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Adding equine chorionic gonadotropin to the G6G protocol improves pregnancy rate of dairy cows under heat stress conditions

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Abstract

We studied 663 cyclic lactating Holstein cows in an industrial farm near Qazvin, Iran, from June 2119 to September 2020. We investigated the effects of equine chorionic gonadotropin (eCG) hormone administration 80 h before the implementation of the Ovsynch and fixed-time artificial insemination (OVS + FTAI) program in the form of the G6G protocol on pregnancy rate and reproductive parameters of dairy cows in the first postpartum insemination under heat stress conditions. The cows were randomly assigned to one of the following protocols and received the following treatments: G6G (prostaglandin F2 alpha (PGF2α)-2d-gonadotropin releasing hormone (GnRH)-6d-OVS+FTAI(GnRH(GnRH1)-7d-PGF2α(PG)-56h-GnRH-18h-FTAI, n = 213), 300 international unit (IU) eCG+G6G (300e+G6G) (PGF2α-2d-GnRH-64h-300 IU,eCG-80h-OVS+FTAI, n = 231), and 500 IU eCG+G6G (500e+G6G) (PGF2α-2d-GnRH-64h-500IU,eCG-80h-OVS+FTAI, n = 219). The overall pregnancy rates of cows in the G6G, 300e+G6G, and 500e+G6G protocols were 29.5 ± 0.03%, 33.7 ± 0.03%, and 35.6 ± 0.03%, respectively. The 500e+G6G protocol increased the pregnancy rate of cows with a body condition score (BCS) ≤ 2.5 compared to the G6G protocol (P = 0.04). The 500e+G6G protocol significantly increased the pregnancy rate of cows that produced > 34 kg milk per day compared to the G6G protocol (P = 0.03). In conclusion, using 500 IU, eCG 80 h before the implementation of the OVS+FTAI program in the form of G6G protocol had beneficial effects on increasing the pregnancy rate of cows that had a BCS ≤ 2.5 or produced > 34 kg milk per day compared to the G6G protocol under heat stress conditions.

Keywords: G6G; eCG; pregnancy rate; Holstein cows; heat stress.

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Study contribution

Heat stress reduces the pregnancy rate in cows. Various actions have been done to increase the pregnancy rate of cows under heat stress conditions, such as the use of ambient cooling devices, increasing the amount of energy in the diet and the use of hormones in the form of Ovsynch and timed insemination programs. In this study, the effect of using equine chorionic gonadotropin before the implementation of the G6G protocol on increasing the pregnancy rate of cows under heat stress conditions was evaluated. The results showed that adding 500 international unit of equine chorionic gonadotropin 80 hours before the implementation of the G6G protocol was an effective way to increase the pregnancy rate of high yielding (> 34 kg/day) cows or cows with a low body condition score (BCS ≤ 2.5).

Introduction

Ovulation synchronization programs have been developed to improve reproductive performance in dairy cows[1] Ovulation synchronization protocols in cows can be accomplished through programs that use GnRH and PGF2α.[2]

The G6G is a fixed-time insemination protocol based on GnRH and PGF2α that is widely used in dairy herds. The presynchronization program of the protocol is designed to allow most cyclic cows to be in the early stages of the diestrus phase and ovulate at the time of the beginning of the Ovsynch and fixed-time insemination protocol.[3]

Equine chorionic gonadotropin is a glycoprotein with a relatively long half-life of about 3 days in cows, which is secreted from endometrial cups of pregnant mares and has both effects of follicule stimulating hormone (FSH) and luteinizing hormone (LH).[4, 5] It has been shown that eCG persists for more than 10 days in the bovine circulation.[4] Treatment of dairy cows with eCG reduces the number of atretic follicles and increases the growth of follicles at any size.[6, 7] The use of eCG in low doses (400–600 IU) alone or in the form of synchronization protocols can induce follicular growth and ovulation while higher doses of eCG (1000 IU), especially in the form of synchronization protocols, can cause superovulation.[8, 9] The luteotrophic[10, 11] and micro vascularization[12] effects of eCG on the corpus luteum (CL) of cows have been reported. It has been demonstrated that the positive effects of eCG to increase the pregnancy rate of dairy cows can be distributed to the following reasons: increasing the diameter of the preovulatory follicle,[13, 14] increasing the ovulation rate,[14] and increasing the plasma concentrations of progesterone (P4).[11] It has been reported that adding eCG to synchronization protocols can increase the ovulation and pregnancy rates in cows, especially in the early postpartum period,[15] anoestrous cows,[15-17] older cows,[17] cows with a low BCS,[18-20] high-yielding dairy cows,[8] physiological, and pathological conditions in which the frequency of LH pulses is decreased,[8] and cows that are under seasonal heat stress conditions.[16]

