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An investigation on the possibility of using eCG for presynchronization prior to ovsynch in dairy cow

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Abstract:

BACKGROUND: Presynchronization could enhance fertility in timed breeding programs. The best presynchronization method has to justify biological requirements and to provide practical and economic advantages. **OBJECTIVES:** Present study investigated the effectiveness of presynchronization with equine chorionic gonadotropin (eCG) before ovsynch (eCG-ovsynch) compared to conventional presynch-ovsynch program (2PG-ovsynch) in lactating cows. **METHODS:** Healthy lactating cyclic Holstein cows (n=110) without any particular pre, peri and post parturient problems were selected for this study. The first insemination following timed breeding program was conducted at 57 days after calving. Ovsynch (timed breeding program) consisted of two GnRH analogue injections, at 7 days before and 56 hours after PG, followed by insemination, 16 hours after the second GnRH. Cows in 2PG-Ovsynch group (n=55) received two PGs, 14 days apart, followed by ovsynch program 12 days later. Cows in eCG-Ovsynch group (n=55) received eCG (500 IU, IM), 84 hours before ovsynch program. Pregnancy was confirmed 40 d after insemination by rectal palpation. Progesterone concentrations were evaluated at presynchronization, at the first GnRH and at PG of ovsynch program. **RESULTS:** The respective first service conception rates, days open and service per conception were similar between 2PG-ovsynch (45.45%; 94.0±6.30 days; 1.95) and eCG-ovsynch (40%; 90.1±4.58 days; 1.81) groups ($p>0.05$). The percentage of cows with high progesterone concentrations increased significantly from the first GnRH (38.2%) until PG (85.4%) in eCG-ovsynch group ($p<0.05$). The percentage of cows with high progesterone concentrations at the time of PG injection of ovsynch program was greater in eCG-ovsynch (85.4%) compared to 2PG-ovsynch (69%). **CONCLUSIONS:** Administration of eCG (500 IU), 84 hours prior to ovsynch could optimize timed breeding program biologically, practically and economically in lactating dairy cows.

Introduction

Ovsynch program provided the basis for timed breeding in cattle (Pursley et al., 1995). The program initiates with synchronization of follicular wave using GnRH injection followed by inducing luteolysis using prostaglandin F2α and inducing ovulation using GnRH prior to blind insemination (Pursley et al., 1995). Success of ovsynch program depends on (1) the degree of follicle wave synchronization following the first GnRH (Bello et al., 2006; Chebel et al., 2006; Stevenson et al., 2012; Vasconcelos et al., 1999), (2) the presence of functional corpus luteum at the first (Galvão and Santos, 2010; Stevenson et al., 2012) and the second GnRH (Bello et al., 2006; Rutigliano et al., 2008) and (3) greater ovulatory response to the second GnRH (Galvão and Santos, 2010; Rutigliano et al., 2008).

The follicular wave was not synchronized in some cows (approx. 30%) following the first GnRH of ovsynch program (Navanukraw et al., 2004; Vasconcelos et al., 1999). Therefore, several approaches (presynchronization programs), were suggested to overcome this problem including: 2 PG (14 days apart), 10-14 days prior to ovsynch (Moreira et al., 2001; Navanukraw et al., 2004), PG-GnRH, 2 days apart, 6 days prior to ovsynch (G6G) (Bello et al., 2006), two successive ovsynchs, 7 days apart (double ovsynch) (Souza et al., 2008), PG+GnRH, 7 days prior to ovsynch (Martins et al., 2017) and GnRH, 7 days prior to ovsynch (Carvalho et al. 2014; Lopes et al. 2013). Fertility could be enhanced following presynchronization-ovsynch program compared to ovsynch alone (Bello et al., 2006; Galvão et al., 2007; Navanukraw et al., 2004).

Besides the biological aspects and outcomes of the presynchronization before ovsynch program, the cost of medicine and labor and the duration of timed breeding program (economic aspects) are important and indicate the superiority of one program to another. Therefore, introducing a program that along with simplicity could incorporate both biological and economic aspects is demanded by cattle industry.

