Comparison of the effects of Presynch-Ovsynch, Presynch-Ovsynch+CIDR, and G6G protocols on the reproductive performance of lactating Holstein cows in the postpartum period

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Abstract

This study was performed on an industrial dairy farm near Tehran province, Iran, on 666 lactating Holstein cows from September 2019 to June 2020 in order to eliminate the harmful effects of heat stress on the reproductive performance of the cows. The hypothesis of the recent study was that by increasing the serum concentration of progesterone (P4) in the luteal phase before insemination in the form of Presynch-Ovsynch+CIDR (POC) and G6G protocols, the pregnancy rate of cows would be increased compared to the Presynch-Ovsynch (PO) protocol. The aim of the present study was to evaluate the effects of Presynch-Ovsynch (PGF$_{2\alpha}$-14d-PGF$_{2\alpha}$-12d-Ovsynch (OVS: GnRH(GnRH1)-7d-PGF$_{2\alpha}$(PG)-2d-GnRH)-18h-Fixed-time artificial insemination (FTAI), *n*=212), Presynch-Ovsynch+CIDR (similar to the PO protocol, plus the use of a CIDR for 7 days, from the start of the OVS+FTAI protocol to the time of PG injection, *n*=230), and G6G (PGF$_{2\alpha}$-2d-GnRH-6d-OVS+FTAI, *n*=224) protocols on the reproductive performance and pregnancy rate of lactating Holstein cows. The average body condition score (BCS) for the cows was about 2.5 at the time of the onset of the OVS+FTAI program. The average daily milk production for cows at the time of the start of the OVS+FTAI program was 38.3 kg/day. On average, the studied cows were inseminated 86 days postpartum. Implementation of the G6G protocol in comparison to the PO and POC protocols increased the serum concentration of P4 at GnRH1 (*P*=0.04). The cows that received the G6G protocol had a greater number of corpuses luteum (CL) on their ovaries at PG in comparison to the cows in the PO and POC protocols (*P*=0.03 and *P*=0.05, respectively). For all treatment protocols, the pregnancy rate

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of cows with >2 CLs on their ovaries at PG was significantly higher than for cows with ≤2 CLs on their ovaries at this time. The overall pregnancy rate for all cows studied was 42%. Separately, pregnancy rates for cows in PO, POC, and G6G protocols were calculated at 36.7%, 41.7%, and 47.3% respectively. The highest and the lowest pregnancy rates were obtained in the G6G and PO protocols, respectively (P=0.03). It can be concluded that the G6G protocol increased the pregnancy rate of cows in comparison to the PO but not to the POC protocol. This result can be attributed to the increase in serum concentration of P4 at GnRH1 and the greater number of CLs at PG following implementation of the G6G compared to the PO protocol. Although CIDR administration in the POC protocol led to an increase in the pregnancy rate of cows compared to the PO protocol, possibly by increasing the synchrony rate and the quality of the ovulatory follicle, this increase was not statistically significant (P=0.09).

Key words: *Presynch-Ovsynch; Presynch-Ovsynch+CIDR; G6G; reproductive performance; Holstein cows*

Introduction

Ovulation synchronization programs in cows have been developed to help dairy farmers use more effective reproduction strategies (Ayad et al., 2015). Presynch-Ovsynch, Presynch-Ovsynch+CIDR, and G6G protocols are the most common ovulation synchronization programs based on GnRH and $PGF_{2\alpha}$ (Pursley et al., 1995; Vasconcelos et al., 1999; de Sousa Sales et al., 2019; Borchardt et al., 2020).

Implementation of the Presynch-Ovsynch protocol allows the greatest number of cyclic cows to be on days 5–10 of the oestrous cycle at the time of the 1st GnRH injection, which is the best time to start the Ovsynch and fixed-time insemination program (Keskin et al., 2010; Bisinotto et al., 2015). Normally, the Presynch-Ovsynch protocol is started at 25–28 days postpartum, and over 25–28 days, three oestruses are produced (Abdel Aziz and Abdel-Wahab, 2017).

The Presynch-Ovsynch+CIDR protocol has the features of the Presynch-Ovsynch protocol plus a CIDR that is administered in the interval between the 1st GnRH and $PGF_{2\alpha}$ injections in the Ovsynch and fixed-time insemination program (Bisinotto et al., 2015). Using a CIDR in the latter protocol inhibits premature oestrous, increases the synchrony rate, improves oocyte and embryo quality and decreases uterine $PGF_{2\alpha}$ secretion in response to oxytocin stimulation in the post insemination luteal phase in cows (Bisinotto and Santos, 2011; Dadarwal et al., 2013; Bisinotto et al., 2015). It has been reported that high serum concentrations of P4 during the preinsemination luteal phase increase the pregnancy rate in dairy cows due to decreased secretion of LH (Bisinotto et al., 2010; Bisinotto and Santos, 2011; Wiltbank et al., 2011; Dadrawal et al., 2013). Mihm et al. (1994) and Bridges and Fortune (2003) stated that persistent preovulatory follicle and extended duration of dominance are yielded by high levels of circulatory LH, which are induced by low serum concentrations of P4 during the luteal phase of the reproductive cycle. Higher serum concentrations of LH also induce oocytes to undergo premature resumption of meiosis (Revah and Butler, 1996). The results of a study showed that the fertility of cows that ovulated persistent follicles was compromised (Melo et al., 2018). Reduction in pregnancy rate has been expressed in cows that had no active CL on their ovaries at the beginning of the Ovsynch protocol compared to cows that had an active CL on their ovaries at this time (Wiltbank et al., 2011). Some studies suggest that high producing dairy cows with an active CL on their ovaries may have insufficient serum concentrations of P4 to support the growth of the qualified dominant follicle or pregnancy, and this phenomenon is due to the high rate of catabolism in these cows (Sangsritavong et al., 2002; Bisinotto et al., 2015). Progesterone supplementation is useful in such cows to increase the pregnancy rate following the implementation of fixed-time insemination programs (Stevenson et al., 2006; Colazo et al., 2013).