Body condition scoring is one of the most useful tools for assessing the nutritional status of cows.[21] The recommended BCS for dairy cows at early lactation is about 2.75 (in the range of 2.5 to 3.25) based on a 5-point scale.[22] It has been demonstrated that in cows with a lower BCS (< 2.5), the pregnancy rate is

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reduced[23] due to defects that occur in the estrous cycles, the reduction of the quality of the follicles, and inappropriate intrauterine environment to support embryonic survival. The results of a study showed that following the implementation of the Ovsynch protocol in the early lactation period, the pregnancy rate of cows with a BCS ≥ 2.5 was significantly higher than that of cows with a BCS < 2.5.[22] The results of two studies showed that there was a positive relationship between BCS and pregnancy rates of cows following the implementation of the Ovsynch and Presynch-Ovsynch protocols.[18, 24]

Heat stress has negative effects on the pregnancy rate of cows.[25] There are several methods for measuring heat stress. The most common heat stress indicator is the temperature-humidity index (THI).[26] A THI value < 86 units generally does not cause safety problems for healthy cows. At 68 ≤ THI ≥ 74 units, heat stress begins reducing milk yield and reproductive efficiency and for THI > 75 units cows can show noticeable decreases in performances.[27] One study reported a reduction in pregnancy rates of cows in summer compared to winter months in the range of 20-30 %.[28] Moreover, it has been shown that heat stress alters follicular dynamics and delays LH peak formation, which results in aging of the dominant follicle that can lead to a lack of ovulation in cows.[25, 28] Another effect of heat stress on follicular function is the suppression of ovarian follicle dominance.[28] Heat stress can affect the secretion of the uterine PGF2α, leading to premature luteolysis and embryonic death.[28] The circulatory prolactin levels are increased in cows in summer compared to winter months. Prolactin is a hormone that inhibits the release of gonadotropins, thereby inhibiting follicular growth and development.[28] Negative energy balance after parturition or during high milk production will be increased under heat stress conditions due to reduced food intake.[28]

The results of various studies on the effect of milk production on the pregnancy rate in cows are contradictory. Several studies have found a negative relationship between milk production and pregnancy rates in cows.[29-31] The catabolism rate is high in high-yielding cows, so, the serum concentrations of P4 and estrogen (E2) hormones, which have positive effects on pregnancy rate, are lower in these cows.[29, 30] Another study suggests that the secretion of opioids from the mammary gland during lactation inhibits the secretion of gonadotropins.[31]

The major strategy to reduce the effects of heat stress on reproductive performance of the cows has been to alter the environmental conditions by using shade, fans or evaporative cooling. However, this approach has not eliminated all problems associated with heat stress. As a result, additional reproductive strategies are needed to counteract the adverse effects of heat stress. Various treatments have been used to increase the pregnancy rate of cows under heat stress conditions, including the use of fixed-time insemination programs,[32, 33] the use of antioxidants in the diet,[34, 35] and the use of hormones such as GnRH,[36, 37] human chorionic gonadotropin (hCG),[38] eCG,[18-20] P4,[19] and melatonin[39] at different times of the estrous cycle.

The hypothesis of the recent study was that injecting 300 or 500 IU eCG, 80 h before the implementation of the OVS+FTAI program in the form of the G6G protocol can increase the pregnancy rate of cows compared to the G6G protocol in the first postpartum insemination under heat stress conditions.

