well as pluriparous animals in different lactations.

3.1 Feeding and Management

The animals at the dairy farm were fed according to the normal feeding schedule. All the cows were supplied with adequate amount of green fodder depending upon the seasonal availability and concentrate ration containing 70-75% TDN, 16 -18% DCP and 3% mineral mixture. The concentrate mixture was fed according to milk production to individual animal at the time of milking. The animals were allowed to take water *ad-libidum*. All the animals were vaccinated and dewormed at the appropriate time as per the recommended schedule fixed at the farm. The animals in the field were provided seasonal feed and fodder and as per suitability and economic/social status of the farmer/owner. Vaccinations and deworming under field conditions were done as per the protocol followed by the state veterinary health services.

3.2 Detection of estrus

The animals were observed for signs of estrus on the basis of behavioral symptoms *viz.* bellowing, restlessness, mounting, standing to be mounted, vulvar oedema and mucus discharge. Cows with apparently normal cervico-vaginal mucus discharge, tone of the uterine horns and without any abnormality of genital tract were selected for the study following clinicogynaecological examination. All the animals were inseminated at standing heat with good quality frozen thawed semen procured from bulls maintained in Sperm Station, Palampur. These bulls were being used for the preparation of frozen semen straws for routine supply in the field veterinary institutions.

3.3 Treatment and insemination

In all, 278 normal cows were inseminated during this study. Detail of various treatment and insemination groups of normal cows has been shown in Table M1.

Table M1. Schematic distribution of normal cows in different insemination groups.

Dose	Day of Administration post-AI	Parity	Cows inseminated	Total
Human chorionic gonadotropin 1500 units (Chorulon 5 ml)	0	Heifer	31	67
		Pluriparous	36	
	5	Heifer	18	65
		Pluriparous	47	
	12	Heifer	12	55
		Pluriparous	43	
Control		Heifer	23	91
		Pluriparous	68	

These cows were divided in three treatment and one control groups. Depending upon different treatments groups, human chorionic gonadotrophin (Chorulon, Intervet Schering Plough India Ltd.) was injected intra-muscularly (i/m) at the dose of 1500 IU on different days (0, 5 or 12) of estrous cycle in the cows. Day of detection of estrus was considered as day 0.

The animal included in treatment group of human chorionic gonadotrophin administration at the dose of 1500 IU. (n=187) were also sub-divided into three groups viz. the administration of hormone simultaneous to AI (day 0, group1), insemination and exogenous administration of hCG on 5^{th} day post AI (group 2) and insemination and administration of hormone on 12^{h} day post AI (group 3). A total of 67, 65 and 55 cows were inseminated in groups 1, 2 and 3 respectively.

Another 91 cows presented for insemination were selected as control (group 4) and insemination was done in standing heat without any treatment in these animals.

3.4 Pregnancy diagnosis

Pregnancy diagnosis was carried out 60 days post AI by rectal palpation method in cows not returning to estrus within this duration.

3.5 Blood Collection for Progesterone Estimation:

For plasma progesterone estimation, blood was collected from 26 cows.This included 18 animals treated with 1500 IU (5ml)) human chorionic gonadotrophin (Chorulon) either on Day 0 (n=6) along with AI or on Day 5 (n=6) or Day 12 (n=6) post insemination and also from control group cows (n=8).

In all the animals four blood samples were collected on the day 0 (day of insemination) and on days 7, 14 and 21 (post insemination) of estrous cycle. The blood was collected aseptically by jugular venipuncture. The site was prepared aseptically and blood was collected using the 18/20 gauge needle in 5 ml heparinised syringe. Prior to collection the needle was flushed by using heparin @ 0.1– 0.2 mg/ ml. After collection the plasma was separated by centrifugation of the blood sample @3000 r.p.m. for 10 min. and frozen at -20°C in 2 ml capacity micro-centrifuge tubes, pending analysis. Repeated thawing of plasma was strictly avoided.

3.6 Plasma progesterone analysis:

Plasma progesterone was estimated using Radioimmunoassay (RIA) procedure in the RIA laboratory located in the Department of Veterinary Gynaecology and Obstetrics, GADVASU, Ludhiana by an established modified liquid phase RIA procedure (Kamboj and Prakash 1993).

Procedure:

1. **Sample:** In the serially numbered glass test tubes (12X75mm), 100 µl (in duplicate) of plasma samples were pipetted.
2. **Standard:** Eight progesterone standards (0.0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 ng/ml) were used. Each standard was pipetted in the test tubes (in duplicate).

3. **Antiserum:** Polyclonal progesterone antiserum raised in the departmental RIA laboratory (Ghuman *et al.* 2009) was used at 1:1000 dilution. About 100 µl of antiserum was added to all the tubes except non-specific binding tube.
4. **Tracer:** Tritiated progesterone (Amersham Biosciences, UK) was used. About 100 µl of progesterone (8000CPM approx) was added to all the tube. After addition of antiserum and tracer, all the tubes were vortexed for about 15 seconds and thereafter incubated overnight at 4-5°C in refrigerator.

In addition to the samples and standard tubes, the following sets of tubes in duplicate were also run along with the assay;

A. Non-specific binding tubes (NSB) containing 200 µl of PBS gel and 100 ml tracer.
B. Maximum binding tubes (0 standard) containing 100 µl PBS gel, 100 µl antibody and 100 µl tracer.
C. Total count tubes (TC) containing 100 µl tracer.
D. Quality control tubes in each set containing same samples to measure the inter and intra-assay variation.