Implementation of the G6G protocol increases the chance of starting the Ovsynch protocol on days 6–7 of the oestrous cycle (Astiz and Fargas, 2013). In the G6G protocol, the 1st injection of $PGF_{2\alpha}$ degenerates probable CL(s) on the ovaries and the injection of GnRH in the presynchronization program induces a new follicular wave and ovulation of a preovulatory follicle which may be present on the ovaries (Dirandeh et al., 2015a; Yousuf et al., 2016).

In various studies, the pregnancy rates of cows following the implementation of the Presynch-Ovsynch, Presynch-Ovsynch+CIDR, and G6G protocols have been reported in the ranges of 47.7-48.4% (Kawate et al., 2004; Keskin et al., 2010), 40-53% (Bisinotto and Santos, 2011; Dadarwal et al., 2013), and 34.8–57% (Dirandeh et al., 2015a; Yousuf et al., 2016), respectively. These differences may be due to farm management differences, different breeds of cows, different seasons, different intervals between parturition and insemination, differences in the type and dosage of drugs used, and so on.

The hypothesis of a present study was that increasing the serum concentration of P4 in the luteal phase before insemination in the form of Presynch-Ovsynch+CIDR and G6G protocols, and increasing the synchrony rate and quality of ovulatory follicle, would increase the pregnancy rate of cows in these protocols compared to the Presynch-Ovsynch protocol.

The aim of the current study was to compare the effects of the Presynch-Ovsynch, Presynch-Ovsynch+CIDR, and G6G protocols on the reproductive performance and pregnancy rate of lactating Holstein cows in the postpartum period.

Materials and methods

Animals, feeding, housing

This study was performed on an industrial dairy farm near Tehran province, Iran, on 666 lactating Holstein cows from September 2019 to June 2020 in order to eliminate the harmful effects of heat stress on the reproductive performance of the cows. The cows had free access to freshwater and were fed twice a day with a mixed ration consisting primarily of corn and alfalfa silages, soybean meal-based concentrate, minerals and vitamins balanced to meet the requirements for lactating dairy cows. Cows were housed in free stall barns with self-catching head-locks. Free stalls were bedded with mattress and straw. The cows were milked three times daily. At the time of the onset of the OVS+FTAI program, the average BCS for the cows was about 2.5. On average, the cows in the study were inseminated 86 days postpartum. Cows were divided into two subgroups based on days in milk (DIM) at AI with 86 days as the cutoff (i.e., ≤86 d and >86 d). The average daily milk production for cows at the time of the start of the OVS+FTAI program was 38.3 kg/day. In terms of milk yield (kg/day) on the day of AI, cows were divided into two subgroups (≤38 and >38). On average, the number of parities for cows in the study was 2.4, and based on the number of parities, cows were classified into primiparous or multiparous subgroups. Classification of the studied variables (DIM and milk yield) into two subgroups was done based on the median determination. Cows without complications such as dystocia, retained placenta, clinical and puerperal metritis, lameness, clinical mastitis, respiratory and digestive system diseases following the recent parturition entered the study. All procedures including hormonal injections, blood sampling, ultrasonographic examinations, and AIs were carried out on the constrained cows. The pregnancy diagnosis test was performed with a 7.5 MHz linear probe (Aloka SSD-900V, Aloka Co. Ltd., Wallingford, CT) 30 days post-AI. The cows that were diagnosed in standing heat in the AI to pregnancy diagnosis interval were considered nonpregnant and were inseminated at the proper time. The research was conducted in accordance with the local Bioethics Committee of Medical Faculty of Kermanshah University (approval ID: IR.KUMS.REC.1399.444).

Treatments and AI

Each week, a cohort of cows at 50±24 DIM were classified based on parity, milk yield, and DIM, and randomly assigned to one of the treatment protocols. Treatment protocols were defined as follows: Presynch-Ovsynch (PO), Presynch-Ovsynch+CIDR (POC), and G6G. The timing of hormonal injections, blood sampling, ultrasonographic examinations, and fixed-time artificial insemination for the cows is shown in Figure 1.