The aim of the recent study was to investigate the effects of two different doses of eCG injection 80 h before the implementation of the OVS+FTAI program

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in the form of the G6G protocol on reproductive performance and pregnancy rates of dairy cows in the first postpartum insemination under heat stress conditions and comparing the findings with the results of the G6G protocol implementation as the control group.

Materials and methods

Ethical statement

The research was conducted in accordance with the local Bioethics Committee of Medical Faculty of Kermanshah University. Because Ovsynch and timed insemination protocols and uterine ultrasonographic experiments are routine processes on industrial dairy farms, the authors did not consider it necessary to obtain an ethical code.

Animals, feeding, housing

The study was conducted on 663 cyclic lactating Holstein cows in one of the industrial farms near Qazvin province, Iran, from June 2019 to September 2020 to investigate the effects of treatments on the reproductive performance and pregnancy rates of dairy cows in the first postpartum insemination under heat stress conditions.

The cyclicity of the cows under study was confirmed on the basis of history or two consecutive ultrasonographic examinations of the ovaries, 10 days apart, which was started 2 weeks before implementation of the protocols to find a CL at least on an examination. The history was based on the detection of estrous with the aid of tail paint. Detection of estrous was performed 4 times per 24 hour period for 30 minutes (i.e., 6 hour intervals). Cows without complications, such as dystocia, retained placenta, clinical and puerperal metritis, lameness, clinical mastitis, respiratory, and digestive system diseases following the latter parturition were entered the study. The cows had free access to freshwater and were fed twice a day with a total mixed ration consisting of corn and alfalfa silages, hay as forage, soybean meal-based concentrate, vitamins, and minerals balanced to meet requirements for lactating dairy cows. The cows were housed in free stall barns with self-catching head-locks. Free stalls were bedded with mattress and straw. Fans were provided to alleviate the heat stress. We measured ambient temperature and relative humidity at 1-h intervals using ten thermograph devices (8613, B. S. 3231, Classell, London, UK). The termographs were placed in the middle of the barn, approximately 1.6 m above the feeding alley floor, making sure they did not contact the walls, they were protected from direct sunlight, and were placed at a sufficient distance (> 10 m) from recirculating fans. Twenty-four measurements were available for each thermograph for each day for calculating average daily temperatura and relative humidity. The following formula was applied to calculate daily average THI: THI = [1.8 × dry bulb temperature (Tdb) + 32] − [0.55 − 0.0055 × relative humidity (RH)] × [1.8 × Tdb − 26].[40] The average maximum temperature during the months of the implementation of the experiment was 33.2 °C. Average of the temperature-humidity indices were calculated 79–86 during the experiment. The cows were milked twice daily. At the time of the onset of the treatments,

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the average of BCS for the cows was 2.6 ± 0.01 based on a 5-point scale.[41] Cows were divided into two subgroups based on BCS as follows: ≤ 2.5 and > 2.5. At the time of the implementation of the experiments, 116, 132, and 137 cows had a BCS ≤ 2.5 in the G6G, 300e+G6G, and 500e+G6G treatment groups, respectively. The average milk production for the cows under study was 33.8 ± 0.2 kg/day on the day of artificial insemination (AI). In terms of milk yield (kg/day), on the day of AI, cows were divided into two subgroups: ≤ 34 and > 34. On the day of AI, 130, 120, and 125 cows produced ≤ 34 kg milk per day in the G6G, 300e+G6G, and 500e+G6G treatment groups, respectively. The mean number of parities for the study cows was 2.5 ± 0.05. According to the number of parities, cows were classified as primiparous and multiparous. In terms of the number of parities 61, 65, and 62 cows were primiparous in the G6G, 300e+G6G, and 500e+G6G protocols, respectively. The average of serum concentration of P4 (ng/mL) at PG was 2.27 ± 0.02 for the cows studied. From the viewpoint of serum concentration of P4 (ng/mL) at PG, cows were divided into two subgroups: ≤ 2.2 and > 2.2. At PG, 161, 120, and 94 cows had a serum concentration of P4 ≤ 2.2 (ng/mL) in the G6G, 300e+G6G, and 500e+G6G treatment groups, respectively. The study cows were inseminated 62 ± 5 days postpartum.