5. **Charcoaling:** Activated charcoal suspension (Charcoal 0.5 gm + Dextran T-70, 0.05 gm in 100 ml phosphate buffer saline gel, pH 7.0) was freshly prepared and continuously stirred in ice-cold water (4°C) for at least half an hour prior to addition into the incubated test tubes. The assay tubes were shifted in ice-cold water (4°C) and 500 µl activated charcoal under constant stirring was added to all tubes, vortexed and incubated for 10 minutes at 4°C. Thereafter, the tubes were centrifuged (3000 rpm for 10 mins) at 4°C in the refrigerated centrifuge. The supernatant containing the antisera bound hormone was decanted into scintillation vials.
6. **Scintillation fluid** (2,5-Diphenyloxazole (PPO) 10g + 2,2 paraphenylene-bis-5-phenyloxazole (POPOP) 0.25 g in 250 litre toulene): About 5 ml was added into all the scintillation vials, which were tightly capped and numbered for identification. The vials were left over night at room temperature to stabilize the radioactivity.

7. **Counting:** The radioactivity was determined as counts per minute (CPM) for one min in the Beckman 6500 multipurpose scintillation counter.
8. **Assay sensitivity:** The sensitivity of the assay 0.1 ng/ml for progesterone.
9. **Precision:** The mean intra- and inter-assay coefficients of variance were 6.0 and 9.4 percent, respectively.

3.7 Statistical analysis

Statistical analysis was carried out by chi square test and't' test as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

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 (Knoblauch 1975; Hansel *et al.* 1976; Patel *et al.* 2005; Selvaraju *et al.* 2009) or in early (Helmer and Britt 1986; Breuel *et al.*1989; Schmitt *et al.*1996c; Santos *et al.* 2001; Ideta *et al.* 2003) or mid luteal phases (Price and Webb 1989; Lewis *et al.* 1990; Paksoy and Kalkan 2010).

4.1 CONCEPTION RATES

4.1.1 Effect of administration of human chorionic gonadotropin (hCG) on different days of estrous cycle on conception in normal dairy cows.

Effect of administration of human chorionic gonadotropin (hCG) on different days of estrous cycle on conception in normal dairy cows has been shown in Table 1 and Figure 1.

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 (%)
Human Chorionic Gonadotropin 1500 units (Chorulon 5 ml) (n=187)	**0**	67	41	**61.19[ab]**
	5	65	41	**63.07[ab]**
	12	55	37	**67.27[b]**
Control (n=91)		91	45	**49.45[a]**

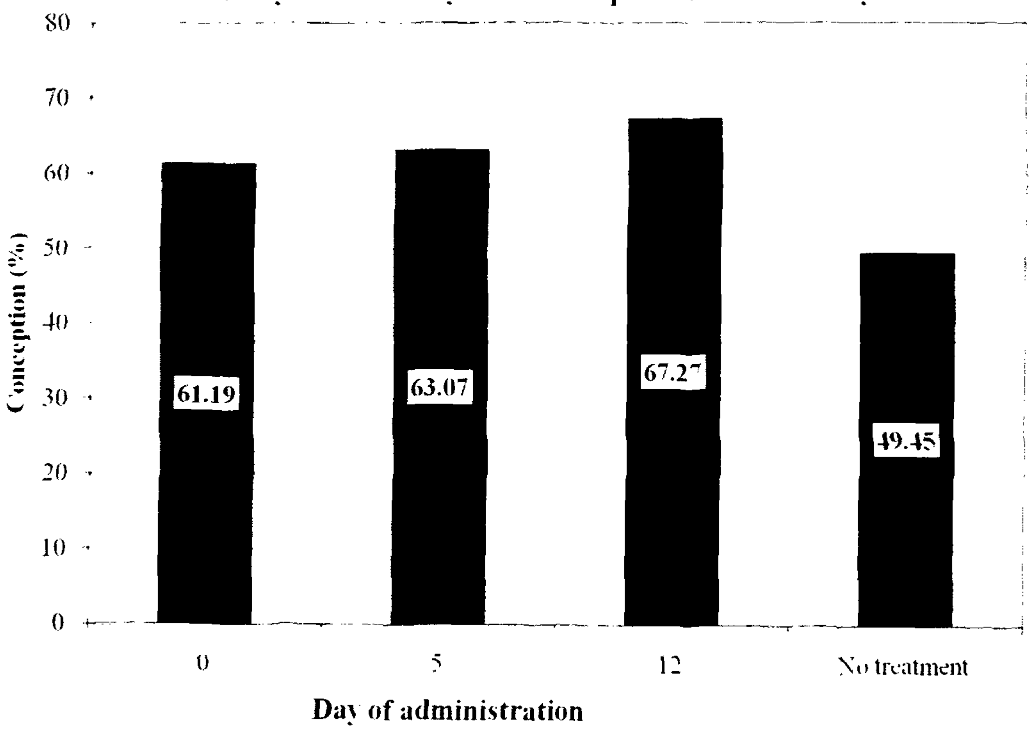
Figures with different superscripts differ significantly

Perusal of the table indicates that out of 67 cows inseminated along with hCG administered simultaneous to AI, 41 were found pregnant. The conception rate achieved was 61.19 per cent. Among 65 cows injected hCG fifth day post AI, 41 conceived with a CR of 63.07 per cent and out of 55 cows inseminated following administration of hCG 12th day post AI, 37 were pregnant. The conception rate was 67.27 per cent.

In the control group, 91 normal animals were inseminated without any treatment and 45 were found pregnant. Conception rate 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 animals (49.45%).

Figure 1: Effect of administration of Human Chorionic Gonadotropin on different days of estrous cycle on conception in normal dairy cows



These results are in agreement with the finding that the use of hCG at the time of AI increased conception rates in dairy cows (Brown *et al.* 1973; Wagner *et al.* 1973; Babler and Hoffman 1974; Breuel *et al.* 1989; Srivastva and Ahlawat 1998; Ingawale *et al.* 2002; Patel *et al.* 2005; Selvaraju *et al.* 2009). Contrary to these findings it has also been suggested that hCG administered at the time of AI has no effect on conception rates (Hansel *et al.* 1960; Hansel *et al.* 1976; Echternkamp and Maurer 1983; Paksoy and Kalkan 2010).