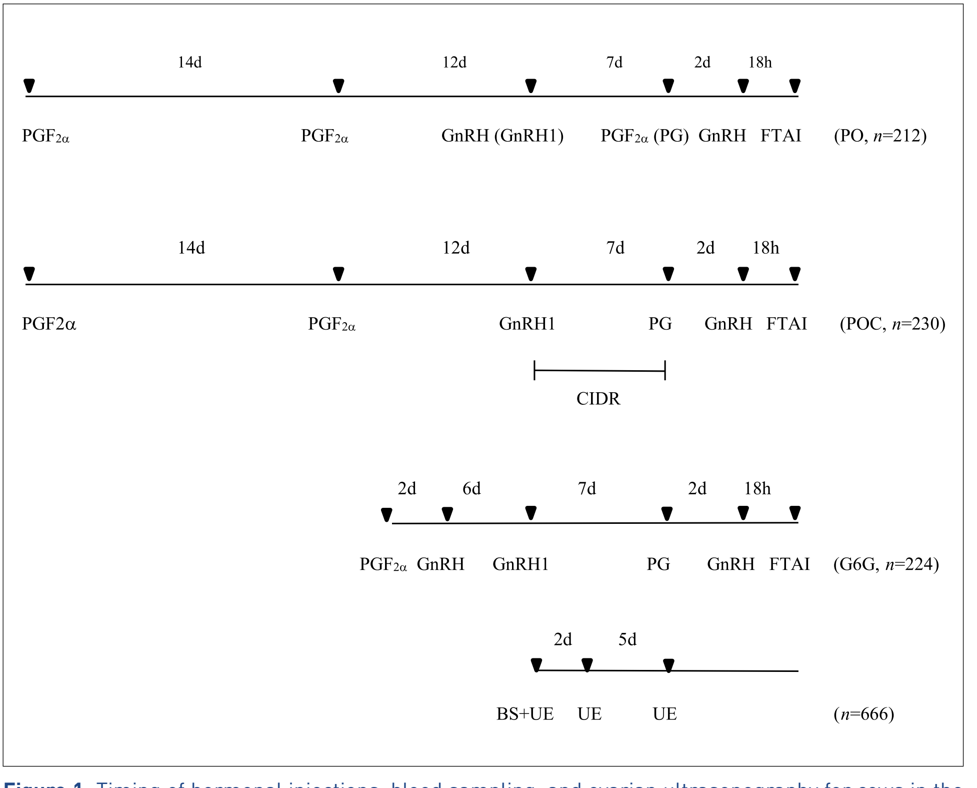

Figure 1. Timing of hormonal injections, blood sampling, and ovarian ultrasonography for cows in the PO, POC, and G6G protocols. BS: Blood sampling. UE: Ultrasonographic examination

The dosage and analogue of GnRH used in this study were 100 µg per injection of gonadorelin acetate (GONAbreed, Parnell, Alexandria NSW 2015, Australia). The dosage and analogue of $PGF_{2\alpha}$ used in this study were 500 µg per injection of cloprostenol sodium (estroPLAN, Parnell, Alexandria NSW 2015, Australia). Two technicians performed the AIs of cows and used two types of conventional semen.

Hormonal assays

Blood sampling was performed (n=666) to measure the serum concentration of P4 at GnRH1. A serum concentration of P4 ≥0.5 ng/mL indicated the presence of an active CL(s) on the ovaries (Wijma et al., 2017). 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 kept at -20°C until analysis. Serum concentrations of P4 (ng/mL) were determined using a commercially available ELISA kit (Competitive EIA-LS-F10072, Lifespan Biosciences, Inc, Seattle, WA). The sensitivity of the assay was 0.15 ng/mL. The intra- and inter-assay coefficients of variation were 3.4% and 5.1%, respectively.

Analysis of ovulatory response and luteal development

Ultrasonographic examination of the ovaries was performed at GnRH1, two days later, and just before PG injection (Figure 1). Ovulatory response was characterized by the absence of a preovulatory follicle(s) on the ovaries two days after GnRH1. The number of CLs present on the ovaries was assessed just before PG injection. Active ovaries were determined by high serum concentrations of P4 (≥0.5 ng/mL) at GnRH1 or ovulation following the GnRH1 injection.

Statistical analyses

Binomially-distributed data (pregnancies per AI, ovulatory response to GnRH1), DIM, milk yield (kg/day), number of parities, serum concentration of P4 at GnRH1 (ng/mL), number of CL(s) at PG were analysed by logistic regression, using the GLIMMIX procedure of SAS (version 9.1, SAS Inst. Inc., Cary, NC, USA). Explanatory variables considered for inclusion in the models were protocol (PO, POC, and G6G), ovulatory response to GnRH1, DIM (categorized as ≤86 or >86 d), milk yield (categorized as ≤38 or >38 kg/day), number of parities (primiparous vs multiparous), serum concentration of P4 (ng/mL) at GnRH1 (categorized as ≤2.8 or >2.8), number of CLs at PG (categorized as ≤2 or >2), technician, type of semen, month of AI, and interactions. The final logistic regression model removed variables by a backward elimination based on the probability value statistics criterion when P>0.1. For analysis of pregnancy status 30 days post AI, the final model included the effects of protocol (PO, POC, and G6G), serum concentration of P4 at GnRH1 (categorized as ≤2.8 or >2.8 ng/mL), number of CLs at PG (categorized as ≤2 or >2), interaction between protocol and parity, and interaction between protocol and number of CLs at PG. A univariable analysis with Proc GLIMMIX was used for analyses of protocol effects on DIM, milk yield, number of parities, serum concentration of P4 (ng/mL) at GnRH1, number of CLs at PG, and ovulatory response to GnRH1 (Table 1).

Results

There was no statistically significant difference between the protocols in terms of DIM, milk yield, number of parities, or ovulatory response to GnRH1 (Table 1).