More recently, we have found that administration of eCG (500 IU) at the random stage of follicular development (follicle emergence, dominant and early static phases) could enhance follicle growth followed by the presence of ovarian follicle responsive to GnRH, 84 hours after eCG injection (Hosseini et al. 2017). We have hypothesized that a single injection of eCG, 84 hours prior to ovsynch program could provide an efficient, simple and cost-effective presynchronization program for dairy cows. The objective of the present study was to compare two timed breeding programs: eCG-ovsynch and 2PG-ovsynch, in lactating dairy cows.

Materials and Methods

Experimental location and animals: This experiment was conducted at a commercial dairy farm located in the south of Tehran-Iran (latitude: 35°23'17.79"N; longitude: 51°17'8.37"E; altitude: 972 m) between November 2016 and April 2017. According to Köppen and Geiger climate classification (Kottek et al., 2006) the farm is classified in a cold semi-arid region in the world map (BSk). The herd consisted of 1388 cows with an annual rolling herd average of 11,977 kg of milk. Cows were milked thrice daily and classified according to their milk production, housed in openshed barn, received TMR according to NRC recommendations (NRC, 2001) thrice daily and had free access to fresh water.

Experimental design: Healthy Holstein dairy cows (n=110) without any problem after parturition such as dystocia, retained placenta, clinical endometritis, clinical mastitis, lameness, displaced abomasum were enrolled in this study. Since Day 22 after calving, cyclicity was confirmed, on a weekly basis, by the presence of CL using a real-time linear array, B-mode ultrasound scanner (Emperor-EMP V9; Shenzhen, China) equipped with a 7.5 MHz rectal probe. Cyclic cows were assigned into two experimental groups including 2PG-ovsynch or eCG-ovsynch, considering their parity and milk production. Treatment of the cows was scheduled such that the first service was initiated from 57 days postpartum. BCS of cows, on the scale of 1 to 5 (Edmonson et al. 1989), were recorded at the time of AI.

Ovsynch program consisted of two GnRH (100 µg, Gonadorelin acetate, GONAbreed®, Parnell, Australia) 224 hours apart, in association with PG, 56 hours before the second GnRH. Artificial insemination was performed 16 hours after the second GnRH (Figure 1). Pregnancy was diagnosed at 40 days after AI by rectal palpation. Non-pregnant cows following first insemination were inseminated based on estrus detection and monitored until 200 days in milk.

Blood sampling and progesterone assay: Blood samples were collected three times: at the start of presynchronization programs, at the first GnRH and at PG injection of ovsynch program and kept at 4 °C. Blood samples were centrifuged within 3 hours after collection, at 3000 rpm for 20 min, and serum samples were stored at -20 °C until assay for progesterone. Progesterone was measured by ELISA using commercial kit (Progesterone ELISA, DRG Instruments, GmbH, Germany). The sensitivity, intra and inter-assay coefficients of variation were 0.045 ng/ml, 5.4% and 9.96%, respectively.

Statistical analyses: Analysis of binary response data was performed by logistic regression in SAS (SAS, 2012). For first service conception rates, the initial model contained the following categorical explanatory variables: parity (primiparous vs. multiparous), treatment (presynch vs. eCG-ovsynch), level of milk production (high vs. low), and BCS (<2.75 vs. ≥ 2.75), as well as the interactions between these variables. The final logistic regression model removed variables by a backward elimination based on the Wald statistics criterion when $p > 0.20$.

Differences in milk production, days in milk, lactation number, BCS and progesterone concentrations at the start of presynchronization, at the first GnRH and PG of ovsynch program, were determined using GLM procedure of SAS. Survival analyses (Proc Lifetest) were used to evaluate the effects of treatment on interval to conception. For interval to conception, the survival analysis regressed the proportion of cows that were not conceived. Data for cattle that were not included in the statistical analyses were considered as censored observations. Differences between the survival curves were tested with the Wilcoxon test.

All progesterone concentrations, measured at different occasions of the experiment, were pooled and analyzed for the normality of distribution. In case that the distribution was not normal according to Shaprio-Wilk criteria, the data was transformed and categorized accordingly. Value

Table 1. Reproductive indices of lactating Holstein cows that received two different presynchronization protocols (eCG vs 2PG) prior to ovsynch program. Data were presented as mean±SEM and percentages.

