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The Effect of Administering Equine Chorionic Gonadotropins (eCG) on Reproductive Performance of Dairy Cows with a CO-Synch + CIDR Protocol and Insemination at a Fixed Time

Research Article

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ABSTRACT

The success of a fixed-time artificial insemination (FTAI) program depends on a high ovulation rate during a short interval. Equine chorionic gonadotropin (eCG) has been used in FTAI protocols to improve follicular growth and thereby to produce larger, more responsive follicles for ovulation with increased pregnancy rates. The aim of the present study was to evaluate the effects of eCG on conception rates of early postpartum dairy cows treated by progesterone-based estrous synchronization with artificial insemination at a fixed time (CO-Synch+CIDR protocols). 144 Holstein cows ≥ 55 days postpartum were used. All cows were enrolled in a 7-day CO-Synch + CIDR protocol which included 100 µg gonadotropin releasing hormone (GnRH) at CIDR insertion (day 0); 500 µg prostaglandin F2α (PGF2α) at CIDR removal (day 7); and 100 µg GnRH at FTAI, 66 hours after PGF2α (day 10). Cows were randomly assigned as control cows (n=69) or treated cows (n=75) which received 500 IU eCG at the time of CIDR removal. All cows were examined by ovarian ultrasound at FTAI. The addition of eCG to CO-Synch + CIDR protocols improved the frequency of estrous response (control cows 51.4%, eCG-treated cows 87.8%; P<0.01). It reduced the interval between the end of treatment and the start of estrus (control cows 56.8±5.52 hours, eCG-treated cows 45.7±5.87 hours; P<0.01). It increased dominant follicular size at FTAI (control cows 15.1±2.41 mm, eCG-treated cows 19.6±1.84 mm; P<0.001). However, eCG had no effect on pregnancy rates (control cows 49.3%, eCG-treated cows 54.6%; P=0.131). The use of eCG, prior to termination of 7-day estrus synchronization when using the CO-Synch + CIDR program alone, could hasten the time of estrous expression and should be considered in FTAI dairy cows.

KEY WORDS CIDR, dairy cows, equine chorionic gonadotropin, estrus synchronization.

INTRODUCTION

Equine chorionic gonadotropin (eCG) is synthesized by endometrial cups in pregnant mares and induces the formation of accessory corpora lutea. In cattle, eCG shows high Luteinizing hormone (LH) and follicle-stimulating hormone (FSH)-like activities and has a high affinity for both LH and FSH receptors in the ovaries. Bcause of the action, eCG is utilized in veterinary mediciene for the control of reproductive activity in the cow (De Rensis and Lopes-Gatius, 2014). eCG improves the development and the ovulation rate of the dominant and pre-ovulatory follicle. In cattle, eCG has been used in fixed-time artificial insemination (FTAI) protocols to improve ovarian follicular growth and thereby to produce a larger, more responsive follicle for ovulation with increased pregnancy rates (Siqueira *et al.* 2009). It has been reported that administration of eCG in lactating dairy cows at the time of luteolysis in a timed artificial insemination (TAI) protocol tended to increase both CL volume and plasma progesterone and increased pregnancy rate in cows induced to ovulate with gonadotrophin-releasing hormone (GnRH) and cows with low body condition score (BCS). Furthermore, it has been reported that eCG administration after the use of a progesterone device may increase the synchrony of ovulation and improve the conception rate in FTAI protocols (Bó *et al.* 2013). The success of a FTAI program is dependent on a high ovulation rate during a short interval. Therefore, the dominant follicle must acquire the capacity to ovulate in response to an induction ovulation treatment. Bovine follicles acquire the capacity to ovulate when they reach a diameter of approximately 10 or 12 mm (Geary *et al.* 2001; Sartori *et al.* 2001). It is noteworthy that the positive efficiency of eCG may or may not be observed in cyclic animals, but the it is evident in animals whose LH secretion and ovarian activity are reduced or compromised, such as during the early period, under seasonal heat stress, in anestrus animals or in animals with low BCS (De Rensis and Lopes-Gatius, 2014; Souza *et al.* 2009). Many studies of beef cows demonstrated that eCG increased the percentage of cows that ovulated to the TAI protocol, increased circulating P4 following FTAI and increased fertility when P4-based FTAI protocols were supplemented with eCG concurrent with removal of the P4 insert (Baruselli *et al.* 2004; Sales *et al.* 2011; Souza *et al.* 2009). Since the early reports have shown that eCG had LH and FSH stimulating effect on follicular development, the possibility of improving the conception rate by including eCG in synchronization and FTAI protocols has been evaluated by many researchers (Dorneles Tortorella *et al.* 2013). This experiment was conducted to evaluate whether eCG at the time of PGF injection and CIDR insert removal had an effect on the estrous response, size of dominant follicles at inseminationTAI and pregnancy rates. The hypothesis was that administration of eCG before removing the progesterone intravaginal device in synchronization and TAI protocols with use of CO-Synch + CIDR will improve reproduction performance in early postpartum lactating dairy cows.