Table 1. Mean (±SEM) effects of studied protocols on DIM, milk yield, number of parities, serum concentration of P4 (ng/ml) at GnRH1, number of CLs at PG, ovulatory response to GnRH1, and overall pregnancy rate in lactating dairy cows

Studied groups	Studied parameters						
	DIM (range)	Milk yield (kg/day) (range)	Number of parities (range)	Serum concentration of P4 (ng/mL) at GnRH1 (range)	Number of CLs at PG (range)	Ovulatory response to GnRH1	Overall pregnancy rate#
PO (*n*=212)	83.2 ± 2.1 (63-92)	38.3 ± 0.51 (22-43)	2.6 ± 0.1 (1-6)	2.5 ± 0.07^{a} (0-4.2)	1.71± 0.04^{ac} (0-3)	185/27 (87.2%)	87/134^{ac} (36.7%)
POC (*n*=230)	91.1 ± 3.2 (62-98)	37.5 ± 0.23 (25-42)	2.5 ± 0.1 (1-7)	2.4 ± 0.06^{a} (0-4.1)	1.98 ± 0.03^{c} (0-3)	204/26 (88.6%)	96/134^{bc} (41.7%)
G6G (*n*=224)	85.6 ± 2.3 (64-99)	38.2 ± 0.44 (21-41)	2.3 ± 0.08 (1-7)	3.1± 0.07^{b} (0-5.3)	2.31 ± 0.03^{b} (0-3)	201/23 (90.9%)	106/118^{b} (47.3%)
Total (*n*=666)	86.6 ± 1.9 (62-99)	38 ± 0.2 (21-43)	2.4 ± 0.04 (1-7)	2.8 ± 0.05 (0-5.3)	1.92 ± 0.02 (0-3)	590/666 (88.5%)	280/386 (42%)
Significance	NS†	NS††	NS†††	*	**	NS††††	***

PO=Presynch-Ovsynch; POC=Presynch-Ovsynch+CIDR; DIM=Days in milk; GnRH1=The 1^{st} GnRH in the Ovsynch+fixed-time insemination protocol; P4=Progesterone; CL=Corpus luteum; PG=$PGF_{2\alpha}$ in the Ovsynch+fixed-time insemination protocol. #This analysis was done with GLIMMIX and accounted for serum concentrations of P4 at GnRH1 and number of CLs at PG. abcDifferent superscripts in each column show significant difference. NS: not significant. †Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.43, *P*=0.65 and *P*=0.55, respectively; †† Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.53, *P*=0.8, and *P*=0.63, respectively; †††Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.73, *P*=0.55, and *P*=0.67, respectively; ††††Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.62, *P*=0.33, and *P*=0.46, respectively; *Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.75, P=0.04, and *P*=0.04, respectively; **Difference between PO & POC, PO & G6G, and POC & G6G at the levels of P=0.81, *P*=0.03 and *P*=0.05, respectively; ***Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.09, *P*=0.03 and *P*=0.08, respectively.

There was a statistically significant difference between the G6G protocol and both the PO and POC protocols in terms of serum concentration of P4 at GnRH1 (*P*=0.04, Table 1).

The cows that received the G6G protocol had a greater number of CLs on their ovaries at PG in comparison to the cows in the PO and POC protocols (*P*=0.03 and *P*=0.05, respectively, Table 1).

The overall pregnancy rate for all cows studied was 42%. Separately, pregnancy rates for cows in PO, POC, and G6G protocols were calculated as 36.7%, 41.7%, and 47.3% respectively. The highest and the lowest pregnancy rates were obtained in the G6G and PO protocols, respectively (*P*=0.03, Table 1). The POC protocol increased the pregnancy rate of cows compared to the PO protocol, although this increase was not statistically significant (*P*=0.09, Table 1). There was a tendency to increase the pregnancy rate of cows following the implementation of the G6G protocol compared to the POC protocol (*P*=0.08, Table 1).

For cows with high serum concentrations of P4 at GnRH1 (>2.8 ng/mL), there was a statistically significant difference between the pregnancy rates of cows in the G6G and PO protocols (*P*=0.03, Table 2).

For the POC and G6G protocols, the pregnancy rate of cows with high serum concentrations of P4 at GnRH1 (>2.8 ng/mL) was significantly higher than cows with low serum concentrations of P4 (≤2.8 ng/mL) at that time (Table 2). The

Table 2. Effect of serum concentration of P4 at GnRH1 (ng/mL) on pregnancies per AI (P/AI) of cows following the implementation of PO, POC, and G6G protocols

Studied groups	Serum concentration of P4 (ng/mL) at GnRH1		*P*-value#
	≤2.8	>2.8	
PO (*n* = 212)	43/85 (33.5%)	35/49[ac] (41.6%)	0.08
POC (*n* = 230)	48/85 (36%)	48/49[cd] (49.4%)	0.04
G6G (*n* = 224)	54/70 (39.1%)	61/48[bd] (55.9%)	0.02
Total (*n*=652)	136/240 (36.1%)	144/146 (49.6%)	<0.01
Significance#	NS†	*	

PO=Presynch-Ovsynch; POC=Presynch-Ovsynch+CIDR; GnRH1=The 1st GnRH in the Ovsynch+fixed-time insemination protocol; P4=Progesterone; CL=Corpus luteum; PG=$PGF_{2\alpha}$ in the Ovsynch+fixed-time insemination protocol. #This analysis was done with GLIMMIX and accounted for number of CLs at PG. abcDifferent superscripts in each column show significant difference. NS: not significant. †Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.63, *P*=0.36, and *P*=0.55, respectively; * Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.32, *P*=0.03, and *P*=0.47, respectively.