	2PG-ovsynch	eCG-ovsynch	p-value	Total
Cows number	55	55		110
Days in milk	61.3±0.54	61.7±0.47	0.53	61.5±0.35
Parity	2.60±0.22	3.05±0.30	0.23	2.82±0.19
BCS	2.68±0.03	2.75±0.04	0.18	2.67±0.02
Milk production (kg)	41.9±1.39	40.9±1.17	0.08	41.4±0.90
First service conception rates				
Total	45.4% (25/55)	40% (22/55)	0.56	42.7 (47/110)
Primiparous	50% (9/18)	40% (6/15)	0.60	45 (15/33)
Multiparous	43% (16/37)	40% (16/40)	0.14	41 (32/77)
High BCS (≥2.75)	56% (18/32)	31% (9/29)	0.06	44 (27/61)
Low BCS (<2.75)	30% (7/23)	50% (13/26)	0.18	40 (20/49)
Days open	94.0±6.30	90.1±4.58	0.78	92.0±3.83
Service per conception	1.95±0.17	1.81±0.11	0.73	1.88±0.10

Table 2. Progesterone concentrations of lactating Holstein cows that received two different presynchronization protocols (eCG vs 2PG) prior to ovsynch program. Data were presented as mean±SEM and percentages.

	2PG-ovsynch	eCG-ovsynch	p-value
At the start of presynchronization	2.86±0.32	3.65±0.43	0.17
At the first GnRH of ovsynch	4.61±0.27	3.67±0.34	0.03
At PG of ovsynch	5.25±0.33	5.91±0.29	0.14
At PG of ovsynch			
Pregnant cows	5.16±0.52	5.76±0.39	0.37
Not pregnant cows	5.20±0.43	6.01±042	0.19
At the start of presynchronization			
Low <1.5	25 (45.45%)	23 (41.82%)	0.72
Medium ≥1.5 - <4	10 (18.18%)	11 (20.00%)	0.54
High ≥4	20 (36.36%)	21 (38.18%)	0.63
At the first GnRH of ovsynch			
Low <1.5	4 (7.27%)	15 (27.27%)	0.05
Medium ≥1.5 - <4	14 (25.45%)	19 (34.55%)	0.96
High ≥4	37 (67.27%)	21 (38.18%)	0.03
At PG of ovsynch			
Low <1.5	4 (7.27%)	3 (5.45%)	0.62
Medium ≥1.5 - <4	13 (23.64%)	5 (9.09%)	0.24
High ≥4	38 (69.1%)	47 (85.45%)	0.02

less than the first interquartile range was considered as low progesterone concentrations. Values between the first and the second interquartile ranges were considered as medium. Values equal to and/or more than the second interquartile range were considered as high progesterone concentration. Data were presented as mean±SEM (Range) and percentages.

Results

Milk production, parity (number of primiparous and multiparous) and BCS

Table 3. Frequency comparison of progesterone categories at the first GnRH and PG injection of Ovsynch program in lactating Holstein cows that received two different presynchronization protocols (eCG vs 2PG) prior to ovsynch program. Data were presented as percentages.

	GnRH	PG	p-value
2PG-ovsynch			
Low <1.5	4 (7.3%)	4 (7.3%)	1.00
Medium ≥1.5 - <4	14 (25.5%)	13 (23.6%)	0.84
High ≥4	37 (67.3%)	38 (69.1%)	0.90
eCG-ovsynch			
Low <1.5	15 (27.3%)	3 (5.4%)	0.01
Medium ≥1.5 - <4	19 (34.5%)	5 (9.1%)	0.009
High ≥4	21 (38.2%)	47 (85.4%)	0.003