The improvement in fertility due to hCG treatment during estrus in the present study could be by influencing a series of events viz; the time of ovulation, fertilization, corpus luteum development, progesterone secretion and embryonic survival. hCG injection, if administered at the time of insemination, increases the chances of pregnancy by inducing ovulation (De Rensis *et al.* 2008) and an increased LH level provides a timely maturation of follicle and ovulation followed by development of a functional corpus luteum able to maintain pregnancy (Kudlac *et al.* 1982).

The positive effects of hCG at the time of AI as a powerful stimulant of ovulation (Kudlac *et al.* 1982; De Rensis *et al.* 2008) is probably mediated by the improved ovulation rate. hCG induces ovulation of a dominant follicle in dairy cows. 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.* 1996a). Since the preovulatory surge of LH normally occurs about 6 hours after onset of estrus (Schams *et al.* 1977), treatment with hCG at insemination may have induced a secondary surge of LH before or after the spontaneous preovulatory surge of LH. This increment in LH may be beneficial for the events associated with conception. The treatment of hCG decreases the time interval between estrus to LH surge, which in turn leads to decreased production of miotic 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 with GnRH-induced LH release in response to hCG's four sites of O-linked glycosylation (Jameson and Hollenberg 1993). De Rensis *et al.* (2008) showed that cows treated with hCG at estrus induced ovulation in timed AI protocol and improve fertility. A delayed interval between ovulation and insemination is the main factor affecting fertility during warm season (De la Sota *et al.* 1998). De Rensis *et al.* (2008) suggested that hCG can to some extent counteract the negative effect of heat stress on fertility, and help in better synchronization before ovulation and insemination.

Progesterone is a vital hormone during early pregnancy that promotes embryo development and controls the luteolytic mechanism (Mann and Lamming 1999). A dose of hCG at AI can increase subsequent plasma progesterone concentration (Machado *et al.* 2008; Geary *et al.* 2001), 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. LH increases blood flow to ovary and cause ovarian hyperemia. High blood flow, increases transmission chance of steroid hormone into systemic blood flow and low density lipoproteins (LDL) into ovary as cholesterol in them is necessary for progesterone synthesis. An increased source of linoleic acid along with hCG, increases progesterone concentration and helps in maternal recognition and increases conception rate in heifers and possibly cows.

Possible interrelationship between low LH surge and low in vitro secretion of progesterone by luteinized granulosa cells has been reported (Less *et al.* 1998; Biger *et al.* 2000). A similar relationship between low LH surge and low post ovulatory progesterone concentration was found in primates (Zelinski-Wooten *et al.* 1997). Also, there is probably a direct relation between low concentration of plasma progesterone reducing LH pulse frequency and low pregnancy rates (Rosenberg *et al.* 2003; Franco *et al.* 2006). Reduction of GnRH concentration decreases LH secretion and progesterone concentration in mid luteal phase (Rosenberg *et al.* 2003). The LH-like effect of hCG on the ovarian cells may last for 30 hours after treatment (Schmitt *et al.*1996a), in contrast, administration of 10 mg buserelin increases LH concentrations in serum for approximately 5 hours (Kinser *et al.* 1983; Chenault *et al.* 1990). Moreover, the CL formed after ovulation induced by GnRH may not be fully functional (Rajamahendran and Sianangama 1992; Schmitt *et al.* 1996c; Diaz *et al.* 1998). Treatment with hCG usually elevates progesterone concentrations more than does GnRH treatment (Fricke *et al.* 1993; Schmitt *et al.*1996a; Stevenson *et al.* 2007; Stevenson *et al.* 2008).

Human chorionic gonadotrophin 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 $PGF_2\alpha$ release more effectively. Consequently, the luteolytic mechanism is either blocked or delayed which gives blastocysts more time to establish and help in maternal recognition of pregnancy in ewes. However, it is yet to be investigated whether the increased blastocyst expansion following hCG treatment leads to increased conceptus growth and improved placentation.

Our results are also in agreement with the findings that the use of hCG during the (early or mid) luteal phase enhances the conception rates in the dairy cows (Breuel *et al.* 1989; Sianangama and Rajamahendran 1992; Rajamahendran and Sianangama 1992; Santos *et al.* 2001; Ideta *et al.* 2003). 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 (Pratt *et al.* 1982; Eduvei and Seguin 1982; Helmer and Britt 1986; Schmitt *et al.* 1996a).

The explanation and rationale for the increased conception rates 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 (Schmitt *et al.* 1996c) and enhancing progesterone production early in the cycle, as an increase in progesterone secretion may facilitate embryonic development. Low progesterone secretion may also compromise fertility in dairy cattle (Mann and Lamming 1999; Lucy 2001). 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). In fact, even follicles smaller than 10 mm in diameter were able to form luteal tissue in response to hCG (Sheffel *et al.* 1982). 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 to7) than during the follicular (Days 0 to 3) or mid luteal (Days 8 to 12) stage of the estrous cycle (Helmer and Britt 1986; Price and Webb 1989). hCG induced three-wave follicular cycles could result in a higher conception rate as some authors have reported a higher conception rate in inseminated cows with three follicular waves compared to the cows with two follicular waves (Ahmad *et al.* 1997). 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).