Table 3. Effect of number of CLs at PG on pregnancies per AI (P/AI) of cows following the implementation of PO, POC, and G6G protocols

Studied groups	Number of CLs at PG		*P*-value#
	≤2	>2	
PO (*n*=212)	63/123[ac] (34.5%)	13/11 (54.1%)	0.03
POC (*n*=230)	84/124[cd] (40%)	12/10 (54.5%)	0.04
G6G (*n*=224)	75/95[bd] (44.1%)	31/23 (57.4%)	0.02
Total (*n*=652)	224/342 (39.5%)	56/44 (56%)	<0.01
Significance#	*	NS†	

PO=Presynch-Ovsynch; POC=Presynch-Ovsynch+CIDR; GnRH1=The 1st GnRH in the Ovsynch+fixed-time insemination protocol; P4=Progesterone; CL=Corpus luteum; PG=$PGF_{2\alpha}$ in the Ovsynch+fixed-time insemination protocol. #This analysis was done with GLIMMIX and accounted for serum concentration of P4 at GnRH1. abcDifferent superscripts in each column show significant difference. NS: not significant. †Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.85, *P*=0.41, and *P*=0.53, respectively; * Difference between PO & POC, PO & G6G, and POC & G6G at the levels of *P*=0.38, *P*=0.05, and *P*=0.42, respectively

same result was achieved for all cows (*n*=666) (*P*<0.01, Table 2). For cows with ≤2 CLs on their ovaries at PG, there was a statistically significant difference between the pregnancy rates of cows in the G6G compared to the PO protocol (*P*=0.05, Table 3).

For all treatment protocols, the pregnancy rate of cows that had >2 CLs on their ovaries at PG was significantly higher than cows that had ≤2 CLs on their ovaries at this time (Table 3). A similar result was achieved for all cows (*n*=666) too (*P*<0.01, Table 3).

Discussion

It was demonstrated in this study that the G6G protocol significantly increased the pregnancy rate of cows compared to the PO protocol (*P*=0.03). Using of GnRH in the pre-synchronisation program in the form of G6G protocol presumably induced cyclicity in some noncyclic cows, while the pre-synchronisation program in the PO and POC protocols likely failed to induce cyclicity in noncyclic cows. The GnRH injected in the pre-synchronisation program of the G6G protocol induced ovulation in some cows, so there will be a mature CL on the ovaries at GnRH1. On the other hand, the injection of GnRH has beneficial effects on the maturation of the corpus luteum being formed and the secretion of P4 (Peters et al., 2000; Howard et al., 2006). Studies have shown that the addition of GnRH to pre-synchronisation programs increases the synchrony rate of cows following the implementation of the OVS+FTAI protocol compared to the Presynch-Ovsynch protocol (Bello et al., 2006; Souza et al., 2008; Herlihy et al., 2012). Acyclic cows do not benefit from pre-synchronisation with PGF2α, because they do not have a CL on their ovaries (Dirandeh et al., 2015b). The results of a study showed that the limited response of cows to the CIDR synch protocol may be associated with inactive ovaries, especially in the early stages of lactation (Moreira et al., 2001). In the present study, the G6G protocol resulted in a greater number of CLs on ovaries at PG in comparison to the PO and POC protocols. As a result, high serum concentrations of P4 in these cows had positive effects on the quality of the preovulatory follicle, oocyte and uterine environment for embryo development. It has been shown that high serum concentrations of P4 in the preinsemination dioestrous phase increases the quality of the ovulatory follicle and oocyte, and the chance of a successful pregnancy is increased following the ovulation of a qualified follicle (Rivera et al., 2011; Bisinotto et al., 2015; Saad et al., 2019).

In the present study, G6G protocol presumably provided greater synchronization rates in ovarian events, increased the quality of the ovulatory follicle, oocyte or embryo, and provided an appropriate uterine environment for embryo development. Therefore, the G6G protocol induced the highest pregnancy rate among the treatment protocols.

In the POC protocol, a CIDR was used in the interval between the GnRH1 and PG injections compared to the PO protocol. The use of a CIDR in the mentioned interval increased the pregnancy rate of cows in comparison to the PO protocol (*P*=0.09, Table 1), presumably by increasing the synchrony rate, quality of the oocyte or ovulatory follicle, and providing a suitable uterine environment for embryo growth. Results from a study showed that the use of Ovsynch+CIDR compared to Ovsynch protocol increased plasma concentrations of P4 in the interval between the the 1st GnRH and $PGF_{2\alpha}$ injections in the OVS+FTAI program, but did not produce a higher pregnancy rate than the Ovsynch protocol in cows that had an active CL on their ovaries at the beginning of the OVS+FTAI program (Bisinotto et al., 2015).

In the present study, subgroup of cows with high serum concentrations of P4 at GnRH1 (>2.8 ng/mL) showed a statistically significant difference between the pregnancy rates of cows in the PO compared to the G6G protocol (*P*=0.03, Table 2). Various studies have shown the beneficial effects of high serum concentrations of P4 in the pre-insemination luteal phase on the pregnancy rate of cows (Rivera et al., 2011; Bisinotto et al., 2015; Saad et al., 2019). In the current study, since the serum concentration of P4 at GnRH1 was significantly higher in cows in the G6G compared to the PO protocol, this could have a positive effect on the pregnancy rate of cows in the G6G compared to the PO protocol. On the other hand, injection of GnRH in the pre-synchronisation program of the G6G protocol can stimulate ovarian cycles in noncyclic cows.