MATERIALS AND METHODS

Animals and treatment

This study was performed on two dairy farms located in Mashhad province in northeastern Iran. 144 multiparous Holstein Friesian cows, at least 55 days in milk (DIM), at unknown stages of the estrus cycle were selected. After gynecological examination by veterinarians, cows with normal uterus and uterine discharge or cows diagnosed as not pregnant were included. Heifers and cows in first parity were excluded.

Cows with a history of lameness, acute mastitis or any systemic illness within ten days before the CIDR insert were excluded. All cows had average BCS of ≥ 2.75, milk production (daily average in the beginning of treatment) of 27.2 ± 0.4 kg/d. Cows were milked twice daily and fed a TMR of corn silage and alfalfa silage as forage, with a corn-soybean meal-based concentrate. The experimental period was September to December 2012. All cows underwent the Cosynch-CIDR protocol with FTAI.

Experimental design

Each cow received a controlled, internal drug release device containing 1.9 g of progesterone (CIDR TM, InterAg, Hamilton, New Zealand) at an unknown stage of the estrous cycle (experimental day 0). The CIDR remained in place for seven days, with an injection of GnRH (250 µg gonadorelin acetate, Gonadobred®, IPA co, Australia) on day 0 and one of PGF2 (500 µg, Cloprostenol sodium, estro-Plan®, IPA co, Australia) on day 7. Following CIDR removal on day 7, cows were randomly assigned into two groups. The control group (CO-Synch-CIDR, n=69) received no further treatment, whereas the eCG group (Co-synch-CIDR+eCG, n=75) received 500 IU eCG (Folligon, Intervet®, Holland). Each cow received TAI with semen of known fertility 64-66 hours after CIDR removal (day 10) with an injection of 0.25 mg gonadorelin. Each cow's ovaries were examined by transectal ultrasonography (Sonoace 600 with 5.0 MHz linear-array transducer; Medison Co. Ltd., Seoul, Korea) on day 10 to observe the size of large follicles. Following CIDR removal, cows were observed for signs of estrus using visual observation every four hours until FTAI was performed. Cows observed to be in estrus in this period were recorded. AI was conducted by the same operator during the course of this study. Pregnancy diagnosis was determined 42-45 days after TAI by ultrasonography and rectal palpation. Pregnancy was confirmed based on the echodensity of the fluid in the uterine horns and the presence of an embryonic heartbeat.

Statistical analysis

Statistical analyses were performed using SAS (2004). Results are shown as mean ± SD. The frequency of estrus response within three days after CIDR removal was calculated separately for the two experimental groups. The highest number of cattle showed signs of estrus within 12 hours after CIDR removal were considered as the tightness of estrus synchrony; it is shown as a percentage. Because the data were not normally distributed the interval from CIDR removal and the onset of estrus and the mean diameter of the largest follicles at the time of FTAI were analyzed by the Mann-Whitney test. Pregnancy rate per TAI was defined as the percentage of cows that were confirmed pregnant at the single pregnancy diagnosis after one TAI. Pregnancy rates following TAI were analyzed using Fisher's Exact test. Results with (P<0.01) were considered significant.

