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Conception Rate of Pre-synchronization and Two Short Term Heat-synch Programs Using Two Doses of PGF2α in Lactating Holstein Dairy Cows

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Abstract

BACKGROUND: Improving the reproductive performance of dairy cows requires estrus manipulation using estrus synchronization protocols.

OBJECTIVES: The current study aimed to assess the effect of reducing the length of estrus synchronization protocol by two doses of prostaglandin injection on the reproductive performance of Holstein dairy cows.

METHODS: At first, all cows received GnRH (day 0) followed by PGF2α on day 7 and GnRH on day 17. Next, cows were assigned randomly to one of the three groups: Group 1) PGF2α injection on days 22 and 23, estradiol benzoate injection on day 24, Group 2) PGF2α injection on days 23 and 24, estradiol benzoate injection on day 25, and Group 3) Control group; injection of PGF2α on day 24, injection of estradiol benzoate on day 25. Estrus detection was performed twice a day up to 48 h (AM/PM) based on the signs of estrus, and fixed time AI protocol was used after 48 h if not previously inseminated.

RESULTS: The conception rate in group 2 was significantly higher than the control group ($P \leq 0.05$) and tended to be higher than group 1 ($P=0.079$).

CONCLUSIONS: Our results confirmed the hypothesis that a decline in the period of follicle dominance by reducing the interval between GnRH and PGF2α through two PGF2α injections improved the fertility of lactating dairy cows.

KEYWORDS: Conception rate, Dairy cows, Heat-synch, Short-term

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Introduction

The use of fertility programs for synchronizing ovulation and timed artificial insemination (TAI) has increased service rate and pregnancies per AI (P/AI) in high-producing lactating dairy cows at first service (Fricke, 2015). Recent research on fertility to TAI after an Ovsynch protocol has focused on incomplete luteal regression after PGF2α treatment during the protocol (Barletta *et al.*, 2018). Administration of a double dose of PGF2α (either clopros=tenol sodium or dinoprost tromethamine) during a 5-day Ovsynch protocol resulted in fewer P/AI and failed to achieve similar rates of luteal regression, compared to cows receiving 2 doses of PGF2α with 24 h interval (Ribeiro *et al.*, 2012). A meta-analysis evaluated the Presynch and Ovsynch protocols reporting that the double Ovsynch protocol benefited primiparous cows but not necessarily multiparous cows (Borchardt, Haimerl, Pohl, & Heuwieser, 2017).

There are several estrus synchronizations programs appropriate for TAI in cows, but most of them need several days to sort the cows to be prescribed regularly and it is difficult to diminish the interval bet-ween the beginning of treatment and AI to <9 days (F. López-Gatius, 2000). The development of short-term synchronization methods led to an acceptable pregnancy rate because TAI provides special benefits for breeders. This relieves breeders of the burden of determining estrus as all the cows that were treat-ed during the voluntary waiting period are inseminated, and the initial completion of the treatment saves time for breeders (F. López-Gatius, 1989; F. López-Gatius & Vega-Prieto, 1990).

Recent research on fertility by TAI after an Ovsynch protocol focused on incomplete luteal regression following PGF2α treatment during the protocol based on progesterone (P4) concentrations at the second GnRH treatment (G2) of the protocol. In these experiments, a subset of cows had slightly elevated P4 (≥0.4 ng/mL) at second GnRH, which is associated with a dramatic decrease in pregnancies per AI (Carvalho, Wiltbank, & Fricke, 2015; Milo C. Wiltbank *et al.*, 2015; M. C. Wiltbank *et al.*, 2014). This observation has led to a modified Ovsynch protocol in which a second PGF2α is administered 24 h after the first one during an Ovsynch protocol to increase luteolysis and P/AI (Archbald *et al.*, 1994; Carvalho, Fuenzalida, *et al.*, 2015; Heidari, Dirandeh, Ansari Pirsaraei, & Colazo, 2017; V. G. Santos *et al.*, 2016; Milo C. Wiltbank *et al.*, 2015).

Synchronizing the estrous cycle of dairy cows with two PGF2α injections or a combination of gonadotropin-releasing hormone (GnRH) and PGF2α augments the proportion of cows that start the AI protocol in early diestrus. Cows at the stage of early diestrus of the estrous cycle in response to exogenous GnRH would have a greater likelihood of ovulation and lower occurrence of spontaneous luteolysis before the timely completion of the AI protocol (Moreira *et al.*, 2001).

