# Effect of prostaglandin F2α and GnRH administration at the time of artificial insemination on reproductive performance of dairy cows

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**Abstract**

The present study aimed to determine the effect of administrating prostaglandin F2α (PGF2α) and GnRH at the time of artificial insemination (AI) on the pregnancy per artificial insemination (P/AI) and the pregnancy survival rate of dairy cows. A number of 830 lactating Holstein cows were randomly divided into four groups. Cows in group 1 (n=200) treated with 150 μg d-cloprostenol. In group 2 (n=212), cows received 10 μg buserelin acetate, and group 3 (n=205) was treated with both 150 μg d-cloprostenol and 10 μg buserelin acetate. In addition, 213 cows were assigned as control group which received normal saline as placebo (group 4). To measure progesterone, milk samples were collected at the insemination day and five days later. Pregnancy diagnosis was performed 28 and 60 days after the insemination, and the size and number of corpus luteum (CL) and twin pregnancies were recorded. Hormone therapies had no effect on the P/AI, pregnancy survival rate, and the size and number of CL. The P/AI ratio in groups 1, 2, 3 and 4 were 38.50%, 42.92%, 41.46% and 40.84%, and the pregnancy survival rates in groups 1, 2, 3 and 4 were 84.42%, 86.81%, 88.23% and 83.91%, respectively. The probability of a twin pregnancy was significantly higher in group 1 (15.58%) than other groups. There was no significant difference between groups in terms of the offspring gender. In conclusion, the administration of d-cloprostenol or buserelin acetate at the time of AI had no effect on P/AI and pregnancy survival rate in dairy cattle under no heat stress condition, while the administration of d-cloprostenol increased the probability of twin pregnancies.

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تأثیر تزریق پروستاگلندین F2α و GnRH در زمان تلقیح مصنوعی بر کارایی تولید مثلی گاوهای شیری

**چکیده**

هدف از این مطالعه ارزیابی تأثیر تجویز PGF2α و GnRH در زمان تلقیح مصنوعی بر شانس گیرایی و بقاء آبستنی در گاوهای شیری بود. در این مطالعه ۸۳۰ رأس گاو شیری به صورت تصادفی به چهار گروه تقسیم شدند. گاوهای گروه ۱ (۲۰۰ رأس) ۱۵۰ میکروگرم دی-کلوپروستول دریافت کردند. گاوهای گروه ۲ (۲۱۲ رأس) ۱۰ میکروگرم بوسرلین و گاوهای گروه ۳ (۲۰۵ رأس) هم زمان ۱۵۰ میکروگرم دی-کلوپروستول و ۱۰ میکروگرم بوسرلین دریافت کردند. گاوهای گروه ۴ (۲۱۳رأس) نیز به عنوان گروه کنترل، نرمال سالین دریافت داشتند. هم زمان با تلقیح و پنج روز پس از آن نمونه شیر جهت اندازه‌گیری غلظت پروژسترون اخذ گردید. تشخیص آبستنی ۲۸ و ۶۰ روز پس از تلقیح صورت گرفت. در زمان تشخیص آبستنی اندازه و تعداد اجسام زرد و آبستنی دوقلو ثبت شد. درمان‌های هورمونی تأثیری بر شانس گیرایی، بقاء آبستنی و اندازه و تعداد جسم زرد نداشتند. شانس گیرایی در گروه های ۱، ۲، ۳ و ۴ به ترتیب ۳۸/۵۰ درصد، ۴۲/۹۲ درصد، ۴۱/۴۶ درصد و ۴۰/۸۴ درصد و میزان بقاء آبستنی در گروه های ۱، ۲، ۳ و ۴ به ترتیب ۸۴/۴۲ درصد، ۸۶/۸۱ درصد، ۸۸/۲۳ درصد و ۸۳/۹۱ درصد بود. احتمال آبستنی دوقلو در گروه ۱ (۱۵/۵۸ درصد) به صورت معناداری نسبت به سایر گروه‌ها بالاتر بود. تفاوت معنا داری از نظر جنسیت جنین متولد شده بین گروه‌های مختلف وجود نداشت. به طور کلی نتایج این مطالعه نشان داد که تزریق دی-کلوپروستول و بوسرلین هم زمان با تلقیح مصنوعی تأثیری بر شانس گیرایی و بقاء آبستنی در گاوهای شیری در شرایط فاقد استرس گرمایی نداشت در حالی که درمان با دی-کلوپروستول هم زمان با تلقیح باعث افزایش احتمال آبستنی دوقلو گردید.