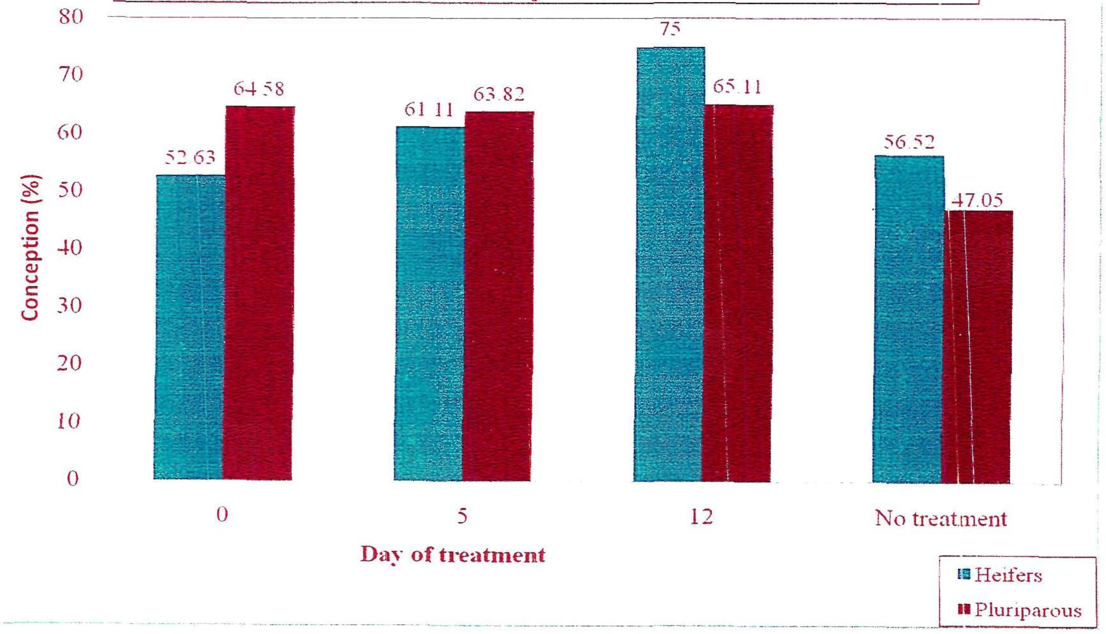
4.1.2 Effect of administration of human chorionic gonadotropin on different days of estrous cycle on conception in normal heifers and pluriparous dairy cows.

Effect of administration of hCG on different days of estrous cycle on conception in normal heifers and pluriparous dairy cows has been shown in Table 2 and Figure 2.

Table 2: Effect of administration of human chorionic gonadotropin on different days of estrous cycle on conception in normal heifers and pluriparous dairy cows.

Dose	Day of Administration post-AI	Parity	Cows inseminated	Pregnant	CR (%)
Human chorionic gonadotropin 1500 units (Chorulon 5 ml)	0	Heifer	19	10	**52.63**
		Pluriparous	48	31	**64.58**
	5	Heifer	18	11	**61.11**
		Pluriparous	47	30	**63.82**
	12	Heifer	12	9	**75.00**
		Pluriparous	43	28	**65.11**
Control		Heifer	23	13	56.52
		Pluriparous	68	32	47.05

Figure 2: Effect of administration of hCG on different days of estrous cycle on conception in normal heifers and pluriparous dairy cows



Perusal of table indicates that following administration of hCG simultaneous to AI, 52.63 per cent heifers (10/19) and 64.58 per cent pluriparous (31/48) cows conceived. Similarly, the conception rates achieved were 61.11 per cent (11/18) and 63.82 per cent (30/47), in heifers and pluriparous cows, respectively, injected with hCG fifth day post AI. Another 75 per cent heifers (9/12) and 65.11 per cent (28/45) pluriparous cows conceived following administration of 1500 IU hCG 12th day post AI.

In control group, 23 normal heifers and 68 pluriparous cows were inseminated without any treatment, of which 13 (56.52%) and 32 (47.05%) conceived, respectively. There was no statistical variation between any of the group.

These studies are in agreement with the studies of Funston *et al.* (2003) showing increase in the conception rates in heifers with hCG treatment, contrary to the other studies (Terma *et al.* 1990; Walker *et al.* 2005; Larson *et al.* 2007) where no increase in conception rates was recorded.

4.1.3 Effect of administration of human chorionic gonadotropin on different days of estrous cycle on conception in normal dairy cows during different lactations.

Effect of administration of human chorionic gonadotropin on different days of estrous cycle on conception in normal dairy cows during different lactations has been shown in Figure 3 and Table 3.

Table 3: Effect of administration of hCG on different days of estrous cycle on conception in normal dairy cows during different lactations

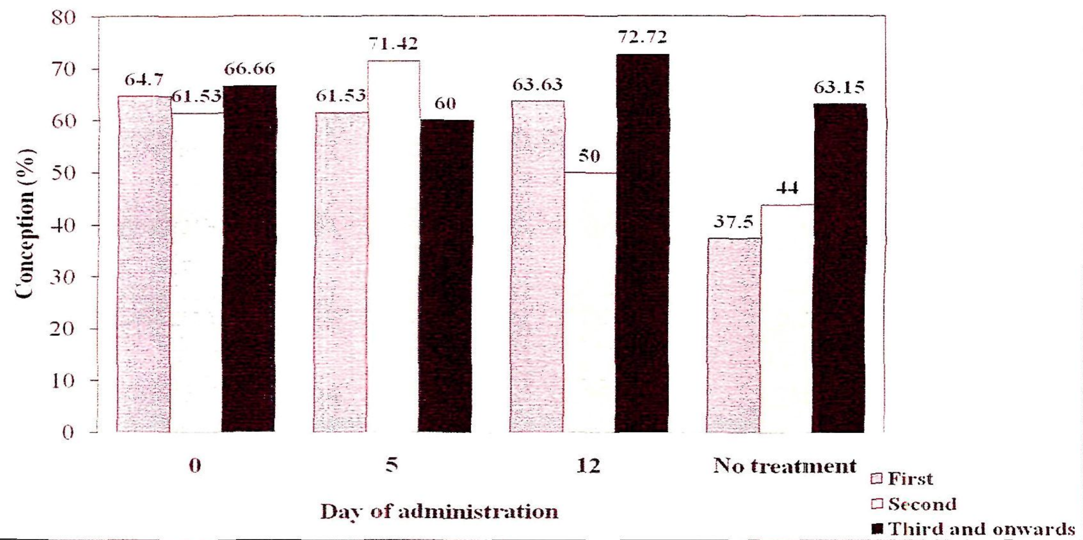


Table 3: Effect of administration of human chorionic gonadotropin on different days of estrous cycle on conception in normal dairy cows during different lactations.