In the present study, the pregnancy rates of cows with high serum concentrations of P4 at GnRH1 (>2 ng/mL) were higher than cows with low serum concentrations of P4 at GnRH1 (≤2 ng/mL) for both the POC and G6G protocols, as for all cows (*n*=666) (Table 2). These results confirm the positive effects of high serum concentrations of P4 in the dioestrus phase before insemination on the pregnancy rate of cows. On the other hand, a number of cows with low serum concentrations of P4 at GnRH1 were likely acyclic.

In this study, the pregnancy rate of cows in the G6G protocol in the subgroup of cows with ≤2 CL at PG was significantly higher than for cows in the PO protocol (*P*=0.05, Table 3). This result is likely due to the fact that there were more acyclic cows in the PO compared to the G6G treatment group at the time of the onset of the OVS+FTAI program. In all treatment protocols and all cows (*n*=666), the pregnancy rate of cows with >2 CLs on their ovaries at PG was higher than for cows with ≤2 CLs on their ovaries at the same time (Table 3). This result is consistent with the results of other studies that have confirmed the positive effects of high serum concentrations of P4 in the luteal phase before insemination on the pregnancy rate of cows (Rivera et al., 2011; Bisinotto et al., 2015; Saad et al., 2019).

Conclusions

The G6G protocol increased the pregnancy rate of cows in comparison to the PO but not the POC protocol. This result can be attributed to the increase in serum concentration of P4 at GnRH1 and the greater number of CLs at PG following implementation of the G6G compared to the PO protocol. Although CIDR administration in the POC protocol led to an increase in the pregnancy rate of cows compared to the PO protocol, possibly by increasing the synchrony rate and the quality of the ovulatory follicle, this increase was not statistically significant.

Acknowledgment

The authors thank Aliabadi dairy farm, and his staff for all the help, covering the cost of hormonal treatments, and the use of his herd to conduct on-farm trials. The authors thank, Dr. Gholamreza Akbari for laboratory work.

References

1. ABDEL AZIZ, R. L. and A. ABDEL-WAHAB (2017): Reproductive responses of primiparous and multiparous Holstein cows submitted to presynch-ovsynch protocol. Beni-Suef. Univ. J. Basic. Appl. Sci. 6, 149-153. 10.1016/j.bjbas.2017.02.005
2. ASTIZ, S. and O. FARGAS (2013): Pregnancy per AI differences between primiparous and multiparous high-yield dairy cows after using Double Ovsynch or G6G synchronization protocols. Theriogenology 79, 1056-1070. 10.1016/j.theriogenology.2013.01.026
3. AYAD, A., M. SALAHEDDINE, K. TOUATI, M. IGUER-OUADA and H. BENBAREK (2015): Evaluation of norgestomet Crestar on oestrus synchronization and reproductive performance of dairy cows in Algeria. Asian. Pac. J. Reprod. 4, 54-60. 10.1016/S2305-0500(14)60059-2
4. BELLO, N. M, J. P. STEIBEL and J. R. PURSLEY (2006): Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. J. Dairy Sci. 89, 3413-3424. 10.3168/jds.S0022-0302(06)72378-5
5. BISINOTTO, R. S. and J. E. SANTOS (2011): The use of endocrine treatments to improve pregnancy rates in cattle. Reprod. Fertil. Dev. 24, 258-266. 10.1071/RD11916
6. BISINOTTO, R. S., I. J. LEAN, W. W. THATCHER and J. E. P. SANTOS (2015): Meta-analysis of P4 supplementation during timed artificial insemination programs in dairy cows. J. Dairy Sci. 98, 2472-2487. 10.3168/jds.2014-8954
7. BORCHARDT, S., A. POHL and W. HEUWIESER (2020): Luteal Presence and Ovarian Response at the Beginning of a Timed Artificial Insemination

Protocol for Lactating Dairy Cows Affect Fertility: A Meta-Analysis. Animals 10, 1551. 10.3390/ani10091551.