All treatments including hormonal injections, blood samplings, AIs, and ultrasonographic examinations were done on the constrained cows.

Treatments

Weekly a cohort of cows at 44 ± 5 days in milk (DIM) were stratified by BCS, milk yield, and parity and randomly assigned to one of the treatment protocols. Treatment protocols were G6G, 300e+G6G, and 500e+G6G. The timing of hormonal injections, blood sampling, ultrasonographic examinations, and FTAI for the cows being studied is shown in Figure 1.

The dosage and analog of GnRH used in the recent study were 100 μg per injection of gonadorelin acetate. The dosage and analog of PGF2α used in the recent study were 500 μg per injection of cloprostenol sodium. The dosage and analog of eCG used in this study were 300 and 500 IU per injection equine chorionic gonadotropin. Two technicians performed the AIs of the cows and used two types of conventional semen.

Ovulatory response and pregnancy diagnosis

Ultrasonographic examination of the ovaries was done with a 7.5 MHz linear probe at GnRH1 and 2 days later to investigate the ovulatory response to GnRH1 injection. Before GnRH1 injection, the presence of a dominant follicle on the ovaries was evaluated. The ovulatory response was characterized by the absence of the preovulatory follicle on the ovaries two days after GnRH1 injection. The pregnancy diagnostic test was done 32 days after AIs. The cows that were diagnosed in standing heat in the AI to pregnancy diagnostic test interval were considered nonpregnant and were inseminated at the proper time based on a.m.-p.m. rule.

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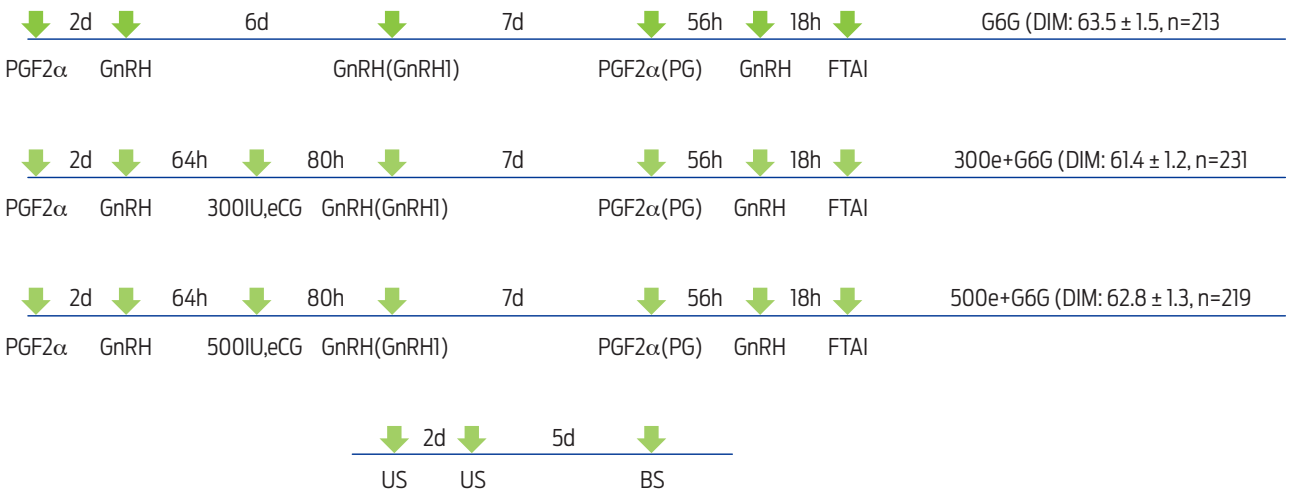

Figure 1. Schematic image of hormonal injections, blood samplings (BS), ultrasonographic examinations (US), and fixed-time artificial insemination (FTAI) for the cows in the G6G, 300 international unit (IU) equine chorionic gonadotropin (eCG)+G6G (300e+G6G), and 500 IU eCG+G6G (500e+G6G) protocols. Days in milk (DIM) = The range of lactation days of cows at the time of the FTAI; PG = prostaglandin f2 alpha (PGF2α) in Ovsynch+FTAI protocol; GnRH1 = The first gonadotropin releasing hormone (GnRH) in Ovsynch+FTAI protocol.