Dose	Day of Administration post-AI	Lactation	Cows inseminated	Pregnant	CR (%)
Human chorionic gonadotropin 1500 units (Chorulon 5 ml)	0	First	17	11	**64.70**
		Second	13	8	**61.53**
		Third and onwards	18	12	**66.66**
	5	First	13	8	**61.53**
		Second	14	10	**71.42**
		Third and onwards	20	12	**60.00**
	12	First	11	7	**63.63**
		Second	10	5	**50.00**
		Third and onwards	22	16	**72.72**
Control		First	24	9	**37.50**
		Second	25	11	**44.00**
		Third and onwards	19	12	**63.15**

Table 3 indicates that conception rates (CR) were 64.70, 61.53 and 66.66 per cent, when 1500 IU hCG was administered along with insemination in first, second and third and onwards lactation animals. Corresponding CR were 61.53, 71.42 and 60.00 per cent following administration of same dose of hCG on 5th day post AI and 63.63, 50.00 and 72.72 per cent following administration on 12th dat post AI, respectively.

In control cows, the CR were 37.50, 44.00 and 63.15 per cent with insemination in first, second and third and onwards lactation, respectively. There was no statistical variation in any group.

4.1.4 Effect of parity on conception in normal cows (Irrespective of treatment)

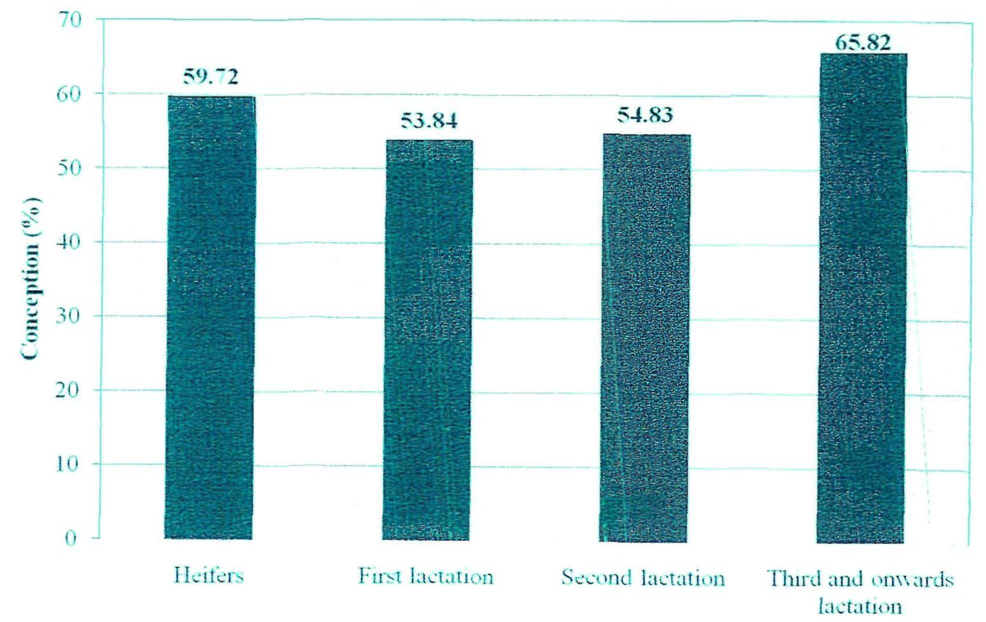
Irrespective of treatment, the effect of lactation on conception in normal dairy cows has been shown in Table 4 and Figure 4

Table 4: Effect of parity on conception in normal cows (Irrespective of treatment)

Reproductive status		Inseminated	Pregnant	CR (%)
Heifer (n=72)		**72**	**43**	**59.72**
Pluriparous (n=206)	First lactation	65	35	53.84
	Second lactation	62	34	54.83
	Third and onwards lactation	79	52	65.82
	Overall	**206**	**121**	**58.73**

Perusal of the table indicates that, out of total 72 heifers inseminated 43 (59.72%) conceived and among 206 cows, 121 (58.73%) animals became pregnant. However, the difference was statistically non significant. Lactation wise CR were 53.84, 54.83 and 65.82 per cent for first, second and third lactation cows, respectively.

Figure 4: Effect of parity on conception in normal cows (Irrespective of treatment)



The CR were comparable between all the lactation groups, irrespective of treatment.

When the results of effect of administration of hCG on different days was compared, the conception rates were non-significantly higher in all the lactation groups as compared to control. Similar studies have been done by Nakao and associates (1983) who reported that GnRH injection at first and third lactation insemination improved conception rates but Mee *et al.* (1990) reported higher conception rates for first lactation than subsequent lactations. Similarly, Kaim *et al.* (2010) recorded that hCG injection improved conception in 1st lactation animals (primiparous). Shabankareh *et al.* (2009) also reported higher CR in first lactation than subsequent lactations in hCG treated primiparous than in pluriparous cows. It may be speculated that hCG treatment eliminates various limiting factors that decrease conception rates and thus allow better realization of the fertility potential.

4.2 PROGESTERONE CONCENTRATION

4.2.1 Plasma progesterone concentration in dairy cows administered human chorionic gonadotropin at estrus or during luteal phase.