**واژه های کلیدی:** بوسرلین، تلقیح مصنوعی، دی-کلوپروستول، گاو شیری، میزان بقاء آبستنی

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## Introduction

In recent decades, along with the increase in milk production, a decline in fertility has been observed in dairy cattle. Nowadays, the poor reproductive performance of dairy cattle has become a major challenge in dairy industry.[1,2] In order to deal with this problem, different therapeutic (hormonal and non-hormonal) regimens have been clinically examined by researchers. Pregnancy per artificial insemination (P/AI) increased by 15.00%, using a reduced dose (50 µg) of cloprostenol at the time of insemination.[3] In addition, the intravenous injection of 500 µg of cloprostenol at the time of insemination, increased the pregnancy rate (odds ratio = 3.60) in primiparous repeat breeder cows.[4] It has been shown that receiving PGF2α at the time of insemination, results in complete corpus luteum (CL) regression and assists with ovulatory process and creates a low progesterone environment conductive for optimal gamete transport, which improves fertility.[5] Several studies showed the effects of PGF2α (cloprostenol) administration at the time of insemination on enhancing ovulation rate in cows and heifers.[6-8] They showed that PGF2α induced ovulation via independent luteolysis mechanism. Moreover, PGF2α analog increases the sensitivity of the pituitary gland to GnRH and enhances LH release independent of progesterone withdrawal, thereby affects the ovulation process.[9] Cruz et al. showed that administration of PGF2α 30 hr before GnRH injection elevated the LH release and ovulation response in both anestrous and cyclic beef cows.[10] Another hypothesis about the effect of PGF2α on reproductive system performance during insemination is related to its impact on the movement and transfer of sperms and the contractions of the oviduct and uterus.[11] In an early study on beef cows,[12] intramuscular (IM) injection of 25 mg dinoprost did neither enhance sperm transport to the oviducts, nor improve P/AI.

The critical role of prostaglandins in follicular rupture and ovulation has been demonstrated.[13] It is clear that the LH surge results in the activation of cyclooxygenase-2 enzyme in the granulosa cells of the preovulatory follicle.[14-16] After activation, the intrafollicular concentrations of prostaglandin F2α and E2 increased till ovulation. Prostaglandin F2α causes contraction of the myoid components of the ovary and also causes lysosomes within the granulosa cells to rupture, releasing their enzymes that lead to further connective tissue deterioration at the apex of the follicle.[17]

The GnRH treatment during the estrous cycle causes LH release, ovulation, and luteinization of large follicles and also synchronizes follicular waves.[18] It was previously suggested that administering GnRH at estrus might prevent ovulation failure or reduce the variation in the time interval to ovulation, thus, provide greater synchrony between the time of insemination and the time of ovulation and the time of ovulation.[18-20] If sufficient GnRH is administered and mature graafian follicles are present, ovulation is induced within 24 to 30 hr.[21] In a meta-analysis by Morgan and Lean it was observed that GnRH treatment at the time of insemination significantly increased the probability of pregnancy (12.50%).[22] Bon Durant et al. proposed that GnRH may reduce early embryonic mortality by enhancing the luteinization of thecal and granulosa cells through the increased LH surge.[23] The subsequent increase in progesterone secretions may increase maternal recognition of the conceptus.[22]

In this study, PGF2α and GnRH were administered at the time of AI in order to evaluate their effects on the P/AI ratios, pregnancy survival rate, milk progesterone concentration at the time of insemination and 5 days after AI and offspring gender of Holstein dairy cows.

## Materials and Methods

**Farm and reproductive management.** The present study was conducted on three different dairy herds under the same management in Mashhad, the east of Iran (1050 m above sea level; 36.1N° 59.3E°), from October 2015 to April 2016. Each herd consisted of 600-800 Holstein dairy cows with three times of milking per day and an average milk production of 11500 kg in each lactation period. Cows were kept using a combination of the free stall and an open shed. Dairy cows were fed formulated diets to provide recommended total dairy nutrients for 670 kg dairy cows producing > 35 kg milk per day.[24] Cows were fed total mixed diets (40.00% forage and 60.00% concentrate; dry matter basis). Cows were fed ad libitum for a daily refusal of approximately 10% of that offered, three times daily at 06:00 am, 2:00 pm and 10:00 pm. The voluntary waiting period in these herds was 45 days. Heat detection was carried out by experienced individuals throughout the day and inseminations were done based on the am/pm rule. All reproductive operations and examinations were performed weekly by veterinarians specialized in the reproductive medicine using the ultrasound technique. In addition, cows were tested regarding the uterine and ovarian health at 5th and 27th days after calving.

