Effect of Additional Dose of Pgf2α Use in Ovsynch During Second Gnrh and at Insemination on Ovulation and Pregnancy Rates in Dairy Cows

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Abstract. *The objective was to investigate the effect of administering an additional dose of a PGF2α analog concurrent with the second GnRH (GnRH2) and at artificial insemination in the Ovsynch protocol on ovulation (OR) and pregnancy rates (PR) in Holstein lactating cows. Multiparous clinically healthy cows (parity: 3–5 lactation and 50 ± 5 days in milk (DIM)) were randomly allocated to three groups: Ovsynch (n = 23): GnRH (Alarelin acetate, 25 µg)-7 days- PGF2α (d-cloprostenol, 500 µg)-2 days-GnRH-16 to 18 h-FTAI plus 5 mL normal saline; OvsynchPG9 (n = 17): as Ovsynch plus administering another dose of PGF2α concurrent with GnRH2; and OvsynchPG10 (n = 28): as Ovsynch plus administering another dose of PGF2α at insemination. The ovaries of all selected animals were scanned by transrectal ultrasonography on days 9, 10, and 11 after the initiation (day 0) of the Ovsynch protocol to record the incidence of ovulation. Pregnancy diagnosis was performed by transrectal ultrasonography 50 ± 2 days after FTAI.*

The results showed that OR was greater in OvsynchPG9 (82.3%) than that in OvsynchPG10 (81.8%) or Ovsynch (81.2%), although the difference was not significant (P = 0.8205). In addition, PR/FTAI was greater in OvsynchPG9 (41.2%) than in Ovsynch (30.4%) and the difference was not significant (P = 0.8288). In conclusion, it was found that administering an additional dose of a PGF2α did not improve OR or PR in Ovsynch protocol in Holstein lactating cows.

Introduction

It has been evidenced that poor fertility in dairy cattle is still a major concern for the dairy industry all over the world and that both oestrus detection rate and conception rate (CR) influence pregnancy rate (PR) of dairy herds (Ambrose et al., 2015). Incorrect oestrus detection is correlated to the profit loss because of long intervals of calving, loss of milk yield, and costs related to veterinary service (Roelofs et al., 2010). During the past decades, new technologies, particularly those related to animal reproduction, have become very important in improving agricultural production worldwide (Bó et al., 2002). The use of fixed-time artificial insemination (FTAI) programs such as the Ovsynch protocol can obviate the necessity for oestrus detection and increase submission rates for artificial insemination (AI) resulting in improvement of overall PRs of herds (Ambrose et al., 2015). Ovulation can be synchronized with the Ovsynch program within an 8-h period from 24–32 h after giving the second GnRH treatment, so that successful AI can be performed at a fixed time without the need for oestrus detection (Keskin et al., 2010). Based on the experimental evidence (Liu et al., 2018), during the last years, interest has been increased in expanding new synchronization protocols with FTAI to enhance reproductive efficiency in the dairy industry. However, it has been indicated that ovulation synchrony rates after the use of the Ovsynch program are not absolute and range between 80% and 90%; therefore, enhancement of ovulation synchrony following the Ovsynch program may enhance the percentage of pregnant cows (Peters & Pursley, 2002).

It has been revealed that prostaglandin is a biologically very powerful material with several applications for controlling reproduction (Pfeifer et al., 2014). It has been reported that improvement of sperm transport to the uterine tube occurred after adding substances such as prostaglandin F2α (PGF2α) to the semen or administering them to the female animals. Therefore, administration of PGF2α at the same time with AI could enhance pregnancy probability due to induction of oxytocin secretion which stimulates contractions of the uterus and supports transport of semen (Sauls et al., 2018). The most common uses of PGF2α in cattle are because of its luteolytic effects, that is, synchronization of oestrus, regression of luteal tissue, inducing abortion and initiating parturition (Gabriel et al., 2011). It