Reducing the timeframe from primary GnRH to the induction of luteolysis from 7 to 5 days elevates pregnancy in AI (P/AI). However, for adequate corpus luteum (CL) regression, two injections of PGF2α were required on days 5 and 6 after the first GnRH. The second PGF2α injection may be of particular importance in pre-synchronized cows due to an increase in ovulation by the first GnRH injection and consequently occurrence of newly formed CL, which may not consistently regress after a single PGF2α injection on day 5 (J. E. Santos, Narciso, Rivera, Thatcher, & Chebel, 2010).

The objective of the present study was to compare the conception rate of lactating dairy cows subjected to a 5-day TAI protocol in the Heat-synch program performed for the first time. We aimed to define a better protocol for luteolysis and shorten the dominance time of follicles and subsequently examine the effects on fertility. The impact of these interventions on luteolysis, shortening of the dominance time of follicles, and pregnancy rate was investigated. The alternative hypothesis is that the conception rate would be better if the 5-day TAI protocol is used along with two injections of the luteolytic dose of PGF2α at 24 h intervals.

Materials and Methods

The present experiment was conducted in a commercial dairy farm located around Tehran province, Iran. The average milk production per cow was approximately 12000 kg/lactation. Cows were fed a

total mixed ration twice a day with *ad libitum* access to feed and water. The diet was formulated to meet or exceed the requirements. A total of 300 lactating dairy cows were enrolled in this study. The experimental design was completely randomized with a parity block. Within each block, primiparous and multiparous cows were entered into each of the three groups (100 cows in each group) in a completely random proportion. Milk production, days in milk (DIM), body condition score (BCS), and season were considered as covariates.

The included cows did not have any postpartum disorders, such as dystocia, retained placenta, metritis, clinical mastitis, clinical ketosis, abomasal displacement, and lameness. Selected cows had follicles smaller than 25 mm, and did not have endometritis or inactive ovaries in clean test (Ferguson, Galligan, & Thomsen, 1994). For clean test (around day 30 postpartum), the following presynchronization program was performed for the three groups: GnRH injection (Vetaroline, Aburaihan Pharmaceutical Co., Iran) on day 0, cloprostenol injection (Vetaprost, Aburaihan Pharmaceutical Co., Iran) on days 7 (500 µg), and GnRH injection on day 17. Next, each of the three groups was entered into one of the programs.

Experiments

Group 1 received 500 µg Cloprostenol injection on days 22 and 23 and 1 mg estradiol benzoate injection (Vetastrol, Aburaihan Pharmaceutical Co., Iran) on day 24. Group 2 was treated with 500 µg Cloprostenol injection on days 23 and 24 and 1 mg estradiol benzoate injection on day 25. Group 3 as the control group received 500 µg cloprostenol on day 24 and 1 mg estradiol benzoate on day 25. Following the injection of estradiol benzoate, estrus detection was performed twice a day up to 48 h (AM/PM) by the visual observation of estrus behavioral signs, and TAI protocol was performed after 48 h (if not previously inseminated). The total duration of the program was 27 days in groups 2 and 3 (control group), but 26 days in group 1. Pregnancy diagnosis was carried out by transrectal ultrasonography (Easi-Scan, BCF Technology Ltd., Livingston, UK) 31±3 days after AI.

Blood samples were collected by venipuncture of the caudal vein into tubes containing EDTA on the day of the clean test. Sera were harvested, frozen, and stored at -20°C until laboratory analysis. Serum progesterone (P_4) concentrations were measured in all samples using radioimmunoassay (Monobind Inc., California, USA). A concentration higher than 1 ng/mL was considered to indicate the presence of a functional CL.

Statistical Analysis

The success of different PG injection methods in CL analysis and fertility rates was analyzed using the GENMOD guideline and the inclusion of a logit link in the statistical model. Analyzes were carried out using SAS statistical software version 9.4. Differences were considered significant at $P<0.05$.

Results

Parity, milk production, DIM, the presence of CL at the beginning of the protocol, BCS, and season did not influence conception rate as covariates in this trial ($P>0.05$; Table 1). Conception rate in group 2 was significantly ($P\leq0.05$) higher than the control group (AOR=2.486, 95% CI=1.219-5.07; $P=0.012$) and tended to be higher than group 1 (AOR=1.873, 95% CI=0.930-3.771; $P= 0.079$; Figure 1).

