



## EFFECTS OF DIFFERENT DOSES OF HUMAN CHORIONIC GONADOTROPIN AT TIMED ARTIFICIAL INSEMINATION ON THE FERTILITY OF LACTATING DAIRY COWS

M. MASHAYEKHI<sup>1</sup> & M. GOLI<sup>2</sup>

<sup>1</sup>DVM graduate, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran; <sup>2</sup>Department of Clinical Science, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran

### Summary

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The aim was to investigate the effects of administering GnRH and low (1650 IU) or high (3300 IU) doses of human chorionic gonadotropin (hCG) at timed artificial insemination (TAI) on the rates of ovulation and pregnancy in lactating dairy cows. Forty-seven multiparous lactating dairy cows (parity: 2 to 5; days in milk: 55±1) received a controlled internal drug release (CIDR) insert and 25 µg of GnRH intramuscularly on Day -10. On Day -3, the CIDRs were removed and 500 µg of cloprostenol sodium was administered intramuscularly. On Day -1, all animals were administered another dose of GnRH and inseminated 16-18 h later (Day 0). At TAI, the animals allocated randomly to the following groups: SALINE (n=12) which received 5 mL of sterile normal saline intramuscularly (i.m.), GNRH (n=11) which received 25 µg of GnRH i.m., L-HCG (n=12) treated with 1650 IU of hCG i.m., and H-HCG (n=12) treated with 3300 IU of hCG i.m.. The animals were examined by transrectal ultrasonography on Days -1, 0 and 1 to identify and record follicle(s) ≥ 8 mm in diameter and ovulation, and on Days 30 and 60 to determine conception and pregnancy rates, respectively. Ovulation was defined as the disappearance (from one scanning session to the next) of a previously identified follicle ≥ 8 mm in diameter. Blood samples were collected from the jugular vein of all animals into vacuum tubes without anticoagulants on Days 0, 6 and 12 for progesterone assay. The results demonstrated no significant differences in the mean progesterone concentrations at days 0, 6 and 12, and also in conception and pregnancy rates to the first insemination among the groups, although administration of gonadotropins resulted in numerically greater conception and pregnancy rates by 16.7–41% compared to SALINE. However, ovulation rate was greater in H-HCG compared to SALINE (P<0.05). In conclusion, it was demonstrated that administering GnRH or hCG at TAI, regardless of low or high doses, resulted in nonsignificant improvement in conception and pregnancy rates in lactating dairy cows synchronised with the Ovsynch protocol combined with CIDR.

**Key words:** controlled internal drug release device, dairy cow, human chorionic gonadotropin, Ovsynch

## INTRODUCTION

Over the last decade, there has been an increasing interest in developing new synchronisation protocols with timed artificial insemination (TAI) to improve reproductive efficiency in the dairy industry (Liu *et al.*, 2018). Despite the enormous amount of research and the rapid adoption of newly developed approaches during the last 20 years, pregnancy per AI (P/AI) in lactating dairy cows has remained low (Motavalli *et al.*, 2017). The Ovsynch protocol is the most prevalent GnRH based protocol for synchronising the time of ovulation which is extensively applied in dairy farms (El-Tarabany & El-Tarabany, 2015). However, 40–60% of treated animals do not become pregnant at the time of TAI (De Rensis *et al.*, 2008a). Some reasons for this could include failure to ovulate, mistiming of AI resulting in reduced fertilisation and pregnancy rates (PRs), reduced secretion of progesterone (P4) in the period after AI, and greater embryonic mortality (Rowe *et al.*, 2019). One strategy that has been employed during TAI protocols, particularly in animals that do not express oestrus prior to AI, is to give GnRH at the time of AI to assure that all animals have a luteinising hormone (LH) surge, theoretically reducing the percentage of animals with no ovulation or delayed ovulation (Madureira *et al.*, 2020). However, another factor that may lead to the failure of TAI could be related to the use of GnRH to induce ovulation (De Rensis *et al.*, 2008a). It has been evidenced that the efficacy of GnRH-based synchronisation protocols is affected by a wide range in interval to ovulation (Zwiefelhofer *et al.*, 2020). In effect, it has been observed that a single dose of a GnRH-analogue (buserelin) induces an LH surge that lasts