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has been already reported that administering PGF2α intramuscularly stimulates ovulation in prepubertal heifers. More lately, intramuscular administration of PGF2α has been used successfully to induce ovulation in FTAI programs in beef and dairy cattle (Pfeifer et al., 2018). In addition, experimental works in cows have demonstrated that intrafollicular prostaglandin during the periovulatory period is necessary for the process of ovulation (Leonardi et al., 2012). Prostaglandin F2α analog augments hypophysial responsiveness to GnRH, thereby increasing LH secretion in a process resulting in ovulation (Pfeifer et al., 2014). Randel et al. (1996) reported that administering alfaprostol, a PGF2α analog to anestrous cows resulted in an increased frequency of LH secretion 6 h after treatment. Additionally, prostaglandin F2α may act on the hypothalamic–hypophysial axis and stimulate LH release and promote ovarian cyclical activity (Weems et al., 2006). Moreover, prostaglandin F2α seems to have a local role in the ovary (Pfeifer et al., 2018). It has been shown that prostaglandin that is secreted by the preovulatory follicles is closely linked to the ovulatory process. Moreover, as ovulation approaches, secretion of PGF2α increases substantially in the follicle; therefore, administering a pharmacologic dose of PGF2α may quicken ovulation (Pfeifer et al., 2014). In addition, intravenous, intramuscular or intrauterine administration of PGF2α to domestic livestock at AI in order to affect conception has been a subject of frequent investigations since the early 1990s (Sauls et al., 2018). Therefore, the objective of this study was to compare the effect of administering an additional dose of a PGF2α analog, d-cloprostenol sodium, at the second GnRH and at insemination on ovulation and pregnancy rates in Holstein lactating dairy cows synchronized with the Ovsynch protocol.

Materials and methods

Ethical approval

All the procedures of the present study were approved (Approval ID: IR.RAZI.REC.1400.059/07-28-2021) by the Research Ethics Committee of Razi University, Kermanshah, Iran.

Animals

The study was conducted on a commercial dairy farm consisting of about 150 Holstein dairy cows located near Kermanshah (with a hot-summer Mediterranean climate (Csa); 34.4576° N, 46.6705° E, altitude: 1,350 m above sea level, average annual precipitation: 478.7 mm, average low and high temperature: −1.7°C and 37.8°C in December and July, and average low and high relative humidity: 23% and 75% in January and July, respectively), the capital city of Kermanshah province, west of Iran in summer, 2021. Cows were housed in barns with open-air and sheltered areas, had free access to fresh water, were fed a mixed diet that had been adjusted to provide their physiological requirements and milked three times daily with an average 8-hour interval and average milk yield of approximately 27 kg/cow/day during the study period. The diet consisted mainly of wheat straw, corn silage, and hay as roughage, and rice bran, soybean meal and, dried grounded corn seeds as concentrates. The reproductive tract and mammary glands of the cows were examined by inspection and manual palpation for the evidence of any clinical abnormalities 30 ± 2 days after calving and again 55 ± 5 days *postpartum* before initiation of the experiment. Those cows that were clinically normal were enrolled in the experiment. Body condition scores (BCS) were determined for all cows at the beginning of the study using a 5-point (1 = thin to 5 = fat) scoring system and those cows with a BCS between 3.25 to 3.50 were selected for the study.

Experimental design

The animals (55 ± 5 days in milk; multiparous) regardless of being observed in oestrus before were randomly allocated to one of the study groups according to their even or odd ear tag numbers and parity, so that an approximately identical distribution of parity occurred in the groups, as follows: Ovsynch (n = 23): cows received 25 µg of a GnRH analog (5 mL of Alarelin acetate, Vetaroline®, 5 µg luliberin A/mL, Abureyhan pharmaceutical Co., Tehran, Iran) on Day 0, 500 µg of d-cloprostenol sodium analog (2 mL of D-Cloprostenol sodium, D-Clo PG®, 250 µg/mL, Royan darou Co., Veterinary Division, Semnan, Iran) on Day 7, and another 25 µg of the GnRH analog plus 5 mL of normal saline solution on Day 9. The cows were inseminated 18 h after the second dose of GnRH and were administered another dose of normal saline at FTAI; OvsynchPG9 (n = 17): as Ovsynch, except that an additional dose of PGF2α was administered concurrent with the second GnRH instead of normal saline; OvsynchPG10 (n = 28): as Ovsynch, except that an additional dose of PGF2α was administered at FTAI. All treatments were given intramuscularly. Those cows that exhibited oestrus signs before the FTAI as well as those that were culled or were treated because of problems such as mastitis, lameness, etc., were excluded from the study. The data of those cows that were not observed in heat until approximately 24 h before FTAI and were inseminated approximately 18 hours after administering the second dose of GnRH were used for the analysis. All cows were inseminated by the same technician with proven commercial frozen-thawed semen from a single bull.

Ovarian ultrasonography

The ovaries of all selected animals were examined by transrectal ultrasound scanning with a 7.5 MHz transducer (ULTRASONIC SCANNER, MODEL: HS-1500V, SN: 70610484, HONDA ELECTRONIC CO. LTD 20 OYAMAZUKA OIWA-CHO, TOYOHASHI, AICHI, JAPAN) on days 9, 10, and 11 after the beginning of the Ovsynch protocol (day 0) in order to record the occurrence of ovulation. The disappearance (from one session of scanning to the next) of a follicle with 8 mm in diameter or greater that had been identified in the previous scanning session was defined as ovulation (Pfeifer et al., 2018). Ovulation rate (OR) was defined as the number of the cows in a group that ovulated until day 11, divided by the total number of the animals, in which ovaries were scanned by ultrasound in the corresponding group × 100.