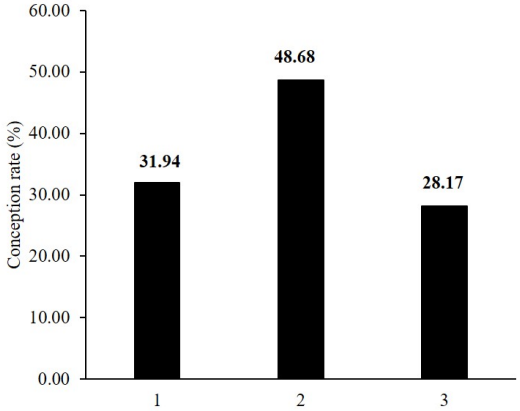

Figure 1. First service conception rate in 3 groups. Conception rate in group 2 were significantly ($P\leq 0.05$) higher than control group and tended to be higher than group 1 ($P= 0.079$).

The conception rates of cows in each group based on progesterone profiles with or without an active CL at the time of clean test were not significantly different (P>0.05; Figure 2). Moreover, the level of serum P_4 at the beginning of the protocol did not affect the conception rate (P>0.05; Figure 3). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for pregnancy prediction based on serum P_4 concentration were 86.4%, 86.27%, 92.86%, and 86.36%, respectively.

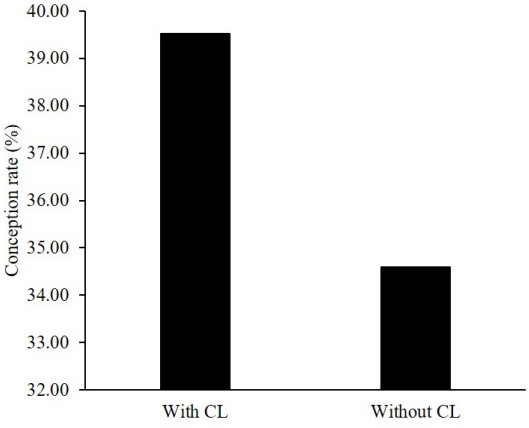

Figure 2. The influence of presence of CL at clean test (based on ultrasonography) on conception rate (P> 0.05)

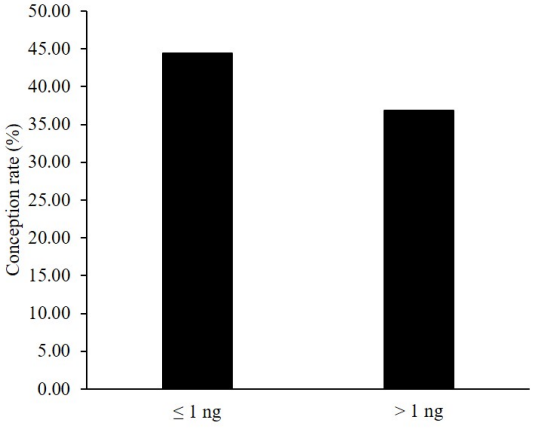

Figure 3. The influence of serum concentration of P4 at clean test on conception rate (P> 0.05)

Table 1. First service conception rate considering estrus synchronization protocol, parity, milk production, DIM at the beginning of protocol, BCS and season.

Effect	Class	Conception rate% (n)	AOR	95% CI	*P*-value
Parity	Primiparous	30.09 (34/113)	0.742	0.351-1.568	0.435
	Multiparous	43.40 (46/107)	—	—	—
Milk production (kg)	—	—	1.013	0.973-1.055	0.537
DIM at the beginning of protocol (day)	—	—	0.983	0.858-1.128	0.811
BCS	—	—	0.753	0.313-1.816	0.528
Season	Spring	30.16 (38/126)	0.618	0.314-1.215	0.163
	Summer	50.00 (5/10)	1.076	0.242-4.787	0.923
	Winter	44.58 (37/83)	—	—	—

Discussion

The results of the current study demonstrated that the conception rate in group 2 had an acceptable rate, compared to the previous studies on the synchronization of ovulation programs (Fricke, 2015; Ribeiro *et al.*, 2012; Milo C. Wiltbank *et al.*, 2015). Furthermore, it was significantly higher than the other two groups. The odds of conception for cows receiving a short Heat-synch program with two injections of PGF2α on days 23 and 24 (days 6 and 7 of the Heat-synch program) were 2.48 (95% CI: 1.21-5.07) times greater than the odds of conception rate in cows receiving the Heat-synch program with one PGF2α injection. Our findings were in line with some other investigations on the short Ovsynch program using 2 doses of PGF2α (Kasimanickam, Day, Rudolph, Hall, & Whittier, 2009). In addition, based on serum P_4 concentration, sensitivity, specificity, PPV, and NPV for the prediction of pregnancy were similar to another study (J. E. Santos *et al.*, 2010).