for about 5 h, which is approximately half the duration of a naturally occurring LH surge (De Rensis *et al.*, 2008a). The reduced LH surge may induce incomplete development of the ovulatory follicle and its oocyte and the formation of a less functional corpus luteum (CL) (De Rensis *et al.*, 2008b). It has been documented that P4 is a crucial hormone in reproductive events related to the establishment and maintenance of pregnancy in cattle (Cunha & Martins, 2022). As a result, there is a great interest to investigate synchrony programmes that may improve P/AI for TAI (Rowe *et al.*, 2019). In this regard, human chorionic gonadotropin (hCG) treatment has been applied at various times relating to AI in order to overcome these drawbacks, but the results have been inconsistent (Stevenson & Pulley, 2012; Alnimer & Shamoun, 2015; Schmitz *et al.*, 2017; Šuluburić *et al.*, 2017; Niles *et al.*, 2019; Zolini *et al.*, 2019; Agarwal *et al.*, 2021; Garcia-Ispierto *et al.*, 2021a; Zheng *et al.*, 2021; Cunha & Martins, 2022) which may be partly due to different hCG doses or days of administration used in different studies and partly to breed differences and other factors including parity, days in milk, and total milk production (Agarwal *et al.*, 2021).

Human chorionic gonadotropin has a potent LH-like effect in cattle, extends the life span of the CL, increases P4 synthesis and induces ovulation throughout the oestrous cycle (Garcia-Ispierto *et al.*, 2018). Human chorionic gonadotropin and GnRH treatments have similar effects on the ovary, inducing ovulation and the formation of accessory CLs with a significant increase in plasma P4 concentrations achieved 7 days after treatment (Canadas *et al.*, 2019). As opposed to the pituitary-

mediated mechanism of action of GnRH, hCG triggers ovulation by binding directly to LH receptors in the follicle (Giordano *et al.*, 2012a; Garcia-Ispierto *et al.*, 2021a), therefore induces ovulation and exerts a luteotropic effect (De Rensis *et al.*, 2010). In dairy cows, plasma LH concentrations are markedly increased for 30 h after hCG administration and do not return to baseline concentrations for 66 h (De Rensis *et al.*, 2010). Thus, hCG seems to be one of the most potent drugs to induce ovulation in cattle and as a luteotropic agonist (Cunha & Martins, 2022). It has been reported that supplementary injection of hCG given at the time of mating in the ewe and cow may improve embryo viability and therefore fertility (De Rensis *et al.*, 2008a).

To our knowledge, there is no comparative study on the effect of administering two different doses of hCG at the time of TAI on the fertility of lactating dairy cows; therefore, the aim of the present study was to evaluate the effect of low and high doses of hCG at TAI on fertility and serum P4 concentrations in lactating dairy cows during the cool months of the year. The hypothesis was that doubling the dose of hCG at TAI will improve the reproductive efficiency of lactating dairy cows.

## MATERIALS AND METHODS

### *Ethical approval*

All the procedures of the present study were approved by the Research Ethics Committee of Razi University, Kermanshah, Iran (Approval ID: IR.RAZI.REC.1400.032/03-03-2021).

### *Animals and location*

Lactating Holstein cows (n=47; parity: 2.4±0.75; days in milk: 55.35±3.25; body

condition score (BCS): 2.45±0.15 on a scale of 1–5) on a commercial dairy farm located in the west of Iran were enrolled in this study regardless of being observed in oestrus or not postpartum. In order to avoid the influence of heat stress on the reproductive performance of the cows, the study was conducted during the regional cool months of the year. Cows were housed in barns with open-air and sheltered areas, had free access to fresh water, fed a mixed diet adjusted to provide their requirements and milked three times daily with average 8-hour interval and had an average daily milk yield of 22.47±3.86 kg during the study. The reproductive tracts and mammary glands of the cows were examined by inspection and manual palpation for evidence of any clinical abnormalities before initiation of the experiment. Cows that were clinically normal were enrolled in the experiment. Milk production, days in milk, BCS and parity on the day of starting treatments did not differ between treatments.