**Experimental design.** A total of 830 Holstein dairy cows were registered as they were in satisfactory health condition regarding to the mammary glands, limbs and reproductive system, with 45 to 365 days of milking (average: 111.40 days), 1 to 7 inseminations (average: 2.30), and the average lactation number of 2.50. After manifesting standing heat at the time of AI, cows randomly assigned to four groups, group 1 (n = 200), treated with IM 150 µg d-cloprostenol (Veteglan®; Laboratorios Calier, Barcelona, Spain); group 2 (n = 212), treated with IM 10 µg buserelin acetate (Vetocept®; Aburaihan Pharmaceutical Co., Tehran, Iran); group 3 (n = 205), treated with IM 150 µg d-cloprostenol plus 10.00 µg buserelin acetate; and

group 4 (n = 213), received IM 2.00 mL normal saline as placebo (control group). Pregnancy diagnosis and confirmation were performed 28 and 60 days after insemination, respectively. At the time of diagnosing pregnancy using the ultrasonography, the size and number of CLs on ovaries and the cases of twin pregnancy were recorded. The gender of calves born to cows conceived in this study were determined at the time of parturition.

**Progesterone assay.** Two milk samples were taken from 25 cows in each group in order to measure progesterone concentration at the time of insemination and five days later. The milk samples were kept frozen at –20 °C and defrosted on the test day at ambient temperature to be analyzed using radioimmunoassay (RIA) method, described by Colazo *et al.*[25]

**Statistical analyses.** All analyses were performed using SAS software (version 9.2; SAS Institute Inc., Cary, USA). The associations between the treatment groups and parity, twinning, offspring gender, mastitis occurrence, estrus expression and CL size were tested using Chi-square test (PROC FREQ). Variables that were significant at $p < 0.20$ were included in the multivariable logistic regression analyses. Continuous variables such as CL size and milk production were tested with analysis of variance (PROC ANOVA). The effect of different treatments on the proportion of cows that became pregnant was evaluated using multivariable logistic regression models (PROC LOGISTIC). Treatment, parity group (primiparous and multiparous), DIM categories, milk yield classes, farm of origin, occurrences of retained fetal membranes, metritis, dystocia, and mastitis were offered to the model for each outcome. Then variables were removed by manual backward stepwise elimination if the $p > 0.2$. Finally, interactions among variables were assessed using multivariable logistic regression (PROC LOSISTIC) modeling through a backward model-selection procedure. Because progesterone concentrations were measured over time, a mixed model (PROC MIXED) was used to evaluate the effects of treatment groups on progesterone levels. For all the statistical analyses, differences with $p < 0.05$ were considered significant.

**Results**

Out of 900 lactating cows entered the study, 70 were excluded due to the lameness, mastitis and general illness. The average daily milk production (41.80 kg, in the range of 15.00 to 70.00 kg), the number of lactations (2.50, in the range of 1 to 9), average number of inseminations and days in milk were not significantly different among the 4 groups at the beginning of the trial.

**Pregnancy per AI and pregnancy survival rate.** Pregnancy/AI ratios among the examined groups were not significantly different. Also, no significant difference in pregnancy survival rate was found among the control and treatment groups (Table 1). Different hormonal treatments at the time of insemination had no effect on P/AI ratios in repeat breeder cows (AI ≥ 3), fresh cows (DIM < 100), and cows with high milk production (> 42.00 kg).