Pregnancy diagnosis

Pregnancy diagnosis was performed in all animals by transrectal ultrasound examination of the uterine horns 50 ± 2 days after FTAI. Those cows that returned to oestrus before pregnancy diagnosis were recorded as non-pregnant. Pregnancy rate per insemination (PR/FTAI) was defined as the number of cows in a group that were confirmed pregnant on days 50 ± 2 after FTAI out of the total number of cows in the corresponding group x 100.

Statistical analysis

Data were analyzed using SAS® software (Statistical Analysis System, Release 9.4. Cary, NC, USA: SAS Inst. Inc.). The analyses were performed in two steps including ORs and PRs to FTAI (PRs/FTAI) by Logistic Regression method using Proc Genmod for determining the probability of significant differences among the groups. χ^2 square statistics was used to determine the degree of difference between the groups. The level of significance was set at $P < 0.05$.

The effects of the average milk production during the study period, BCS and parity on OR and PR were statistically analyzed by Logistic Regression method using Proc Genmod.

Results

The average milk yield, BCS and parity showed no significant interaction.

Ovulation rate

The incidence of ovulation and the number of animals that ovulated between days 9 and 11 after the beginning of the Ovsynch protocol are presented in Table 1. Although the incidence of ovulation in OvsynchPG9 group (82.3%) was numerically greater than those in Ovsynch and OvsynchPG10 groups (81.2% and 81.8%, respectively), the differences were not significant (P = 0.8205).

Pregnancy rate

PRs to FTAI in the study groups are presented in Table 1. Although PR/FTAI in OvsynchPG9 group (41.2%) was higher than those in Ovsynch (30.4%) and OvsynchPG10 (35.7%) groups, and the differences were not significant (P = 0.8288).

Discussion

According to the results of the present study, there was no significant effect of parity, BCS, and milk yield of the cows on the PR. In a previous study, Momcilovic et al. (1998) reported no effect of BCS and number of lactation on pregnancy rate in dairy cows synchronized with GnRH and/or PGF2α for oestrus and ovulation, which is in agreement with the finding of the present study. In addition, in agreement with the finding of the present study, the results of a recent research (Chenault et al., 2014) have demonstrated that dam parity had no significant effect on pregnancy to FTAI in dairy cows that underwent synchronization of ovulation and FTAI. Lajili et al. (1991) also reported no significant effect of parity and milk yield on the conception rate. Moreover, the findings of the present study revealed that parity and milk yield had no significant effect on OR. In agreement with this finding, a more recent research (Liu et al., 2018) has reported that parity had no effect on OR. By contrast, Gümen and Seguin (2003) reported that parity influenced ovulation after administration of GnRH, because it was demonstrated that ovulation was much less likely to occur in first parity cows.

According to the results of the present study, administering an additional dose of PGF2α concurrent with the second GnRH and at insemination in Ovsynch does not improve OR significantly in lactating dairy cows synchronized with the Ovsynch protocol compared with those that did not receive the additional dose of PGF2α. In agreement with these findings, Sauls et al. (2018) reported that following administration of PGF2α at FTAI, an average ovulation risk was higher than 90% in lactating dairy cows, but did not differ between treatments. Pfeifer et al. (2018) reported no difference in the proportion of cows ovulating between cows that received an extra dose of d-cloprostenol and those that did not receive it. Contrary to these findings, López-Gatius et al. (2004) reported that a single injection of PGF2α

Table 1. Ovulation and pregnancy rates at insemination in Holstein lactating dairy cows synchronized with the Ovsynch protocol with or without an additional dose of PGF2α.

Variables	Ovsynch1	OvsynchPG92	OvsynchPG103	P-value
OR (%)*	13/16 (81.2)[a]	14/17 (82.3)[a]	9/11 (81.8)[a]	0.8205
PR/FTAI (%)**	7/23 (30.4)[a]	7/17 (41.2)[a]	10/28 (35.7)[a]	0.8288

[*] OR: ovulation rate; [**] PR/FTAI: pregnancy rate to fixed-time artificial insemination.
[1] Ovsynch protocol (GnRH-7 days-PGF2α-2 days-GnRH-18 hours-FTAI);
[2] OvsynchPG9: GnRH-7 days-PGF2α-2 days-GnRH+PGF2α-18 hours-FTAI;
[3] OvsynchPG10: GnRH-7 days-PGF2α-2 days-GnRH-18 hours-FTAI+ PGF2α;
[a] Means with different superscripts in the same row are significantly different ($P < 0.05$).

intravenously at the time of AI increased OR in dairy cows.