### *Experimental design*

Estrous cycles were synchronised by intravaginal insertion of a controlled internal drug release (CIDR; Abureyhan pharma Co., Veterinary Division, Tehran, Iran) device containing 1.9 g of P4, which was remained in place for 7 days, an intramuscular administration of 25 µg of a GnRH analog (Alarelin acetate, Vetaroline, 10 mL vial, 5 µg/mL, Abureyhan pharma Co., Veterinary Division, Tehran, Iran) at device insertion, an intramuscular administration of 500 µg of a PGF2α analog (D-cloprostenol sodium, D-clo PG, 10-mL vial, 0.025 mg/mL, Royan darou Co., Veterinary Division, Semnan, Iran) at device removal, and another intramuscular administration of 25 µg of GnRH 56 h after the device removal. Cows were in-

seminated (TAI) 16 to 18 h after the second GnRH. At TAI (d 0), cows were randomly allocated to receive 5 mL of normal saline (SALINE), 25 µg of GnRH (GnRH), 1650 IU of hCG (L-HCG; Chorulon; Intervet, Dublin, Ireland) or 3300 IU of hCG (H-HCG) via intramuscular injection. The hCG vials used in the present experiment contained 5000 IU hCG, so a solution of each vial was made by adding 10 mL of sterile distilled water and 3.3 mL of the solution (1650 IU hCG) was administered to each cow allocated to L-HCG group. For the cows allocated to H-HCG group that received 3300 IU hCG, 10 mL of sterile distilled water was added to two hCG vials and 3.3 mL of the solution (3300 IU hCG) was administered to each cow.

#### *Ovarian ultrasonography*

The ovaries of all animals were scanned by transrectal ultrasonography (EMP V9 Veterinary Portable Diagnostic Ultrasound System, China) using a 7.5 MHz transducer on days -1, 0, and 1 in order to record the occurrence of ovulation. Ovulation was defined as the disappearance (from one scanning session to the next) of a previously identified follicle  $\geq 8$  mm in diameter in either ovary (Núñez-Olivera *et al.*, 2019; Cabrera *et al.*, 2021). Ovulation rate (OR) was defined as the number of the cows in a group that ovulated until day 1, divided by the total number of the animals in the corresponding group  $\times 100$ .

#### *Blood sampling and hormone assays*

Blood samples (8 mL each) were collected from the jugular veins of all animals by jugular venipuncture into blood collection vacuum tubes without anticoagulant on days 0, 6 and 12 for P4 assay, transmitted to a laboratory within 1 h after collection in cool condition and allowed

to coagulate for 2 h. Then, the samples were centrifuged at  $3000\times g$  for 15 minutes, the sera were loaded into 2-mL microtubes and stored at  $-20^{\circ}\text{C}$  until hormone assay. Serum P4 concentrations assay was performed by ELISA method using commercial P4 kits (assay range: 0.2–40 ng/mL; analytical range: 0.2–40 ng/mL; sensitivity: 0.05 ng/mL; DiaMetra S.r.l., Spello – Perugia, Italy). The intra- and inter-assay coefficients for the kits were 6.5% and 7.2%, respectively.

#### *Pregnancy diagnosis*

Uterine horns of all animals were scanned by transrectal ultrasound on days  $30\pm 1$  and  $60\pm 1$  post TAI to record pregnancy status. Conception rate (CR) was defined as the percentage of the cows in a group diagnosed as pregnant on day  $30\pm 1$  post TAI divided by the total number of the animals in the corresponding group  $\times 100$ . Pregnancy rate was defined as the percentage of the pregnant cows on day  $60\pm 1$  divided by the total number of the animals in the corresponding group  $\times 100$ .

#### *Statistical analysis*

Data were analysed using SAS® software (Statistical Analysis System, Release 9.4. for Windows SAS Institute Inc., USA) by logistic regression method using PROC GENMOD to determine the probability of significant differences between treatments and occasions. Chi-square ( $\chi^2$ ) statistics was used to assess the difference between the groups. The level of significance was  $P\leq 0.05$ . Differences with  $0.05 < P \leq 0.10$  were interpreted as tendencies.

## RESULTS

The results of the blood P4 concentration assay are presented in Table 1. The differ-

ences in mean±SD concentrations did not differ between the groups (P>0.05).

The ovaries of all cows were scanned by ultrasound on days -1, 0, and 1 relative to TAI (day 0). The incidence of ovulation and the number of animals that ovulated between days -1 and 1 are presented in Table 2. The least and greatest incidence of ovulations were observed in GNRH and L-HCG groups (45.4% and

75.0%, respectively), which differed significantly (P=0.0296). However, there was no significant differences between each of these two groups and the others (P>0.05).

As seen from Table 2, the lowest and highest CRs confirmed by transrectal ultrasound on day 30±1 post TAI were observed in SALINE (33.3%, 4/12) and H-HCG (75.0%, 9/12) groups, respectively (P>0.05).