**Table 1.** Pregnancy/ artificial insemination ratios (%) and pregnancy survival rate (%) in the treatment groups.

| Treatment | Pregnancy/AI | | Pregnancy Survival |
|---|---|---|---|
| | Treated | Pregnant | Pregnant |
| PGF2α | 200 | 77 (38.50) | 65 (84.42) |
| GnRH | 212 | 91 (42.92) | 79 (86.81) |
| PGF2α + GnRH | 205 | 85 (41.46) | 75 (88.23) |
| Control | 213 | 87 (40.84) | 73 (83.91) |
| Total | 830 | 340 (40.90) | 292 (85.88) |

**Twin pregnancy.** The probability of a twin pregnancy was higher in cows received d-cloprostenol (15.58%) than the other treated and control groups ($p = 0.04$), (Table 2). The average amount of milk production at the time of insemination in cows with twin and single pregnancy were 45.00 and 40.30 kg, respectively. There was a significant relationship between the twin pregnancy and the milk production of cows ($p < 0.01$). However, twin pregnancy showed no relationship with days in milk at the time of insemination, number of inseminations, and parity.

**Table 2.** Effect of different treatments on twinning rate at the time of pregnancy diagnosis test (28-34 days after artificial insemination).

| Treatments | Pregnant | Twin (%) |
|---|---|---|
| PGF2α | 77 | 12 (15.58)[a] |
| GnRH | 91 | 6 (6.59)[b] |
| PGF2α + GnRH | 85 | 4 (4.71)[b] |
| Control | 87 | 6 (6.90)[b] |
| Total | 340 | 28 (8.23) |

[ab] Different superscripts indicate significant difference ($p < 0.05$).

**Progesterone concentration.** The mean concentration of milk progesterone at the time of insemination and five days later were $0.51 \pm 0.35$ and $4.93 \pm 1.80$ ng mL$^{-1}$, respectively (Table 3). The progesterone concentration on the 5th day of sampling tended to be significantly different between PGF2α + GnRH and PGF2α groups ($p = 0.07$). Moreover, there was a significant difference in progesterone concentration on the 5th day of sampling between the PGF2α + GnRH and control group ($p = 0.03$).

**Estrus expression, number and size of the CL.** There was no difference in estrus expression before the first pregnancy test among treatment groups. Also, no difference was observed among the groups regarding the number and size of CL at the first pregnancy test (Table 4).

**Table 3.** Milk progesterone concentration (ng mL$^{-1}$) at the time of insemination (day 0) and five days (day 5) after insemination.

| Treatment | No. | Day 0 | Day 5 |
|---|---|---|---|
| PGF2α | 25 | $0.52 \pm 0.39$ | $3.70 \pm 2.20$[ab] |
| GnRH | 25 | $0.47 \pm 0.28$ | $5.69 \pm 1.90$[ab] |
| PGF2α + GnRH | 25 | $0.53 \pm 0.38$ | $7.06 \pm 1.60$[b] |
| Control | 25 | $0.74 \pm 0.59$ | $3.27 \pm 1.70$[a] |

[ab] Different superscripts indicate significant difference ($p < 0.05$).

**Table 4.** Corpus luteum (CL) numbers and dimensions (cm; mean ± SD) at the time of pregnancy diagnosis test (28-34 days after artificial insemination).

| Treatments | No. | One CL (%) | Two CLs (%) | CL dimensions |
|---|---|---|---|---|
| **PGF2α** | 97 | 79 (81.44) | 18 (18.56) | 3.04 ± 0.86 |
| **GnRH** | 100 | 88 (88.00) | 12 (12.00) | 3.02 ± 0.66 |
| **PGF2α + GnRH** | 100 | 83 (83.00) | 17 (17.00) | 2.96 ± 0.75 |
| **Control** | 102 | 91 (89.22) | 11 (10.78) | 2.87 ± 0.60 |
| **Total** | 399 | 341 (85.46) | 58 (14.54) | - |

**Offspring gender.** There was no significant difference among the groups in terms of the offspring gender. In addition, days in milk, number of inseminations, number of lactations and milk production did not have any effect on offspring gender.

## Discussion

To the best of our knowledge, this was the first study which investigates the effect of d-cloprostenol and buserelin acetate administered alone and in combination at the time of artificial insemination on P/AI ratios and pregnancy survival rate. The effects of d-cloprostenol and buserelin acetate were also evaluated on the progesterone concentration, the size and number of CL, the gender of offspring, and twin pregnancies in the examined cows.