Hormonal assays

Blood sampling was performed (n = 663) to measure the serum concentration of P4 at PG. All blood samples were taken from the coccygeal vein using tubes without an anticoagulant agent. Refrigerated samples were centrifuged (3000 × g for 20 min) within 1 hour after collection and then were kept at -20 °C until P4 concentration was measured. Serum concentrations of P4 (ng/mL) were determined using a commercially available ELISA kit. The intra– and inter-assay coefficients of variation were 3.21 % and 5.43 %, respectively. The sensitivity of the assay was 0.15 ng/mL.

Statistical Analyses

Binomially-distributed data (i.e., ovulation following GnRH1 and pregnancies per AI) and concentration of P4 at PG were analyzed by logistic regression, using the GLIMMIX procedure of SAS (SAS Institute, 2002–2003). Explanatory variables considered for inclusion in the models were treatment, ovulation at GnRH1, serum concentration of P4 at PG (categorized as ≤ 2.2 or > 2.2 ng/mL), BCS (categorized as ≤ 2.5 or > 2.5), parity (primiparous vs multiparous), milk yield (categorized as ≤ 34 or > 34 kg/day), DIM (categorized as ≤ 62 or > 62 days), technician, month of AI, semen type, and interactions. Classification of the variables under study (serum concentration of P4 at PG, BCS, milk yield, and DIM) into two subgroups was done based on the median determination. The final logistic regression model removed variables by a backward elimination. Probability values ≤ 0.05 were considered significant, whereas those between 0.051 and 0.1 were considered trends. The variables that were included in the final model for the analysis of fertility were treatment, BCS, milk yield, and interaction between treatment and milk yield. A univariable analysis with PROC GLIMMIX was used for analyses of treatment effects on DIM, milk yield, parity, BCS, ovulation at GnRH1, and serum concentration of P4 at PG (Table 1).

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Table 1. Effects of study protocols on Days in milk (DIM), milk yield, number of parities, body condition score (BCS), ovulation at GnRH1, serum concentration of P4 (ng/mL) at PG, and overall pregnancy rate in lactating dairy cows (mean ± SEM)

Study groups	n	Studied parameters						
		Days in milk (DIM)	Milk yield (kg/day)	Number of parities	Body condition score (BCS)	Ovulation at GnRH1, %	Serum concentration of progesterone(P4) (ng/ml) at PG	Overall pregnancy rate, %#
G6G	213	63.5 ± 1.5	34.3 ± 0.44	2.44 ± 0.09	2.63 ± 0.02	76.0[a]	1.71 ± 0.04[a]	29.5
300e+G6G	231	61.4 ± 1.2	32.1 ± 0.29	2.47 ± 0.1	2.6 ± 0.02	84.4[ab]	2.4 ± 0.04[b]	33.7
500e+G6G	219	62.8 ± 1.3	33.9 ± 0.35	2.53 ± 0.09	2.58 ± 0.02	89.0[b]	2.6 ± 0.05[bc]	38.3
Total	663	62.3 ± 0.09	33.8 ± 0.2	2.5 ± 0.05	2.6 ± 0.01	83.2	2.27 ± 0.02	32.1
Significance		NS†	NS††	NS†††	NS††††	*	**	NS†††††

300e+G6G = Injection of 300 international unit (IU), equine chorionic gonadotropin (eCG) 80 h before the first gonadotropin releasing hormone (GnRH1) in the Ovsynch+ fixed-time artificial insemination (OVS+FTAI) program of the G6G protocol, 500e+G6G = Injection of 500 IU, eCG 80 h before GnRH1 in the OVS+FTAI program of the G6G protocol; PG = prostaglandin f2 alpha (PGF2α) in the OVS+FTAI program of the G6G protocol.

#This analysis was done with GLIMMIX and accounted for BCS and milk yield. NS: not significant.