**Table 1.** Serum progesterone concentrations (mean ± SD; ng/mL) on different occasions relative to timed artificial insemination (day 0) in lactating dairy cows synchronised with the Ovsynch protocol plus normal saline (SALINE), GnRH (GNRH), 1650 IU (L-HCG) or 3300 IU of human chorionic gonadotropin (H-HCG) at timed artificial insemination

Groups	Day 0	Day 6	Day 12
SALINE (n=12)	4.03 ± 2.03	5.40 ± 1.78	1.31 ± 0.64
GNRH (n=11)	0.34 ± 0.09	5.02 ± 0.77	4.44 ± 0.82
L-HCG (n=12)	2.86 ± 0.68	3.70 ± 1.82	4.01 ± 1.56
H-HCG (n=12)	0.20 ± 0.00	4.29 ± 2.76	10.80 ± 2.64

Legend: SALINE: Cows received the Ovsynch protocol (GnRH, 7 days, PGF2α, 56 hours, GnRH) and an intravaginal insertion of a controlled internal drug release (CIDR) device plus TAI and i.m. injection of 5 mL of normal saline 16–18 h after the second GnRH (GnRH2) of the Ovsynch protocol; GNRH: As SALINE except that the cows received 25 µg of GnRH i.m. instead of normal saline 16–18 h after GnRH2; L-HCG: As SALINE except that the cows received 1650 IU of hCG i.m. instead of normal saline 16–18 h after GnRH2; H-HCG: As SALINE except that the cows received 3300 IU of hCG i.m. instead of normal saline 16–18 h after GnRH2.

**Table 2.** Ovulation rate, conception rate (30±1 days after timed artificial insemination), and pregnancy rate (60±1 days after timed artificial insemination) in lactating dairy cows synchronised with the Ovsynch protocol plus normal saline (SALINE), GnRH (GNRH), 1650 IU (L-HCG) or 3300 IU of human chorionic gonadotropin (H-HCG) at timed artificial insemination

Groups	Ovulation (%)	Conception (%)	Pregnancy (%)
SALINE* (n=12)	58.3 (7/12) <sup>ab</sup>	33.3 (4/12)	33.3 (4/12)
GNRH (n=11)	45.4 (5/11) <sup>a</sup>	54.5 (6/11)	54.5 (6/11)
L-HCG (n=12)	75.0 (9/12) <sup>b</sup>	50.0 (6/12)	50.0 (6/12)
H-HCG (n=12)	66.7 (8/12) <sup>ab</sup>	75.0 (9/12)	75.0 (9/12)

Legend: SALINE: Cows received the Ovsynch protocol (GnRH, 7 days, PGF2α, 56 hours, GnRH) and an intravaginal insertion of a controlled internal drug release (CIDR) device plus TAI and i.m. injection of 5 mL of normal saline 16–18 h after the second GnRH (GnRH2) of the Ovsynch protocol; GNRH: As SALINE except that the cows received 25 µg of GnRH i.m. instead of normal saline 16–18 h after GnRH2; L-HCG: As SALINE except that the cows received 1650 IU of hCG i.m. instead of normal saline 16–18 h after GnRH2; H-HCG: As SALINE except that the cows received 3300 IU of hCG i.m. instead of normal saline 16–18 h after GnRH2. <sup>a,b</sup> Different superscript letters within a column denote significant difference (P≤0.05).

Ultrasound scanning of the uterine horns was performed on day 60±1 after TAI to determine PR. As presented in Table 2, the highest and lowest PRs were observed in H-HCG (75.0%, 9/12) and SALINE groups (33.3%, 4/12), respectively (Table 2), but the difference was not statistically significant ( $P>0.05$ ). In other words, no pregnancy loss has occurred between days 30±1 and 60±1 after TAI in all animals.

## DISCUSSION

In the present study, the average milk yield had no significant effect on the rates of ovulation and pregnancy, which was in agreement with that of several other studies (Momcilovic *et al.*, 1998; Chenault *et al.*, 2014). It has been documented that milk yield and reproductive performance have no genetic correlation (Pryce *et al.*, 2004). Cunha & Martins (2021) reported that parity had no effect on ovulation response in lactating Holstein and Jersey cows. According to the results of another study (Momcilovic *et al.*, 1998), BCS and lactation number had no effect on CR and PR of lactating dairy cows underwent oestrus or ovulation synchronisation programs, which confirms the findings of the present study. Heuwieser *et al.* (1994) found conflicting results in CR of Holstein dairy cows received GnRH at breeding due to different parity and BCS. The authors reported that all cows, regardless of parity, with a BCS<3.0 (on a 5-point scale; 1=emaciated, 5=obese) at the first breeding will benefit from a GnRH injection. The authors concluded that efficacy of GnRH may not be consistent in all parity and body condition groups. Ryan *et al.* (1991) reported that administering a GnRH analogue treatment to dairy cows at AI increased first service PRs and that the

response was most evident in first parity cows. In the present study, administration of GnRH improved PR by 21.2% in lactating cows compared to saline treatment, although the difference was not significant.