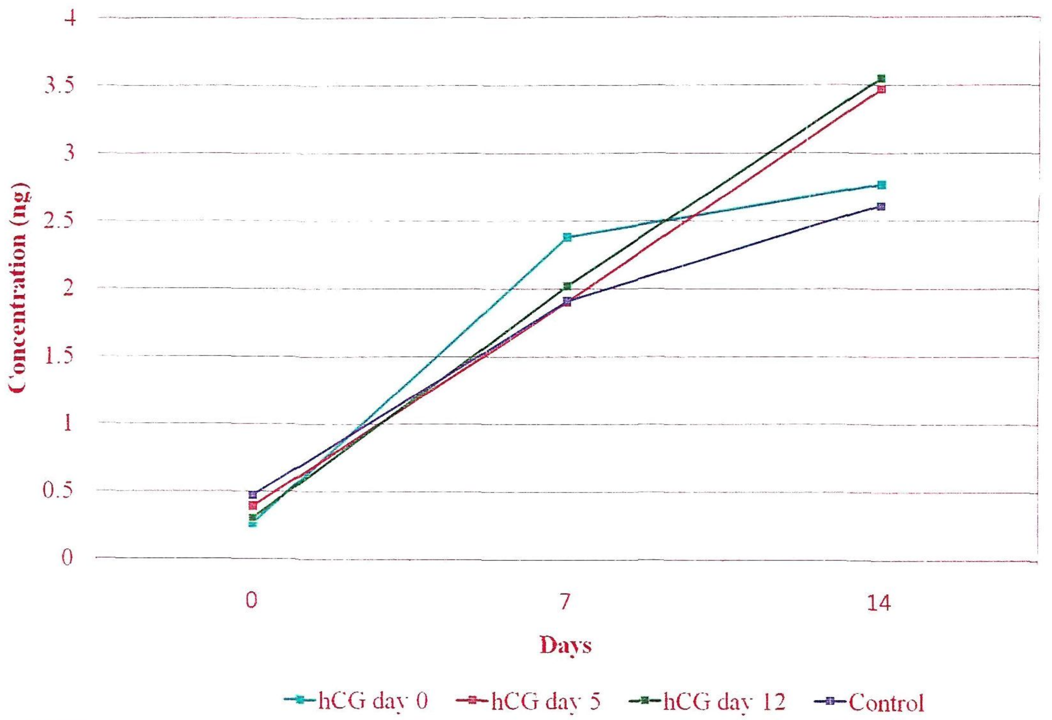
Mean (±SE) plasma progesterone concentration (ng/ml) in dairy cows administered with human chorionic gonadotropin at estrus or during luteal phase has been documented in Table 5 and Figure 5.

Table 5: Mean (±SE) plasma progesterone concentration (ng/ml) in dairy cows administered human chorionic gonadotropin at estrus or during luteal phase.

Treatment	Administration	Progesterone concentration (ng/ml)		
		Day 0	Day 7	Day 14
Human chorionic gonadotropin 1500 units (n=18)	Simultaneous to AI (n=6)	0.26±0.06 (0.1-0.46)	2.15±0.51 (0.98-4.2)	2.77±0.41 (1.6-4.6)
	5th day post AI (n=6)	0.39±0.09 (0.1-0.68)	1.9±0.37 (1.2-3.7)	3.47±0.42 (1.8-4.8)
	12th day post AI (n=6)	0.30±0.07 (0.01-0.6)	2.02±0.16 (1.7-2.7)	3.55±0.43 (2.2-5.2)
Control (n=8)		0.47±0.34 (0.1-1.1)	1.91±0.61 (0.8-2.6)	2.61±0.90 (1.7-4.4)

The mean plasma progesterone concentration on Day 0, 7 and 14 in cows treated with hCG on day 0 was 0.26±0.06, 2.15±0.51 and 2.77±0.41 ng/ml, respectively. Similarly, the mean plasma progesterone concentration on Day 0, 7 and 14 in cows treated with hCG 5th day post AI was 0.39±0.09, 1.9±0.37 and 3.47±0.42 ng/ml, respectively. Corresponding values on Day 0, 7 and 14 were 0.30±0.07, 2.02±0.16 and 3.55±0.43 ng/ml, respectively, when hCG was injected on Day 12th post AI. In the control cows, the mean plasma progesterone concentration was 0.47±0.34, 1.91±0.61 and 2.61±0.90 on Day 0, 7 and 14, respectively.

Figure 5: Mean (±SE) plasma progesterone concentration (ng/ml) in dairy cows administered Human Chorionic Gonadotropin at estrus or during luteal phase



The increase in plasma progesterone concentration on Day 7 following treatment with hCG on day 0 was non-significantly higher than other treatment groups. These observations are in agreement with the findings of Kaim *et al.* (2003) and 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 (Helmer and Britt 1986; Geary *et al.*

2001; Machado *et al.* 2008) or no effect on progesterone concentration by other researchers (Paksoy and Kalkan 2010).

Similarly, an increase in plasma progesterone concentration on Day 14 following treatment with 1500 IU hCG on day 5 or 12 was non-significantly higher than other groups. The non-significant increase in progesterone concentration following hCG administration 12th day post AI is in agreement with the findings of Paksoy and Kalkan (2010). However, Macmillan *et al.* (1985) and Nephew *et al.* (1994) reported increased progesterone concentration in cattle and sheep, respectively, following the same treatment.

Treatment with hCG increases serum concentrations of progesterone in at least two ways: first by induction of ancillary CL (Schmitt *et al.* 1996a; Santos *et al.* 2001; Stevenson *et al.* 2007) and second by an increase in number of progesterone-secreting luteal cells in existing CL (Nishwender *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. Concentration of progesterone increased between day 7 and 12 in treated cows but not in control cows suggesting that a new corpus luteum was formed.

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. Follicular structure changes with ovulation and theca granulosa cells transform to corpus luteum, increasing the proportion of large and decreasing the proportion of small luteal cells in corpus luteum (Farin *et al.* 1988; Sianangama and Rajamahendran 1992) thereby increasing progesterone concentration and hence embryo viability.