[abc]Different superscripts in each column show significant difference.

†Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.35$, $P = 0.54$, and $P = 0.42$, respectively;

††Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.33$, $P = 0.52$, and $P = 0.48$, respectively;

†††Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.65$, $P = 0.34$, and $P = 0.44$, respectively;

††††Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.87$, $P = 0.82$, and $P = 0.91$, respectively;

†††††Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.12$, $P = 0.07$, and $P = 0.11$, respectively;

*Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.07$, $P = 0.04$, and $P = 0.23$, respectively;

**Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.04$, $P = 0.02$, and $P = 0.32$, respectively.

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Results and discussion:

A comparison of the means of the DIM, milk yield, number of parity, BCS, and overall pregnancy rate between the study protocols showed no statistically significant difference (Table 1).

In the recent study, the injection of 300 and 500 IU eCG, 80 h before the implementation of the OVS+FTAI program in the form of the G6G protocol, did not significantly increase the pregnancy rate of cows compared to the G6G protocol in the first postpartum insemination under heat stress conditions. However, when the comparison of the pregnancy rates of cows was done based on the BCS or milk production, it was found that in cows with a low BCS (≤ 2.5) or high milk yield (> 34 kg/day), the implementation of the 500e+G6G protocol significantly increased the pregnancy rate of cows compared to the G6G protocol ($P = 0.04$, $P = 0.03$, respectively).

In the recent study, 500e+G6G increased the ovulation rate at GnRH1 compared to the G6G protocol ($P = 0.04$, Table 1). There was a tendency to increase the ovulation rate at GnRH1 for the 300e+G6G compared to the G6G protocol ($P = 0.07$, Table 1). Equine chorionic gonadotropin has the effects similar to FSH and LH, and stimulates follicular growth and ovulation in a dose dependent manner.[4, 5] In cows that ovulate at the beginning of the Ovsynch protocol, the synchrony rate and serum concentrations of P4 in the luteal phase before insemination is increased, which can increase pregnancy rates compared to the cows that do not ovulate at the mentioned time.[42] The result of the recent study indicates the effect of eCG injection on increasing the growth and development of the preovulatory follicle in cows under heat stress conditions and in this regard, injection of 500 IU eCG is more effective than injection of 300 IU eCG. The beneficial effects of using eCG on the growth and development of ovarian follicles in cows under heat stress conditions have been documented.[5]

In the recent study, serum concentrations of P4 at PG were increased significantly following the implementation of the 300e+G6G and 500e+G6G protocols compared to the G6G protocol ($P = 0.04$ and $P = 0.02$, respectively, Table 1). This result is due to the increase in ovulation at GnRH1 and possibly the formation of larger and more qualified ovulatory follicles and CLs following eCG administrations. Equine chorionic gonadotropin administration stimulates follicular growth in cows.[6, 7] The larger follicle creates larger CL after ovulation and in turn, P4 production will be increased. It has been demonstrated that under heat stress conditions, the profile of the gonadotropins in the serum, which has direct effects on the growth of ovarian follicles, is changed, and this phenomenon negatively affects the growth and development of the ovulatory follicles.[28, 43] With effects similar to those of FSH and LH, eCG has beneficial effects on the growth and development of ovarian follicles.[5] The grown follicles secrete more amounts of E2 and are more likely to produce the LH peak to induce ovulation.[5] The effects of eCG injection remain in the blood for more than 10 days,[4] and because eCG has the same effects as LH, it increases the secretion of P4 by affecting the CL.[5] Findings of a study showed that serum concentrations of P4 were increased significantly on day 5 post insemination in cows that received 600 IU eCG at the time of PGF2α injection in the Ovsynch protocol compared to the cows that did not receive eCG at the mentioned time.[44] It was demonstrated in a study that injection of 400 IU eCG at the time of the last PGF2α injection in the Presynch-Ovsynch protocol increased the number of CLs on

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Table 2. Association of different equine chorionic gonadotropin (eCG) concentrations with different levels of body condition score (BCS) on the pregnancy rates of dairy cows (%, mean ± SEM)