RESULTS AND DISCUSSION

The addition of eCG to a CO-Synch + CIDR improved the frequency of estrous response (control 51.4%, eCG 87.8%, P<0.01; Table 1). It decreased the interval between CIDR removal and the onset of estrous (control 56.8±5.5 hours, eCG 45.7±5.9 hours, P<0.001; Table 1).

Table 1 Effects of eCG at CIDR removal on reproductive performance of dairy cows with a CO-Synch protocol and insemination at fixed time

Variables	Groups		
	Control	eCG	P-value
Estrus response ≤ 66 h (n/n, %)	(35/69) 51.4%	(65/75) 87.8%	P < 0.01
Beginning of estrus (hrs)	45.7±5.9	56.8±5.5	P < 0.001
Intensity of estrus response (hrs)	48-60	36-48	P < 0.01
Tightness of estrus (n/n, %)*	(30/35) 88.2%	(49/65) 75.4%	P > 0.01
Largest follicle at TAI (mm)	15.1±0.29	19.6±0.21	P < 0.001
Pregnancy ate (n/n, %)	(41/75) 54.6	(34/69) 49.3	P= 0.131

* Tightness of estrus synchrony was calculated within 12 hours between 48.60 and 36-48 for control and equine chorionic gonadotropin (eCG) groups, respectively.

It increased the intensity of the estrus response after CIDR removal (control 36-48 hours, eCG 48-60 hours; Figure 1). However, it had no effect on the tightness of estrus synchrony within 12 hours (control 75.8%, eCG 88.2%, P>0.05; Figure 1). It increased the final follicular size at the time of FTAI (control 15.1±2.41 mm, eCG 19.6±1.84 mm, P=0.001; Figure 2). It did not improve pregnancy rates (control 49.3%. eCG 54.4%, P=0.13). However, it improved the conception rate by 1.73 (P=0.04; 95% CI=0.89-3.36; Figure 3).

Previous authors reported that eCG administration after the use of a progesterone device increased follicular development and ovulation (Garcia-Ispierto *et al.* 2013; Meng Chao *et al.* 2010; Sheldon and Dobson, 2000). In the present study, eCG reduced the interval between the end of treatment and the onset of estrus. These findings agree with a finding that treatment with PGF2α plus eCG and GnRH 48 hours later followed by FTAI in dairy cows with silent ovulation, improved the frequency of spontaneous estrus (Garcia-Ispierto *et al.* 2013). Another study showed that eCG administration during the post-partum period stimulated relatively early oestradiol production and increased the intensity of the oestrus response (Sheldon and Dobson, 2000). Meng Chao *et al.* (2010) showed that oestrus intensity differed between natural oestrus and induced oestrus and also between the methods of synchronization.

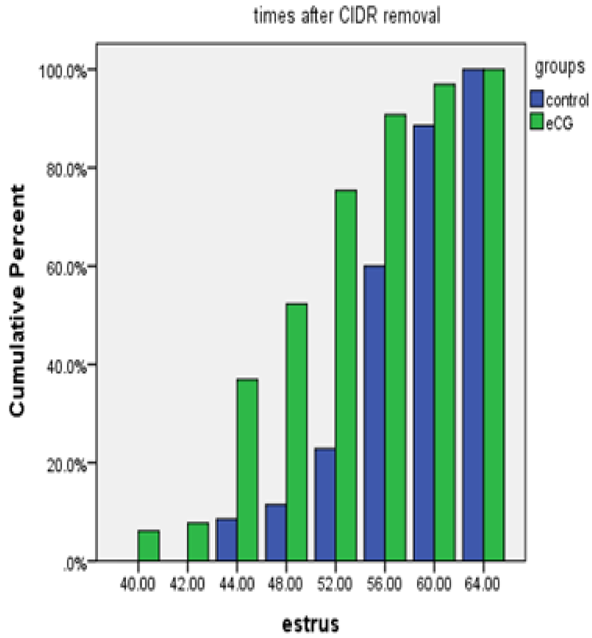

Figure 1 Histogram of cumulative percent the interval between the end of treatment and the start of estrus in control and eCG groups