were similar between experimental groups (p>0.05; Table 1). First service conception rates (FSCR) were similar between 2PG-ovsynch (25/55; 45.45%) and eCG-ovsynch (22/55; 40%) groups (p>0.05; Table 1). There was no effect of parity and BCS on FSCR (p>0.05; Table 1). There was no effect of parity in each experimental group on FSCR (p>0.05; Table 1). Cows with high BCS (≥2.75) in 2PG-ovsynch (56%) tended to have greater FSCR than those in eCG-ovsynch group (31%; p=0.06; Table 1). In 2PG-ovsynch group, FSCR tended to decrease in cows with low BCS (30%) compared to those with high BCS (56%; P=0.06). However, such tendency was not observed in eCG-ovsynch group (p=0.16). After 200 days in milk, days open (2PG-ovsynch: 94.0±6.30 days; eCG-Ovsynch: 90.1±4.58 days; Table 1), survival curves (Fig. 2) and the number of service per conception (2PG-ovsynch: 1.95; eCG-ovsynch: 1.81) were similar between experimental groups (p>0.05; Table 1).

Progesterone concentrations were similar between experimental groups at the start of presynchronization and at the time of PG administration (p>0.05; Table 2). However, at the first GnRH of the ovsynch program, the mean concentration of progesterone was greater in 2PG-ovsynch (4.61 ng/mL) than eCG-ovsynch program (3.67 ng/mL; p= 0.03; Table 2). According to distribution analysis of the data, progesterone concentrations were categorized into 3 classes: low (<1.5 ng/ml), medium (1.5 - 3.99 ng/ml) and high (≥4 ng/ml). Accordingly, at the first GnRH administration, the percentage of cows with high progesterone concentrations was greater in 2PG-ovsynch (67.3%) than eCG-ovsynch (38.2%) group (p=0.03; Table 2). However, such difference was reversed at the time of PG injection (2PG-ovsynch: 69.1%; eCG-ovsynch: 85.4%; p=0.02; Table 2). At the time of GnRH, the number of cows with low progesterone concentrations tended to be greater in eCG-ovsynch (27.3%) than in 2PG-ovsynch (7.3%; p=0.05; Table 2). In eCG-ovsynch group, from the first GnRH to PG injection, the percentage of cows with low (GnRH: 27.3% to PG: 5.4%; p=0.01) and medium (GnRH: 34.5% to PG: 9.1%; p=0.009) progesterone concentrations decreased (Table 3); whereas, the percentage of cows with high progesterone concentrations (GnRH: 38.2% to PG: 85.4%; p=0.002) increased. Such difference was not observed in 2PG-ovsynch group (p>0.05; Table 3). At the time of PG for ovsynch program, proges-

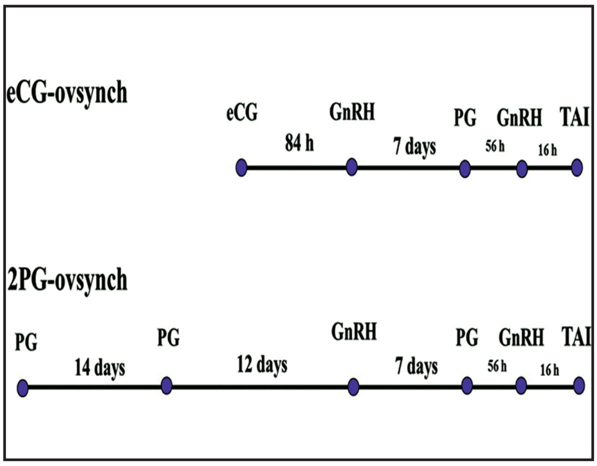

Figure 1. Experimental design of two timed breeding protocols that involved two different presynchronization programs (eCG or two prostaglandin F2α) followed by ovsynch in Holstein dairy cows.

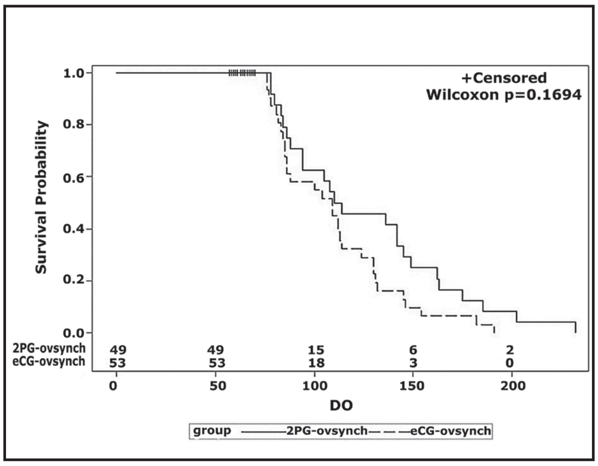

Figure 2. Survival analysis of interval from parturition to pregnancy for lactating Holstein cows that received two different presynchronization protocols (eCG vs 2PG) prior to ovsynch program.

terone concentrations were not different between pregnant and not pregnant cows in both groups (p>0.05; Table 2).