8. BRIDGES, P. J. and J. E. FORTUNE (2003): Characteristics of developing prolonged dominant follicles in cattle. Domest. Anim. Endocrinol. 25, 199-214. 10.1016/s0739-7240(03)00053-5
9. COLAZO, M. G., A. DOUREY, R. RAJAMAHENDRAN and D. J. AMBROSE (2013): Progesterone supplementation before timed AI increased ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. Theriogenology 79, 833-841. 10.1016/j.theriogenology.2012.12.011
10. DADARWAL, D., R. J. MAPLETOFT, G. P. ADAMS, L. F. PFEIFER, C. CREELMAN and J. SINGH (2013): Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. Theriogenology 79, 859-866. 10.1016/j.theriogenology.2013.01.003
11. DE SOUSA SALES, J. N, L. M. S. SIMOE, R. E. ORLANDI, E. A. LIMA, A. P. C. SANTOS, M. P. BOTTINO, L. A. C. L. DA SILVA, J. C. DE SOUZA, M. M. DIAS, J. P. M. MASSONETO, L. A. SCANDIUZZI JR, B. G. FREITAS, B. M. GUERREIRO and M. R. BASTOS (2019): Pre-TAI protocol strategies to increase reproductive efficiency in beef and dairy cows. Anim. Reprod. 16, 402-410. 10.21451/1984-3143-AR2019-0041
12. DIRANDEH, E., A. R. ROODBARI, M. GHOLIZADEH, H. DELDAR, R. MASOUMI, M. KAZEMIFARD and M. G. COLAZO (2015a): Administration of prostaglandin F2α 14 d before initiating a G6G or G7G timed artificial insemination protocol increased circulatory progesterone prior to artificial insemination and reduced pregnancy loss in multiparous Holstein cows. J. Dairy Sci. 98, 5414-5421. 10.3168/jds.2015-9417
13. DIRANDEH, E., A. REZAEI ROODBARI and M. G. COLAZO (2015b): Double-Ovsynch, compared with presynch with or without GnRH, improves fertility in heat-stressed lactating dairy cows. Theriogenology 83, 438-443. 10.1016/j.theriogenology.2014.10.011
14. HERLIHY, M. M., J. O. GIORDANO, A. H. SOUZA, H. AYRES, R. M. FERREIRA, A. KESKIN, A. B. NASCIMENTO, J. N. GUENTHER, J. M. GASKA, S. J. KACUBA, M. A. CROWE, S. T. BUTLER and M. C. WILTBANK (2012): Presynchronization with double-ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J. Dairy Sci. 95, 7003-7014. 10.3168/jds.2011-5260
15. HOWARD, J. M., R. MANZO, J. C. DALTON, F. FRAGO and A. AHMADZADEH (2006): Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormone 5 days after artificial insemination. Anim. Reprod. Sci. 95, 224-233. 10.1016/j.anireprosci.2005.10.010
16. KAWATE, N., T. ITAMI, T. CHOUSHI, T. SAITOH, T. WADA, K. MATSUOKA, K, UENAKA, N. TANAKA, A. YAMANAKA, M. SAKASE, H. TAMADA, T. INABA and T. SAWADA (2004): Improved conception in timed-artificial insemination using a progesterone-releasing intravaginal device and Ovsynch protocol in postpartum suckled Japanese black beef cows. Theriogenology 61, 399-406. 10.1016/s0093-691x(03)00239-5
17. KESKIN, A., G. YILMAZBAS-MECITOGLU, A. GUMEN, E. KARAKAYA, R. DARICI and H. OKUT (2010): Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology 74, 602-607. 10.1016/j.theriogenology.2010.03.009
18. MELO, L. F., P. L. J. MONTEIRO JR, A. B. NASCIMENTO, J. N. DRUM, C. SPIES, A. B. PRATA, M. C. WILTBANK and R. SARTORI (2018): Follicular dynamics, circulating progesterone, and fertility in Holstein cows synchronized with reused intravaginal progesterone implants that were sanitized by autoclave or chemical disinfection. J. Dairy. Sci. 101, 3554-3567. 10.3168/jds.2017-13570
19. MIHM, M., A. BAGUISI, M. P. BOLAND and J. F. ROCHE (1994): Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 102, 123-130. 10.1530/jrf.0.1020123
20. MOREIRA, F., C. ORLANDI, C. A. RISCO, R. MATTOS, F. LOPES and W.W. THATCHER (2001): Effects of pre-synchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy. Sci. 84, 1664-1659. 10.3168/jds.S0022-0302(01)74600-0
21. PETERS, A. R., T. A. MARTINEZ and A. J. C. COOK (2000): A meta-analysis of studies of the effect of GnRH 11-14 days after insemination on pregnancy rates in cattle. Theriogenology 54, 1317-1326. 10.1016/S0093-691X(00)00438-6
22. PURSLEY, J. R., M. O. MEE and M. C. WILTBANK (1995): Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44, 915-923. 10.1016/0093-691X(95)00279-H
23. REVAH, I. and W. R. BUTLER (1996): Prolonged dominance of follicles reduced viability of bovine oocytes. J. Reprod. Fertil. 106, 39-47. 10.1530/jrf.0.1060039
24. RIVERA, A. F., L. G. D. MENDONCA, G. LOPES JR, J. E. P. SANTOS, R. V. PEREZ, M. AMSTALDEN, A. CORREA-CALDERON and R. C. CHEBEL (2011): Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reproduction 141, 333-342. 10.1530/REP-10-0375
25. SAAD, M., Z. SARWAR, M. SALEEM, U. ARSHAD, M. SHAHZAD, M. HASSAN MUSHTAQ, A. HUSNAIN, A. RIAZ and N. AHMAD (2019):

Effect of plasma progesterone on oocyte recovery, oocyte quality, and early in-vitro developmental competence of embryos in Bos indicus dairy cows. Anim. Reprod. Sci. 202, 80-86. 10.1016/j.anireprosci.2019.02.001