4.2.2 Plasma progesterone concentration in pregnant and non pregnant dairy cows.

Mean (±SE) plasma progesterone concentration (ng/ml) in pregnant and non pregnant dairy cows on different days post AI has been shown in Table 6 and Figure 6.

Table 6: Mean (±SE) plasma progesterone concentration (ng/ml) in pregnant and non pregnant dairy cows on different days post AI.

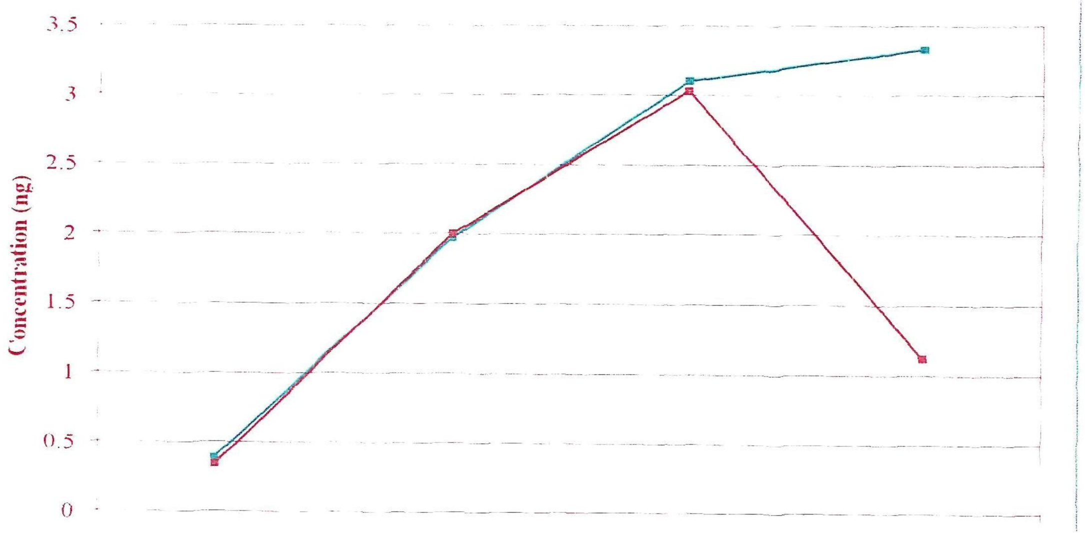
Reproductive Status	Progesterone concentration (ng/ml)			
	Day 0	Day 7	Day 14	Day 21
Pregnant (n=12)	0.39±0.08 (0.01-0.68)	1.97±0.20 (0.98-3.7)	3.1±0.34 (1.6-5.2)	3.33±0.24[a] (2.0-4.8)
Non pregnant (n=14)	0.34±0.53 (0.1-0.84)	2.0±0.23 (0.8-4.2)	3.03±0.24 (1.7-4.6)	1.12±0.34[b] (0.01-3.6)

Figures with different superscripts differ significantly ($P<0.05$)
(Figures within parenthesis indicate range)

The mean plasma progesterone concentrations in pregnant cows (n=12) were 0.39±0.08, 1.97±0.20, 3.1±0.34 and 3.33±0.24 ng/ml on day 0, 7, 14 and 21 of the cycle, respectively. In non pregnant (n=14) cows the corresponding values were 0.34±0.53, 2.0±0.23, 3.03±0.24 and 1.12±0.34 ng/ml on day 0, 7, 14 and 21, respectively.

In this study, there was no significant difference in the progesterone levels in pregnant and non pregnant animals on Day 0, 7 and 14 day post AI. However, the levels of progesterone on day 21 post AI were much higher ($p<0.0001$) in pregnant cows.

Figure 6: Mean (±SE) plasma progesterone concentration (ng/ml) in pregnant and non pregnant dairy cows on different days post AI



Progesterone concentration in the peripheral circulation during the first 14 days of gestation is similar to those of dioestrus; there is sharp decline in these concentrations from about Day 18 in non-pregnant cows. In the pregnant cow there is normally only a slight fall at this stage with rapid recovery. Thereafter the concentration increases slightly during pregnancy until it starts to decline at about 20-30 days prepartum.

Batra *et al.* (1979) reported changes in progesterone concentration of blood in Murrah buffaloes up to 40 days after insemination. Progesterone concentration in blood plasma at estrus was 0.1 ng/ml which rose to a peak of 3.6 ng/ml on day 13. It continued to increase in animals that conceived; progesterone in pregnant animals did not drop after day 13 but was maintained and elevated a little to an average of 4.13 ± .73ng/ml on day 25 after insemination. But it declined gradually followed by a rapid fall on day 2, thereby attaining a low of 0.10 ± .07 ng/ml at the onset of subsequent estrus in those that failed to conceive. It generally is agreed that concentrations of progesterone in blood and milk provide estimates of luteal function during the reproductive cycle. The mid cycle rise of progesterone in plasma and milk appears to result from luteinization after ovulation and subsequent progesterone secretion by the corpus luteum.

SUMMARY AND CONCLUSIONS

CHAPTER 5

SUMMARY AND CONCLUSIONS

Treatment and insemination

The work was conducted at the university livestock farm, CSK Himachal Pradesh Krishi Vishvavidyalya, Veterinary clinical complex of Dr.G.C.Negi college of Veterinary and Animal Sciences Palampur and in field veterinary institutions from February 2008 to March 2010.

In all, 278 normal cows were inseminated during this study. These cows were divided in three treatment and one control group. Depending upon different treatments groups, human chorionic gonadotrophin (Chorulon Intervet Schering Plough India Ltd.) was injected at the dose of 1500 *IU.* on different days (0, 5 or 12) of estrous cycle in these cows.