Conflicting results have been reported regarding the effect of GnRH or hCG on the reproductive efficiency in dairy cows. Some studies reported that administration of hCG resulted in greater ovulatory responses compared to GnRH (Stevenson *et al.*, 2007; Buttrey *et al.*, 2010), but others did not find such an improvement (Burns *et al.*, 2008; Keskin *et al.*, 2010). In a meta-analysis on 107 different trials from 52 publications, data from 18,082 cows treated with hCG and GnRH between days 4 and 15 after AI were meta-analysed. The results did not show any difference in P/AI between cows treated with hCG and cows treated with GnRH. Treatment with hCG at a dose above 2,500 IU was associated with increased chances of P/AI compared with lower doses. The meta-analysis showed that the use of GnRH and hCG after AI should be focused on cows expected to have low or moderate fertility. Day and dose of treatment have to be considered as well (Besbaci *et al.*, 2020). In the present study, PR improved by 20.5% and 25% in cows received a high dose of hCG (3300 IU) compared to those received GnRH or a low dose of hCG (1650 IU), respectively, although the differences were not significant. The study by De Rensis *et al.* (2008a) reported that PR to AI failed to differ between the hCG treatment regime compared to the GnRH treatment regime; however, the cumulative PR was higher for hCG compared to GnRH during the warm period. Mohammadi *et al.* (2019) demonstrated that administration of buserelin acetate at the time of AI had no ef-

fect on P/AI and pregnancy survival rate in dairy cattle under no heat stress condition. By contrast, Alves *et al.* (2021) affirmed that administering GnRH (busarelin acetate) at TAI improved P/AI in Nelore (*Bos indicus*) cows. Madureira *et al.* (2020) and Prata *et al.* (2020) also found improvement of fertility with administering GnRH at AI in Nelore cows. In another study (Aboul-Ela & El-Keraby, 1986), administration of a GnRH analog to Frisian cows at AI significantly improved CR to the first insemination compared to those receiving saline. Drew & Peters (1994) found that administering GnRH on the day of AI had no significant effects on fertility in comparison with untreated control cows. On the other hand, Madureira *et al.* (2020) reported that GnRH treatment at TAI increased fertility of cows that had been synchronised with a 7-day P4-based program. Garcia-Ispierto *et al.* (2021b) reported that hCG treatment at AI can substantially improve CR of multiparous dairy cows at day 28 after TAI, even during heat stress periods compared to GnRH treatment and no treatment. In contrast, De Rensis *et al.* (2002) found similar PR for GnRH or hCG after a synchronisation protocol in cyclic dairy cows. Perry & Perry (2009) found no improvement in CR or overall PR in beef heifers or cows after GnRH treatment at AI. Moreover, López-Gatius *et al.* (2006) reported a decreased probability of pregnancy after a single treatment of GnRH at AI in high-producing dairy cows when compared to double treatment (at AI and 12 days later). It was elucidated that although hCG and GnRH have similar effects on the ovary, the good conception response to hCG could mean that this hormone not only acts directly on the ovarian follicle, as opposed to GnRH, which acts via the hypophyseal-ovarian

axis, but also on the CL, reducing early embryo losses. This may suggest that hCG, besides appropriately inducing ovulation, also improves CL health, increasing plasma P4 concentrations (Garcia-Ispierto *et al.*, 2021a).