Discussion

The objective of the present study was to compare two presynchronization programs (eCG and 2PG) prior to ovsynch in Holstein dairy cows. The result of first service conception rates was similar between eCG-ovsynch (40%) and 2PG-ovsynch (45.4%) programs. Different kinds of presynchronization strategies have been evaluated to optimize response to ovsynch protocol. Some of these strategies with the respective conception rates were: two PG, 14 days apart, with the last GnRH 10-14 days prior to Ovsynch [36.9-49.6%] (Galvão et al., 2007; Moreira et al., 2001; Navanukraw et al., 2004), PG and GnRH, 2 days apart, with the interval of 6 days between GnRH and ovsynch [50%] (Bello et al., 2006), preparatory ovsynch, 7 days prior to the main ovsynch [49.7%] (Souza et al., 2008), simultaneous administration of PG and GnRH, 7 days before ovsynch [43%] (Martins et al., 2017), single GnRH, 7 days prior to ovsynch [28-37%] (Bruno et al., 2014; Lopes et al., 2013). The rationale behind all of these programs was to have responsive ovarian follicle to the first GnRH of ovsynch and synchronization of follicular wave prior to the second ovsynch in association with functional CL at the time of PG of ovsynch. The result of the present study as far as conception rates is concerned is within the range of previous results.

To the best of our knowledge, this is the first study in which eCG was used for presynchronization prior to ovsynch program. eCG is a glycoprotein with a relative long half-life of about 3 days secreted by the endometrial cups of pregnant mares and has both FSH and LH-like activity in cattle (Murphy and Martinuk, 1991). Treatment of dairy cattle with eCG resulted in fewer atretic follicles, recruitment of smaller follicles (≤ 5 mm) with an increased growth rate, and sustained growth of medium (6-8 mm) and large (≥9 mm) follicles (Driancourt et al., 1991; Macmillan and Peterson, 1993; Monniaux et al., 1984; Newcomb et al., 1979; Păcală et al., 2010). Administration of eCG can increase ovulation rate (Garcia-Ispierto et al., 2012; Păcală et al., 2010; Rostami et al., 2011), especially in early postpartum cows (Rostami et al., 2011), in cows suffering long anestrus or under seasonal heat stress (Garcia-Ispierto et al., 2012) and in cows with low body condition score (Souza et al., 2009). Fertility could be improved in cows that received eCG at the end of estrus synchronization programs in dairy (Ferreira et al., 2013; Pulley et al., 2013; Souza et al., 2009) and beef cows (Cutaia et al., 2003). The latter effect of eCG could be attributed to three main effects: a) increase in the diameter of the preovulatory follicle (Dias et al., 2009; Marques et al., 2005; Peres et al., 2009; Sá Filho et al., 2010b), b) improvement of ovulation rate and pregnancy (Dias et al., 2009; Marques et al., 2005; Peres et al., 2009; Sá Filho et al., 2010a), c) improvement of plasma progesterone concentrations during the next luteal phase (Baruselli et al., 2004).