Study groups	n	BCS		
		≤ 2.5	> 2.5	*P*-value[#]
G6G	213	23.2 ± 0.04[a]	37.1 ± 0.05	0.03
300e+G6G	231	28.7 ± 0.04[ab]	40.0 ± 0.05	0.04
500e+G6G	219	33.5 ± 0.04[b]	39.0 ± 0.05	0.23
Total	663	28.8 ± 0.02	38.8 ± 0.03	0.02
Significance[#]		*	NS[†]	

300e+G6G=Injection of 300 international unit (IU), eCG 80 h before the first gonadotropin releasing hormone (GnRH1) in the Ovsynch+ fixed-time artificial insemination (OVS+FTAI) program of the G6G protocol; 500e+G6G = Injection of 500 IU, eCG 80 h before GnRH1 in the OVS+FTAI program of the G6G protocol.

[#]This analysis was done with GLIMMIX and accounted for milk yield. NS: not significant.

[abc]Different superscripts in each column show significant difference.

[†]Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.56$, $P = 0.61$, and $P = 0.83$, respectively;

*Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.12$, $P = 0.04$, and $P = 0.25$.

the ovaries compared to the cows that did not receive eCG at this time.[45] Various studies have highlighted the positive effects of high serum concentrations of P4 in the luteal phase before insemination to increase pregnancy rates in cows.[46-49]

The effect of BCS on pregnancies per AI of cows following the implementation of G6G, 300e+G6G, and 500e+G6G protocols is shown in Table 2.

The results of the recent study showed that for cows with a low BCS (≤ 2.5) at AI, the pregnancy rate of cows in the 500e+G6G protocol was significantly higher than that of cows in the G6G protocol ($P = 0.04$, Table 2). It was demonstrated in several studies that fertility was decreased in cows with a low BCS at the time of AI.[24, 50, 51] In cows with a low BCS, due to the low concentration of gonadotropins in the serum, the quality of follicles and CL is low, resulting in low P4 production.[51-53] Presumably, an injection of eCG 80 h before starting the OVS+FTAI program in the form of G6G protocol can stimulate the growth of ovarian follicles and dose-dependently increase the quality of follicles and CLs. Therefore, a qualified follicle produces a high-quality CL that will produce enough P4 to support the survival of pregnancy.[47, 48] The results of the recent study are consistent with the results of several other studies that found, using eCG in the 400–500 IU dose range to increase pregnancy rates in the form of ovulation synchronization protocols had the greatest effect on cows with a BCS < 2.75.[18-20] In the recent study for G6G and 300e+G6G protocols, there was a statistically significant difference between the pregnancy rates of cows with a high (> 2.5) versus a low (≤ 2.5) BCS ($P = 0.03$, $P = 0.04$, respectively, Table 2). However, no such difference was found for the 500e+G6G protocol ($P = 0.23$, Table 2). This result confirms that the implementation of the 500e+G6G protocol has beneficial effects on increasing the pregnancy rate in cows with a low BCS (≤ 2.5) and has little effects on increasing the pregnancy rate in cows with a high BCS (> 2.5, Table 2). In cows with a low BCS, the secretion of gonadotropins may not be sufficient to produce qualified follicles,[51] whereas injection of eCG, with FSH like effects, can induce the growth and development of the preovulatory follicles. The grown preovulatory follicle, by secreting

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Table 3. Association of different equine chorionic gonadotropin (eCG) concentrations with different levels of milk yield on pregnancy rates of dairy cows (%, mean ± SEM)

Study groups	n	Milk yield, kg/day		*P*-value[#]
		≤ 34	> 34	
G6G	213	35.3 ± 0.04	20.4 ± 0.04[a]	0.04
300e+G6G	231	37.5 ± 0.04	29.0 ± 0.04[ab]	0.09
500e+G6G	219	35.2 ± 0.04	36.1 ± 0.05[b]	0.5
Total	663	36.0 ± 0.02	29.1 ± 0.03	0.06
Significance[#]		NS[†]	*	