The animal included in treatment group of human chorionic gonadotrophin (hCG) administration (n=187) were further sub-divided into three groups viz. the administration of hormone simultaneous to AI (day 0, group1), insemination and exogenous administration of hCG on 5^{th} day post AI (group 2) and insemination and administration of hormone on 12^{h} day post AI (group 3). A total of 67,65and 55 cows were inseminated in groups 1, 2 and 3 respectively.

In addition 91 cows presented for insemination were selected as control and these were inseminated in standing heat without any treatment. Pregnancy diagnosis was carried out 60 days post AI. Conception rates (CR) were calculated and statistically analyzed.

Blood Collection for Progesterone Estimation

For plasma progesterone estimation, blood was collected from 26 cows.This included 18 animals treated with 1500 IU (5ml) hCG (Chorulon) either on Day 0 (n=6) along with AI or on Day 5 (n=6) or Day 12 (n=6) post insemination and also from control group cows (n=8). In all the animals four blood samples were collected on the day 0 (day of insemination) and on days 7, 14 and 21 (post insemination) of estrous cycle.

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

Out of 67 cows inseminated along with hCG administered simultaneous to AI, 41 were found pregnant. The conception rate achieved was 61.19 per cent. Among 65 cows injected hCG fifth day post AI, 41 conceived with a CR of 63.07 per cent and out of 55 cows inseminated following administration of hCG 12th day post AI, 37 were found pregnant. The conception rate was 67.27 per cent. In the control group, 91 normal animals were inseminated without any treatment and 45 (49.45%) were found pregnant. Clinically, 1500 IU hCG administered 12th day post AI (67.27%) significantly ($P<0.05$) improved the conception as compared to control animals (49.45%).

Effect of administration of hCG on different days of estrous cycle on conception in normal heifers and pluriparous dairy cows.

Following administration of hCG simultaneous to AI, 52.63 per cent heifers (10/19) and 64.58 per cent pluriparous (31/48) cows conceived. Similarly, the conception rates achieved were 61.11 per cent (11/18) and 63.82 per cent (30/47), in heifers and pluriparous cows, respectively, injected with hCG fifth day post AI. Another 75 per cent heifers (9/12) and 65.11 per cent (28/45) pluriparous cows conceived following administration of hCG 12th day post AI. In control group, 56.52 per cent heifers and 47.05 per cent pluriparous cows conceived, respectively. There was no statistical variation between any of the groups.

Effect of administration of hCG on different days of estrous cycle on conception in normal dairy cows during different lactations .

Conception rates (CR) were 64.70, 61.53 and 66.66 per cent, when hCG was administered along with insemination in first, second and third and onwards lactation animals. Corresponding CR following administration of hCG on 5th day post AI were 61.53, 71.42 and 60.00 per cent and were 63.63, 50.00 and 72.72 per cent following administration on 12th day post AI, respectively.

In control cows, the CR were 37.50, 44.00 and 63.15 per cent with insemination in first, second and third and onwards lactation, respectively. There was no statistical variation in any group.

Effect of parity on conception in normal cows (Irrespective of treatment)

Out of total 72 heifers inseminated 43 (59.72%) conceived and among 206 cows, 121 animals became pregnant and conception rate was (58.73%). However, the difference was statistically non significant. Lactation wise CR were 53.84, 54.83 and 65.82 per cent for first, second and third lactation cows, respectively. The conception rates were non-significantly higher in all the lactation groups as compared to control.

Plasma progesterone concentration in dairy cows administered hCG at estrus or during luteal phase.

The mean plasma progesterone concentration on Day 0, 7 and 14 in cows treated with hCG (n=6) on day 0 was 0.26±0.06, 2.15±0.51 and 2.77±0.41 ng/ml, respectively. Similarly, the mean plasma progesterone concentration on Day 0, 7 and 14 in cows treated with hCG (n=6) 5th day post AI was 0.39±0.09, 1.9±0.37 and 3.47±0.42 ng/ml, respectively. Corresponding values on Day 0; 7 and 14 were 0.30±0.07, 2.02±0.16 and 3.55±0.43 ng/ml, respectively, when hCG was injected on Day 12th post AI (n=6). In the control cows, the mean plasma progesterone concentration was 0.47±0.34, 1.91±0.61 and 2.61±0.90 on Day 0, 7 and 14, respectively. The increase in plasma progesterone concentration on Day 7 following treatment with hCG on day 0 was non-significantly higher than other treatment groups. Similarly, an increase in plasma progesterone concentration on Day 14 following treatment with hCG on day 5 or 12 was non-significantly higher than either Day 0 or control groups.

Plasma progesterone concentration in pregnant and non pregnant dairy cows.

The mean plasma progesterone concentrations in pregnant cows (n=12) were 0.39±0.08, 1.97±0.20, 3.1±0.34 and 3.33±0.24 ng/ml on day 0, 7, 14 and 21 of the cycle, respectively. In non pregnant (n=14) cows the corresponding values were 0.34±0.53, 2.0±0.23, 3.03±0.24 and 1.12±0.34 ng/ml on day 0, 7, 14 and 21, respectively.

In this study, there was no significant difference in the progesterone levels in pregnant and non pregnant animals on Day 0, 7 and 14 day post AI. However, the levels of progesterone on day 21 post AI were much higher ($p<0.0001$) in pregnant cows.

CONCLUSIONS

1. hCG administration (along with AI or during luteal phase) improved conception in treated than control animals.
2. Highest conception rates were recorded when hCG was administered on day 12 post AI.
3. The response to hCG was better in heifers than cows, when supplemented on day 12.
4. Consistently higher mean plasma progesterone concentration was recorded during luteal phase in hCG treated cows.
5. The mean plasma progesterone values were highest in cows receiving hCG on day 12 post-AI.
6. The plasma progesterone values remained higher in pregnant cows on day 21, whereas, these values were significantly low in non-pregnant cows on this day.

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LITERATURE CITED

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