Even though in the present study we have not done ultrasonography throughout timed breeding program, the difference in the number of cows with high progesterone concentrations at the first GnRH and PG of ovsynch program indicated that the number of ovulations and formation of functional CL were substantially enhanced in eCG treated group. In eCG-ovsynch group, the percentage of cows with high progesterone concentrations was increased from 38.2 to 85.4% (47.2% increment; $p<0.01$). Whereas such elevation was not evident in 2PG-ovsynch group (67.3 to 69.1%; $p>0.05$). This result confirms our primary findings that single injection of eCG (500 IU) at random stage of follicular development (follicle emergence, dominant and early static phases) could enhance follicle growth followed by the presence of ovarian follicle responsive to GnRH, 84 hours later (Hosseini et al. 2017). Conclusively, eCG could increase the percentage of cows responsive to the first GnRH of ovsynch program. Previous presynchronization protocols were also able to increase the percentage of cows with functional CL at the time of PG injection in ovsynch program including: 2PG, 10 days prior to ovsynch [85-87%] (Stevenson, 2011; Stevenson and Pulley, 2012) and double-ovsynch [84-88%] (Ayres et al., 2013; Souza et al., 2008). Increased concentration of progesterone resulted in decreased LH pulse frequency (Sanchez et al., 1995; Wehrman et al., 1993) followed by improved competency of the dominant follicle that could ovulate at smaller size (Brusveen et al., 2009; Wiltbank et al., 2011) with better quality of the ovulated oocyte (Mihm et al., 1994; Revah and Butler, 1996) and produce good quality embryos (Wiltbank et al., 2011).

Progesterone concentrations at the time of PG administration in ovsynch program is an important indicator for subsequent fertility. Cows with low progesterone concentrations at this time had reduced fertility (Bisinotto et al., 2010; Denicol et al., 2012; Lopes et al., 2013). On the other hands, the greater circulating concentrations of progesterone at this time was associated with improved fertility (Bello et al., 2006; Martins et al., 2011). In the present study, mean progesterone concentration was high in pregnant and non-pregnant cows as well as eCG-ovsynch and 2PG-ovsynch treated cows ($p>0.05$). Therefore, in the present study, any reduction of fertility in experimental groups could not be explained by the hypothesis of low progesterone concentrations at the time PG administration.

The effect of parity on FSCR is controversial. In the current study, parity had no effect on FSCR similar to the study that used double-ovsynch protocol (Brusveen et al., 2009; Giordano et al., 2013; J O Giordano et al., 2012). In contrast, in some studies using double-ovsynch or GnRH, 7 days prior to ovsynch, primiparous cows had better FSCR than multiparous cows (Giordano et al., 2012; Lopes et al., 2013; Souza et al., 2008). DeJarnette and Marshall found advantageous effect of 2PG-ovsynch for multiparous but not primiparous cows (DeJarnette and Marshall, 2003). It is generally accepted that primiparous cows are more likely to be anovular compared with multiparous cows (Chebel et al., 2006; El-Zarkouny et al., 2004; Silva et al., 2007). The lack of effect of parity on FSCR may be due to selection of cyclic cows at the beginning of the present study. Further studies are suggested to elucidate the effect of presynchronization with eCG on primiparous and anestrus cows.

In the present study, cows in 2PG-ovsynch group, with low BCS, tended to have less FSCR (30%) compared to cow with high BCS (56%; P=0.06). This is in agreement with previous result using the same protocol (Souza et al., 2008). In eCG-ovsynch, there was no difference in FSCR in cows with low and high BCS, implying a eCG may have beneficial effect on the cows with low BCS. Advantageous effects of eCG in dairy cows are perhaps restricted to animals with low body condition scores (BCS) at beginning of the protocol for timed breeding (Souza et al., 2009), in anestrus (Bryan et al., 2010; Garcia-Ispierto et al., 2012; Souza et al., 2009; Veneranda et al., 2006) or in older cows (Bryan et al., 2010).

Overall, eCG-ovsynch had similar biological effects compared to 2PG-ovsynch program. Practically, eCG-oysynch involves just single extra injection prior to ovsynch program and requires 14 days to accomplish presynch-ovsynch program compared to 2PG-ovsynch that involves two more injections and 36 days to complete the whole program. Moreover, eCG-ovsynch reduced the final cost of the presynchronization program compared with 2PG-ovsynch. In conclusion, the combination of eCG and ovsynch, 84 hours apart, provides a biologically sound, simple, short and cost effective program for timed breeding in Holstein dairy cows.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۲، شماره ۲، ۹۶-۸۵

بررسی امکان استفاده از eCG در برنامه پیش همزمانی قبل از برنامه اوسینک در گاو شیری