300e+G6G=Injection of 300 international unit (IU), eCG 80 h before the first gonadotropin releasing hormone (GnRH1) in the Ovsynch+ fixed-time artificial insemination (OVS+FTAI) program of the G6G protocol;
500e+G6G = Injection of 500 IU, eCG 80 h before GnRH1 in the OVS+FTAI program of the G6G protocol.
[#]This analysis was done with GLIMMIX and accounted for body condition score. NS: not significant.
[abc]Different superscripts in each column show significant difference.
[†]Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.71$, $P = 0.89$, and $P = 0.63$, respectively;
*Difference between G6G & 300e+G6G, G6G & 500e+G6G, and 300e+G6G & 500e+G6G at the levels $P = 0.09$, $P = 0.03$, and $P = 0.11$.

large amounts of E2 and inducing LH surge, can prevent the follicle from aging and thus increase the pregnancy rate.[5]

The effect of milk yield (kg/day) on pregnancies per AI of cows following the implementation of G6G, 300e+G6G, and 500e+G6G protocols is shown in Table 3.

In the recent study, it was found that the implementation of the 500e+G6G protocol significantly increased the pregnancy rate in high yielding cows compared to the G6G protocol ($P = 0.03$, Table 3). There was a tendency to increase the pregnancy rate in high yielding cows in the 300e+G6G compared to the G6G protocol ($P = 0.09$, Table 3). It has been shown that the serum concentration of reproductive hormones in high producing dairy cows is lower than low producing cows because the rate of catabolism is higher in high producing dairy cows.[29, 30] However, high milk production inhibits the secretion of gonadotropins, through the opioids that are secreted from the mammary gland.[29-31] Due to the lower concentration of gonadotropins in the serum of high producing cows, the growth rate of ovarian follicles is reduced and the possibility of developing a persistent preovulatory follicle is increased.[29] It has been demonstrated that the pregnancy rate of cows is decreased following ovulation a persistent follicle.[49] Nevertheless, hormonal disorders and ovarian cysts are more likely to occur in high yielding cows.[54] In the recent study in the G6G protocol, there was a statistically significant difference between the pregnancy rates of high producing (> 34 kg/day) and low producing (≤ 34 kg/day) cows ($P = 0.04$, Table 3), while no such difference was found in the 300e+G6G and 500e+G6G treatment protocols. This result confirms that the implementation of the 300e+G6G and 500e+G6G protocols to increase the pregnancy rate is more effective on high yielding (> 34 kg/day) dairy cows (Table 3). Equine chorionic gonadotropin, with FSH like effects, helps the growth of the preovulatory follicle and the formation of a qualified follicle, which after ovulation will increase the chance of pregnancy. The ovulation of a qualified follicle produces a qualified CL that can produce enough P4 to support the pregnancy too. Moreover, the presence of eCG in serum for more than 10 days after injection[4], and the fact

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that it has LH like effects,[5] stimulate the secretion of P4 by the CL. The increasing serum concentration of P4 in the luteal phase after AI leads to increasing embryonic growth and provides an appropriate intrauterine environment for survival and growth of the embryo, and thus, a higher likelihood of pregnancy.[55] Some studies have shown that there is a negative relationship between milk yield and pregnancy rates in dairy cows.[29, 30] It has been stated that using eCG to increase the pregnancy rate in the form of ovulation synchronization protocols is more effective in multiparous cows and cows with physiological or pathological disorders in the secretion of adequate amounts of LH.[8, 17]

Conclusions

This study demonstrated that the use of 500 IU eCG, 80 h before the implementation of the OVS+FTAI program, in the form of the G6G protocol, had beneficial effects on increasing the pregnancy rate of cows that had a BCS ≤ 2.5 or produced > 34 kg milk per day compared to the G6G protocol in the first postpartum insemination under heat stress conditions.

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Data availability

Raw data are publicly available at https://data.mendeley.com; doi: 10.17632/3rm57jbf7g.1.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

Author contributions

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Formal analysis: H Kohsari.
Funding acquisition: F Mohammadi.
Investigation: F Mohammadi.
Methodology: Kh Berenjian.
Project administration: Kh Berenjian.
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