26. SANGSRITAVONG, S., D. K. COMBS, R. SARTORI, L. E. ARMENTANO and M. C. WILTBANK (2002): High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17beta in dairy cattle. J. Dairy. Sci. 85, 2831-2842. 10.3168/jds.S0022-0302(02)74370-1
27. SOUZA, A. H., H. AYRES, R. M. FERREIRA and M. C. WILTBANK (2008): A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70, 208-215. 10.1016/j.theriogenology.2008.03.014
28. STEVENSON, J. S., J. R. PURSLEY, H. A. GARVERICK, P. M. FRICKE, D. J. KESLER, J. S. OTTOBRE and M. C. WILTBANK (2006): Treatment of cycling and noncycling lactating dairy cows with progesterone during ovsynch. J. Dairy. Sci. 89, 2567-2578. 10.3168/jds.S0022-0302(06)72333-5
29. VASCONCELOS, J. L., R. W. SILCOX, G. J. ROSA, J. R. PURSLEY and M. C. WILTBANK (1999): Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52, 1067-1078. 10.1016/S0093-691X(99)00195-8
30. WIJMA, R., M. L. STANGAFFERO, M. MASELLO, G. E. GRANADOS and J. O. GIORDANO (2017): Resynchronization of ovulation protocols for dairy cows including or not including gonadotropin-releasing hormone to induce a new follicular wave: Effects on re-insemination pattern, ovarian responses, and pregnancy outcomes. J. Dairy. Sci. 100, 7613-7625. 10.3168/jds.2017-12550
31. WILTBANK, M., P. D. CARVALHO, A. KASKIN, K. S. HACKBART, M. A. MESCHIATTI, M. R. BASTOS, J. N. GUENTHER, A. B. NASCIMENTO, M. M. HERLIHY, M. C. AMUNDSON and A. H. SOUZA (2011): Effect of progesterone concentration during follicle development on subsequent ovulation, fertilization, and early embryo development in lactating dairy cows. Biol. Reprod. 85, 685. 10.1093/biolreprod/85.s1.685
32. YOUSUF, M. R., J. P. N. MARTINS, N. AHMAD, K. NOBIS and J. R. PURSLEY (2016): Presynchronization of lactating dairy cows with PGF2α and GnRH simultaneously, 7 days before Ovsynch have similar outcomes compared to G6G. Theriogenology 86, 1607-1614. 10.1016/j.theriogenology.2016.05.021

Usporedba učinaka Presynch-Ovsynch, Presynch-Ovsynch+CIDR i G6G protokola na reproduktivne pokazatelje holštajnskih krava u laktaciji u razdoblju nakon teljenja

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Novija studija provedena je na industrijskoj mliječnoj farmi blizu Teheran provincije u Iranu, na 666 Holstein krava u laktaciji od rujna 2019. do lipnja 2020. godine u svrhu eliminacije štetnih učinaka toplinskog stresa na reproduktivne pokazatelje krava. Pretpostavka studije koja je prethodila ovom radu bila je da će se postotak gravidnosti krava, povećanjem koncentracije progesterona (P4) u serumu u lutealnoj fazi prije oplodnje putem Presynch-Ovsynch+CIDR (POC) i G6G protokola, povećati u usporedbi s Presynch-Ovsynch (PO) protokolom. Cilj ove studije bio je procijeniti učinke Presynch-Ovsynch ($PGF_{2\alpha}$-14d-$PGF_{2\alpha}$-12d-Ovsynch (OVS: GnRH(GnRH1)-7d-$PGF_{2\alpha}$(PG)-2d-GnRH)-18h-fiksno vrijeme umjetnog osjemenjivanja (FTAI), *n*=212), Presynch-Ovsynch+CIDR (slično kao PO protokol, plus uporaba CIDR tijekom 7 dana, od početka OVS+FTAI protokola do vremena ubrizgavanja PG, *n*=230) te G6G ($PGF_{2\alpha}$-2d-GnRH-6d-OVS+FTAI, *n*=224) protokola na reproduktivne pokazatelje i postotak gravidnosti Holstein krava u laktaciji. Prosječno bodovanje stanja tijela (BSC) za krave bilo je oko 2,5 u vrijeme početka OVS+FTAI programa. Prosječna dnevna proizvodnja mlijeka za krave na početku OVS+FTAI programa bila je 38,3 kg/dan. U prosjeku, istraživane krave bile su osjemenjene 86 dana nakon teljenja. Implementacija G6G protokola u usporedbi s PO i POC protokolima povećala je koncentraciju P4 u serumu pri GnRH1 (P=0,04). Krave koje su primale G6G protokol imale su veći broj žutih tijela (CL) na svojim jajnicima kod PG u usporedbi s kravama u PO i POC protokolima (*P*=0,03, odnosno *P*=0,05). Za sve protokole liječenja, postotak gravidnosti krava koje su imale >2 žuta tijela na jajnicima kod PG bio je značajno veći od krava koje su u to vrijeme imale ≤2 žuta tijela na svojim jajnicima. Sveukupni postotak gravidnosti svih proučavanih krava bio je 42 %. Zasebno, postotci gravidnosti za krave u PO, POC i G6G protokolima izračunati su kako slijedi: 36,7 %, 41,7 %, odnosno 47,3 %. Najveći i najniži postotak gravidnosti postignuti su G6G, odnosno PO protokolom (*P*=0,03). Može se zaključiti da je G6G protokol povećao postotak gravidnosti krava u usporedbi s PO protokolom, ali ne i POC protokolom. Ovaj rezultat moguće je pripisati povećanju P4 koncentracije u serumu pri GnRH1 i većem broju žutih tijela kod PG nakon implementacije G6G protokola u usporedbi s PO protokolom. Premda je primjena CIDR u POC protokolu dovela do povećanja postotka gravidnosti krava u usporedbi s PO protokolom, moguće povećanjem sinkronizacijske stope i kvalitete ovulacijskog folikula, to povećanje nije statistički značajno (*P*=0,09).

Ključne riječi: *Presynch-Ovsynch Presynch-Ovsynch+CIDR, G6G, reproduktivni pokazatelji, Holstein krave*