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چکیده

زمینه مطالعه: برنامه‌های پیش همزمانی باعث بهبود میزان باروری در برنامه‌های تلقیح در زمان ثابت می‌شوند. بهترین برنامه پیش همزمانی در گاوهای شیری روشی است که بتواند علاوه بر تکیه بر اصول بیولوژیک، از لحاظ اقتصادی و کاربردی بر سایر روش‌ها برتری داشته باشد. **هدف:** مطالعه حاضر به بررسی کارایی دو روش پیش همزمانی شامل یک تزریق eCG و یا دو تزریق پروستاگلندین (PG، روش متداول) قبل از برنامه اوسینک (eCG-Ovsynch و ۲PG-Ovsynch) می پردازد. **روش کار:** در این مطالعه از ۱۱۰ رأس گاو شیری هولشتاین سالم دارای چرخه فحلی مناسب که فاقد هر گونه مشکلی قبل، در حین و پس از زایش بودند انتخاب شدند. برنامه ریزی به ترتیبی انجام شد تا تمامی گاوها دارای اولین تلقیح در زمان ثابت در فاصله ۵۷ روز پس از زایش باشند. برنامه اوسینک (تلقیح در زمان ثابت) شامل دو تزریق آنالوگ GnRH (۱۰۰ µg، عضلانی) در فواصل ۷ روز قبل و ۵۶ ساعت بعد از تزریق آنالوگ PG (۵۰۰ µg، عضلانی) و سپس تلقیح دام ها در فاصله ۱۶ ساعت پس از دومین تزریق GnRH صورت پذیرفت. گاوهای موجود در گروه ۲PG-Ovsynch (تعداد= ۵۵ رأس) دو تزریق PG به فاصله ۱۴ روز دریافت داشته و سپس برنامه اوسینک، ۱۲ روز پس از دومین تزریق PG آغاز شد. گاوهای موجود در گروه eCG-Ovsynch (تعداد= ۵۵ رأس) یک تزریق eCG (۵۰۰ واحد بین المللی، عضلانی) در فاصله ۸۴ ساعت قبل از برنامه اوسینک دریافت داشتند. تشخیص آبستنی در فاصله ۴۰ روز پس از تلقیح به روش آزمون راست روده‌ای صورت پذیرفت. ارزیابی غلظت پروژسترون در روز شروع برنامه پیش همزمانی، در روز شروع برنامه اوسینک و در روز تزریق PG برنامه اوسینک صورت گرفت. **نتایج:** بترتیب نرخ آبستنی در اولین تلقیح، روزهای باز آبستنی و تعداد تلقیح به ازاء آبستنی در گروه ۲PG-Ovsynch (۴۵/۴۵٪؛ ۹۴/۰±۶۳/۳۰ days؛ ۱/۹۵) و eCG-Ovsynch (۴۰٪؛ ۹۰/۱±۴/۵۸ days؛ ۱/۸۱) یکسان بود (p<۰/۰۵). در گروه eCG-Ovsynch، فراوانی دام‌های با غلظت بالای پروژسترون از زمان شروع برنامه اوسینک (۳۸/۲٪) تا روز تزریق PG برنامه اوسینک (۸۵/۴٪) به طور معنی‌داری افزایش یافت (p<۰/۰۵). فراوانی دام‌های با غلظت بالای پروژسترون در زمان تزریق PG برنامه اوسینک در گروه eCG-Ovsynch (۸۵/۴٪) بالاتر از گروه ۲PG-Ovsynch (۶۹/۱٪) بود (p<۰/۰۵). **نتیجه‌گیری نهایی:** یک تزریق eCG (۵۰۰ واحد بین المللی) در فاصله ۸۴ ساعت قبل از برنامه اوسینک می‌تواند برنامه پیش همزمانی مناسبی قبل از آغاز برنامه تلقیح در زمان ثابت فراهم سازد که نه تنها از لحاظ بیولوژیکی بلکه بدلایل کوتاه نمودن برنامه و در بر داشتن هزینه کمتر، از نظر کاربردی و اقتصادی برای گاوهای شیری مناسب‌تر باشد.

واژه‌های کلیدی: eCG، اوسینک، پیش همزمانی، پروستاگلندین، تلقیح در زمان ثابت

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