It was previously hypothesized that the injection of PGF2α on day 8 and 24 h after day 7 in the Ovsynch program would increase the likelihood of complete CL regression and a decrease in P_4 concentration before day 9 of GnRH injection (Brusveen, Souza, & Wiltbank, 2009). The half-life of PGF2α is very short, and once absorbed into the bloodstream, it is rapidly inactivated by oxidation after passing through the lungs, and thus, returns to plasma concentrations 90 min after injection. The new CL induced by the first GnRH treatment of the ovulation synchronization program may be difficult to regress with a single treatment of PGF2α, particularly important if a shortened protocol is used. Therefore, PGF2α injection 24 h later, providing complete CL regression increased pregnancies per AI (Milo C. Wiltbank *et al.*, 2015).

Ovulation following the first GnRH protocol results in the recruitment of new follicles (4-5 mm in size) 24 h later, and these follicles would have 6 and 9 days of development since the day of AI in 5-day Co-Synch with 2 PG and Co-Synch 72 h, respectively. Such follicles are expected to be highly responsive to ovulation induction with GnRH on days 6-9 (Bello, Steibel, & Pursley, 2006; Cerri, Rutigliano, Chebel, & Santos, 2009). Santos *et al.* (2010) showed that cows under short Co-synch with 2 doses of PGF2α had greater P/AI compared with those receiving routine Co-synch protocol, while Rabaglino *et al.* (2010) observed no significant differences in luteolysis 24 h after the first injection.

Others demonstrated that in cows that ovulated at the time of the first GnRH, AI protocol augmented ovulation by the final GnRH (Rutigliano *et al.*, 2008). Similarly, cows without a CL on the day of the first GnRH injection in the Ovsynch protocol had reduced ovulation by the final GnRH, compared to cyclic cows (Galvão & Santos, 2010). The estrous response (Archbald *et al.*, 1994) and luteal regression (Brusveen *et al.*, 2009) were improved in dairy cows with an additional PGF2α treatment 8-24 h apart. It was reported that 2 PGF2α injections with 24 h interval in the Ovsynch protocol increased luteal regression from 84.6% to 95.6% in comparison with one injection; however, P/AI did not improve (Brusveen *et al.*, 2009; Fernando LÓPez-Gatius, 2021).

The limiting factor to reduce the period of follicle dominance in TAI programs is the regression of the newly formed CL resultant from ovulation by the first GnRH injection of the protocol. The newly formed CL responds less to the luteolytic functions of PGF2α on the first 5 days of growth (Miyamoto, Shirasuna, & Sasahara, 2009). Primary CL was suggested to have distinct molecular responses to PGF2α compared to the mid-cycle CL, which is usually sensitive to PGF2α (Miyamoto *et al.*, 2009). Because luteolysis rate elevates with CL age, a second dose of PGF2α is expected to coincide with the response time of CL to PGF2α in the PGF2α state 24 h after the first (Xu, Burton, & Macmillan, 1996).

Previous studies have indicated that the standard luteolytic dose of PGF2α is not sufficient to optimize luteal regression and P/AI in the 5-day timed AI (Kasimanickam *et al.*, 2009; J. E. Santos *et al.*, 2010). To solve this problem, the second dose of luteolytic PGF2α is administration 7-24 h after the first dose leading to improved luteolysis and fertility in dairy cattle (Santos *et al.,* 2010), heifers (Rabaglino *et al.,* 2010), and beef cows (Kasimanickam *et al.*, 2009). Therefore, the higher dose of PGF2α administered as a single injection did not overcome the

refractory response of the early CL to luteolysis observed in previous studies that used the conventional luteolytic dose (Santos *et al.,* 2010).