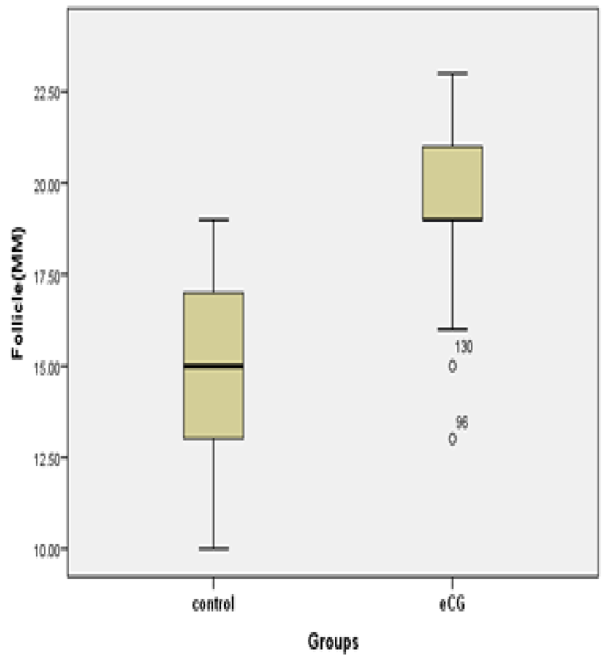

Figure 2 Histogram of the comparison of final follicular size (mm) at the time of FTAI in the control and eCG groups

The present study clearly indicates that eCG injection, before the termination of seven-days estrus synchronization when using the CO-Synch + CIDR program, could hasten the onset of estrous.

It should be considered in FTAI programs. Previously it was reported that one of the most difficult problems of TAI for lactating dairy cows was a decrease in circulating estradiol concentrations prior to TAI (Souza *et al.* 2009).

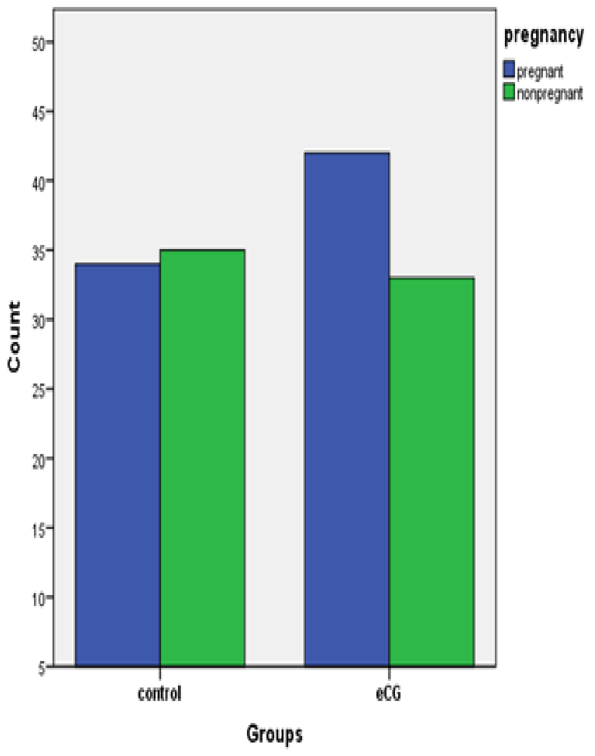

Figure 3 Pregnancy rates in dairy with Co-Synch + CIDR protocol and insemination at fixed time in control and eCG groups

It seems that an addition eCG to a Co-Synch + CIDR protocol improves the frequency of estrous response due to an effect on follicular development. It is noteworthy that eCG shows long-lasting LH and FSH-like activity on the theca and granulosa cells of the follicle-inducing oestradiol secretion.