It has been documented that hCG use for synchronisation of ovulation in dairy and beef cattle thus far has produced variable results, yielding increased, decreased, or similar rates of conception or pregnancy (Binversie *et al.*, 2012). The effect of a single hCG dose on fertility is variable and the authors in this field of work have reported an improved CR to first service or no improvement (De Rensis *et al.*, 2008a). In the present study, the Ovsynch protocol was initiated regardless of the cyclicity of the cows or the stage of the ovarian cycle. Ovulation rate in the SALINE group was 58.3% but not significantly different in relation to those in GNRH, L-HCG, and H-HCG groups (45.4%, 75%, and 66.7%, respectively). Liu *et al.* (2018) reported that although OR was not correlated with treatment, the rate within 48 h of GnRH injection (93.3%) tended to be higher compared with that in the Saline group (60.0%) and that the average time of ovulations was  $29.3 \pm 0.5$  h after administering GnRH. In the study by Giordano *et al.* (2012b), most of the ovulations occurred between 28 and 30 h after administering the second GnRH of the Ovsynch protocol. Giordano *et al.* (2012a) reported similar results for OR (90.90%) and ovulation time (28 h after GnRH injection) in lactating dairy cows. In the present study, 58.3% of ovulations in the SALINE group occurred 48 h after the second GnRH of the Ovsynch protocol. The lowest and highest CRs or PRs were observed in the SALINE and H-HCG groups, respectively; however, the differences between the groups were not

significant. In a review article by De Rensis *et al.* (2010) variable results of experiments on the effect of hCG at breeding on CRs were documented. De Rensis *et al.* (2008a) demonstrated that hCG had a positive effect after synchronisation in dairy cows only during the warm period of the year and under the negative effects of heat stress. According to Stevenson & Pulley (2012), pregnancy rate per AI at day 32 or 60 after first AI was lower in lactating Holsten cows treated with 1000 IU of hCG 7 days after TAI than saline-treated cows. By contrast, Alnimer & Shamoun (2015) reported that applying hCG 6 days after TAI was beneficial in improving P/AI either in summer or winter as a result of reducing pregnancy loss and increasing P4 concentration in repeat breeding dairy cows. In the present study, the lowest and highest ORs were occurred in the GnRH (45.4%) and L-HCG (75.0%) groups, which differed significantly. The lowest and highest PRs were obtained in the SALINE (33.3%) and H-HCG (75.0%) groups, respectively; however, the differences in PRs among the groups were not significant. In all studies where the hCG dose ranged widely from 1000 to 10,000 IU, the dose did not seem to affect the ovarian response. In addition, the hCG and GnRH treatments have similar effects on the ovary (De Rensis *et al.*, 2010). In a study, administration of hCG to lactating dairy cows on day 2 post-AI increased P4 production by the nascent CL; however reproductive performance was not improved. The authors hypothesised that the effect of hCG administration varies depending on the physiological status of the animal and the exact timing of hCG administration (Sánchez *et al.*, 2017).

Progesterone is essential for the maintenance of pregnancy and embryo devel-

opment. Therefore, techniques have been assessed to increase fertility by increasing CL function. One proposed method was to treat with GnRH at time of insemination. However, there have been conflicting results with this method. Treatment with GnRH at the time of insemination resulted in an increase, decrease, or no change in subsequent concentrations of progesterone compared to controls (Fields *et al.*, 2009).

The results of the present study demonstrated that administration of hCG at TAI caused an increase in serum P4 concentrations on days 6 and 12 after TAI, although there was no significant difference between the groups that received or not received GnRH or hCG at TAI. It has been reported that hCG administration at insemination increases plasma P4 levels on days 3, 5 or 7 of the estrous cycle, and cows with higher P4 concentrations have more developed embryos (De Rensis *et al.*, 2008a). By contrast, the results of Agarwal *et al.* (2021) on healthy Indian crossbred cows indicated that while hCG administration post AI may enhance primary luteal dimensions or induce the formation of accessory corpora lutea, it apparently had no beneficial effect on luteal function or pregnancy.

## CONCLUSIONS

In summary, the findings of this study demonstrated that administering a single dose of human chorionic gonadotropin at timed artificial insemination, regardless of being low or high, resulted in no significant improvement in the rates of conception and pregnancy in lactating dairy cows synchronised with the Ovsynch protocol combined with an intravaginal progesterone releasing insert regardless of being cyclic or not. The major drawback of the study was the limited number of the ani-

mals allocated to the experiment, so a large scale study is warranted to illustrate more exactly the effects of administering low or high doses of human chorionic gonadotropin on the fertility of lactating dairy cows.

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**Correspondence:**

M. Goli, DVM, DVSc  
Department of Clinical Science,  
Faculty of Veterinary Medicine,  
Razi University, P.O. Box 6715685414,  
Kermanshah, Iran  
tel: +98 83 38329540,  
fax: +98 83 38320041;  
mobile: +98 914 341 8361  
e-mail: mojtabagoli@razi.ac.ir;  
mojtabagoli@yahoo.com;