Some researchers reported that the addition of a second PGF treatment on day 8 during a 7-day Ovsynch protocol increased P/AI, compared to the traditional 7-day Ovsynch, including a single PGF dose on day 7 and a double PGF dose on day 7 (Borchardt, Pohl, Carvalho, Fricke, & Heuwieser, 2018; Rheinberger *et al.*, 2020; Tippenhauer *et al.*, 2021). Treatment with a second PGF increased the percentage of cows with complete CL regression at the end of the Double-Ovsynch protocols (Barletta *et al.*, 2018; Borchardt *et al.*, 2018; Milo C. Wiltbank *et al.*, 2015). Progesterone status at the onset of the synchronization program is critical for pregnancy outcomes in primiparous but not multiparous cows (Stevenson, Hill, Bridges, Larson, & Lamb, 2015).

According to our study, the higher conception rate in group 2 compared to the control group is probably due to the shorter exposure of follicles in group 2 to progesterone after the dominance phase resulting in enhanced quality. Furthermore, the lower conception rate in group 1 in comparison with group 2 can be attributed to an immature follicle at the time of fixed AI, because fixed AI was performed 1 day earlier (day 26 of the protocol) in group 1, while this duration was 27 days in groups 2 and 3.

Conclusion

The results of the present study confirmed a decline in the period of follicle dominance by diminishing the interval between GnRH and PGF2α using two injections of PGF2α that improved the fertility of lactating dairy cows. Cows that were treated with a short Heat-synch protocol and received a second injection of PGF2α on day 7 of Heat-synch had significantly higher conception rates than the other 2 groups. Economic analyses to determine the cost-benefit of the short Heat-synch protocol with two injections of PGF2α would be a useful area of future research.

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Conflict of Interest

The authors declared no conflict of interest.

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میزان آبستنی متعاقب برنامه پیش هم‌زمانی و دو برنامه هیتسینک کوتاه مدت با استفاده از ۲ تزریق $PGF2_{\alpha}$ در گاوهای شیری

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زمینه مطالعه: بهبود عملکرد تولید مثل گاوهای شیری نیاز به دستکاری فحلی با استفاده از پروتکل‌های هم‌زمانی فحلی دارد.

هدف: هدف از انجام این مطالعه بررسی تأثیر کاهش طول پروتکل هم‌زمانی فحلی با استفاده از دو تزریق پروستاگلاندین برعملکرد تولید مثل گاوهای شیری بود.

روش کار: درزمان تست پاکی، به تمامی گاوها در روزصفر گنادرلین، در روز ۷ کلوپروستنل (۵۰۰ میکروگرم)، در روز ۱۰ گنادرلین، و در روز ۱۷ گنادرلین تزریق شد. از روز ۱۷ به بعد گاوها با در نظر گرفتن شکم زایش به شکل تصادفی وارد یکی از سه گروه زیر شدند: گروه۱) تزریق کلوپروستنول در روز ۲۲ و ۲۳، تزریق استرادیول بنزوات در روز ۲۴؛ گروه۲) تزریق کلوپروستنول در روز ۲۳ و ۲۴، تزریق استرادیول بنزوات در روز ۲۵؛ گروه۳) گروه شاهد: تزریق کلوپروستنول در روز ۲۴ و تزریق استرادیول بنزوات در روز ۲۵. به دنبال تزریق استرادیول بنزوات، تشخیص فحلی دو بار در روز تا ۴۸ ساعت با مشاهده علائم رفتاری فحلی انجام و پروتکل تلقیح اجباری پس از ۴۸ ساعت انجام شد.

نتایج: میزان آبستنی در گروه۲ به صورت معنی‌داری بیشتر از گروه شاهد ($P\leq$۰/۰۵) و متمایل به معنی‌داری ($P=$۰/۷۹۰) نسبت به گروه ۱ است.

نتیجه‌گیری نهایی: نتایج آزمایش حاضر فرضیه ما را تأیید کرد که کاهش اندکی در دوره غالبیت فولیکول با کاهش فاصله بین تزریق گنادورولین و $PGF2_{\alpha}$ با استفاده از ۲ تزریق $PGF2_{\alpha}$، باروری در گاوهای شیری را بهبود بخشیده است.

واژه‌های کلیدی: تولیدمثل، گاو شیری، هیت سینک، نرخ آبستنی، هم‌زمانی

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