Fricke et al. (1998) reported that the incidence of ovulation after injection of the second GnRH was 84% when Ovsynch was initiated at various stages of the estrous cycle. In another study (Vasconcelos et al., 1999), it was reported that the incidence of ovulation induced by the second GnRH was 81% to 94% and that the total mean synchronization rate was 87%. Yilmazbaś-Mecitoglu et al. (2014) found an 84.5% OR in dairy cows after the second GnRH of the traditional Ovsynch protocol. In a study by Liu et al. (2018), 93.3% of ovulations were successfully induced by GnRH mainly within 36 h of injection in dairy cows synchronized with a modified Ovsynch protocol, in which two low doses of PGF2α were given on days 7 and 8 of the protocol. In the present study, the Ovsynch protocol was initiated at random stages of the oestrus cycle and ultrasonic scanning of the ovaries was performed three times with a 24 h interval beginning at the time of administration of the second dose of GnRH. The results showed 81.2% ovulation within 48 h after administration of the second dose of GnRH in the Ovsynch group, which did not differ significantly with those in the OvsynchPG9 or OvsynchPG10 groups (82.3% and 81.8%, respectively), in which the animals received an additional dose of PGF2α concurrent with the second dose of GnRH or at FTAI, respectively.

According to Pfeifer et al. (2014), for achieving excellent fertility to FTAI, synchronous ovulations must happen (within a few hours) in most of the treated cows, with the least number of failures. It has been shown that exogenous PGF2α can be successfully used as an ovulatory stimulus for FTAI in parous and nulliparous cows (Pfeifer et al., 2014). Ovulation may be successfully induced and synchronized by PGF2α similar to treatments with estradiol benzoate or estradiol cypionate (Sauls et al., 2018). Prostaglandins can increase the response to GnRH (Pfeifer et al., 2014). However, the ovulation process is influenced by many factors such as the environment and diet (Sauls et al., 2018).

The results of the present study showed no significant difference in PR to FTAI between the treatment groups, although the low number of animals in each group was a limitation of the study. Conflicting results have been reported regarding the effect of administering PGF2α or its analog at the time of AI on PRs. Some studies (López-Gatius et al., 2004; Neglia et al., 2008) have reported improved PRs after administration of PGF2α at the time of insemination, but some others (Archbald et al., 1992; Kauffold et al., 2009; Gabriel et al., 2011; Mohammadi et al., 2019) have reported the opposite. Neglia et al. (2008) reported positive results on PRs when 500 μg cloprostenol were administered intramuscularly to Italian Mediterranean buffaloes. Ambrose et al. (2015) found that 10 mg, but not 5 mg, of PGF2α given intramuscularly concurrent with FTAI resulted in a significant increase in conception rate in lactating dairy cattle. López-Gatius et al. (2004) reported that administration of a single dose of PGF2α intravenously at AI improved PR in dairy cows. Meanwhile, in agreement with the results of the present study, Sauls et al. (2018) reported no increase in PR when a single dose of cloprostenol (500 μg) was injected intramuscularly at AI. In addition, in another study (Archbald et al., 1992), it was reported that administering standard luteolytic doses of PGF2α at AI had no positive effect on PR. Similarly, Gabriel et al. (2011) reported that PRs were unaffected by intramuscular injection of 25 mg dinoprost at AI in dairy cows and heifers. In a very recent study (Mohammadi et al., 2019), it has been demonstrated that treatment with d-cloprostenol or buserelin acetate at the time of AI did not have any effect on pregnancy per AI in dairy cows under a condition without heat stress. Kauffold et al. (2009) reported that intramuscular injection of 500 μg cloprostenol immediately after AI had no effects on ovulation and PR in primiparous and multiparous cows and, therefore, cannot be recommended as a means of improving fertility. Pfeifer et al. (2018) reported that an additional injection of PGF2α to FTAI beef cows postpartum did not enhance pregnancy per AI. Sauls et al. (2018) suggested that according to the inconsistent results of treatment with PGF2α, the positive effects seem to be herd specific and depend on unknown factors for success.

Conclusion

In conclusion, the results of the present study showed that administering an additional dose of a PGF2α analog, d-cloprostenol sodium, concurrent with the second GnRH and at insemination of Ovsynch, did not improve ovulation rates on Days 9, 10, and 11 after the initiation of the Ovsynch program or pregnancy rates on Days 50 ± 2 after insemination significantly in Holstein lactating dairy cows. However, further studies with a larger number of cows are needed.

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