Thus eCG improves the development of the dominant and pre-ovulatory follicles (De Rensis and Lopes-Gatius, 2014; Marquezini *et al.* 2013; Souza *et al.* 2009). Furthermore, it has been reported that eCG injection may be an important tool for the enhancement of follicular growth, ovulation, size and function of the subsequent corpus lutea and pregnancy rates in progestin-based FTAI protocols in *Bos indicus* heifers (Sá Filho *et al.* 2010). The present study shows that eCG increased the size of dominant follicles at the time of insemination. This finding might be explained by a different rate of follicular development.

In contrast to these results, the addition of 400 or 600 IU eCG to progestin-based FTAI protocols has been found inefficient in altering follicular and luteal dynamics (Ferreira *et al.* 2013). Also in that study, an increase in pregnancy rate followed AI in highly productive dairy cows that were more than 150 DIM.

However, in other studies, supplementation of eCG at the end of progestin-based treatment increased the diameter of the ovulatory follicle in anestrous beef cattle (Bryan *et al.* 2013). Physiological effects of eCG seem to be evident in beef cows with a relatively low BCS (Sales *et al.* 2011).

Interestingly, the results of the present study showed that eCG addition failed to increase pregnancy rates in dairy cows with a CO-Synch + CIDR protocol and FTAI. These results are in close agreement with two further experiments, that eCG addition failed to increase pregnancy rates either in cows receiving a presynch-ovsynch protocol or in herds with a low percentage of cows with poor BCS (Ferreira *et al.* 2013; Pulley *et al.* 2013).

Earlier reports have shown that the FTAI using protocols combining GnRH, progesterone, PGF2α and estrogens normally produce consistent conception rates (De Rensis and Lopes-Gatius, 2014; De Rensis and Peters, 1999; Thatcher *et al.* 2001). Furthermore, it has been reported that the positive effects of eCG are clearly detectable in cows in anestrus or under nutritional stress or in cows with low BCS particularly when BCS < 2.75 (Garcia-Ispierto *et al.* 2013).

It is pertinent to mention that all cows in the experiment reported here had average BCS of ≥ 2.75 and were cyclic and had not high daily milk production. The present study showed that eCG administration when using the CO-Synch + CIDR program had no effect on pregnancy rates in dairy cows.

In contrast to our results, other studies showed that eCG administration after the use of a progesterone device increased the synchrony of ovulation and improved the conception rate in FTAI protocols (Garcia-Ispierto *et al.* 2013; Geary *et al.* 2001).

In this Experiment an eCG dose of 500 IU was used. It seems that, this amount of eCG is insufficient to cause multiple ovulation in dairy cows. The effect of eCG on the development of medium and large follicles is variable and depends on the dose administered and the stage of the oestrus cycle at which eCG is given. Generally, it is accepted that the 'standard' doses of eCG required to promote single ovulation should range between 200 and 1000 IU, while the doses required to induce superovulation should be approximately 2500 IU (Bellows and Short, 1972; Yaniz *et al.* 2004).

CONCLUSION

In general, results of this investigation show eCG administration at CIDR device removal when using the CO-Synch + CIDR program only increased the growth rate of the largest follicle (LF) from implant removal to TAI, the diameter of the LF at TAI, and rates of ovulation and did not any effect on pregnancy rates in dairy cows. We conclude that eCG is a viable alternative to increase follicle growth rate and diameter before FTAI. The variability of pregnancy outcomes in response to eCG may be related to the dose and timing of eCG treatment, BCS and days postpartum of the cows. More research is warranted to clarify the effect of eCG in low BCS cows.

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