The results showed that the administration of these hormones to primiparous and multiparous dairy cows in cold and temperate weather conditions (no heat stress) did not affect P/AI ratios and pregnancy survival rates. Previous study showed that the intramuscular injection of 500 μg cloprostenol did not increase the P/AI ratios.[26] Gabriel *et al.* reported that the intramuscular administration of 25 mg dinoprost did not affect the P/AI.[11] López-Gatius *et al.* showed that intravenous injection of 500 μg cloprostenol to primiparous repeat breeder cows under heat stress condition increased the P/AI,[4] but it had no effect on cows that were under a positive energy balance and were expected to have a high fertility (90 to 120 days in milk at the time of AI) in cold temperatures.

Reportedly, 500 μg cloprostenol was administered to Mediterranean buffalos both intravenously and intramuscularly.[27] Regardless of the route of injection, P/AI ratios and pregnancy survival rates in buffalos treated with cloprostenol were increased. They also reported that the size of the CL and milk progesterone concentration were increased in buffalos treated with cloprostenol 11 days after insemination.

In the present study, progesterone concentration five days after the insemination was higher in the GnRH + PGF2α group than the PGF2α and control groups. In fact, progesterone concentration was generally higher in two groups received GnRH compared to the PGF2α and control groups. A number of previous studies reported the increase of progesterone in cows treated with GnRH at the time of artificial insemination during the post-insemination cycle.[28-31] In contrast, Ryan *et al.* showed that administration of 10 μg buserelin at AI, reduced serum concentrations of progesterone 3 to 5 days after the insemination and did not affect P/AI.[32]

López-Gatius *et al.* reported that P/AI was increased and the possibility of the presence of an additional CL was doubled in the case of administration of 100 μg GnRH at the time of insemination.[33] In the present study, the probability of twin pregnancy in cows treated with PGF2α was higher than the other groups. It seems that the administration of PGF2α induces double ovulation. In high producing dairy cows, high levels of FSH after follicular deviation causes the dominance of more than one follicle.[4] In the present study, it was revealed that the amount of milk production at the time of AI was a risk factor for increasing twin pregnancy, as the possibility of a twin pregnancy was higher in cows with the average daily milk production of 45.00 kg than those with an average production of 40.00 kg.

López-Gatius *et al.* reported that days in milk and parity are the risk factors for the occurrence of twin pregnancies.[33] The probability of twin pregnancy in the multiparous cow is 2.90 times more than a primiparous cow. Twin pregnancies were 2.70 times more likely to occur during the mid- than during the early-lactation period.[33] In addition, it was mentioned that the administration of GnRH at the time of insemination has no impact on the occurrence of a twin pregnancy. In two comprehensive studies comprising 11951 and 52362 lactations records in dairy cows, it was showed that parity and milk production are the most important factors affecting the occurrence of a twin pregnancy.[34,35]

In a study conducted in 2004, intravenous administration of 500 μg cloprostenol at the time AI increased double ovulation by 2.60 times, which can underlie twin pregnancy.[4] In a recent study, the authors sustained that cows treated with 10 mg of dinoprost at the time of insemination had 3.60 times more twins than the control group.[36] The results of the above studies are in consistent with the findings of the present study regarding the effect of cloprostenol on double ovulation and subsequently increasing the probability of a twin pregnancy.

In the present study, the size and number of CL were evaluated at the time of pregnancy test (28-34 days post AI) and the results showed no significant difference among the four groups. Neglia *et al.* reported that the size of CL

was larger in Mediterranean buffalos 11 days after AI in groups treated with cloprostenol.[27] In another study, it was observed that the administration of GnRH increases the possibility of the presence of an additional CL by two times 11 days after AI.[33] The differences between the results of these studies with the findings of the present study may be attributed to the time of assessment of the CL and the ovaries.

Several factors affect the gender of offspring in ruminants, such as the parity of dam, hormonal profile, stress, season, temperature, body condition score, nutritional conditions at conception, time of insemination relative to the onset of estrous, site of semen deposition, and sire.[37] Emadi *et al.* showed that the treatment with estradiol before the insemination in the Heatsynch program could increase the likelihood of the birth of male calves (sex ratio: 63.80%).[37] In the present study, the simultaneous administration of PGF2α and GnRH during the insemination had no effect on sex ratio.

In conclusion, under the conditions of this study, 150 μg d-cloprostenol and 10.00 μg buserelin acetate administered intramuscularly to cows immediately after AI did not have any effects on P/AI ratios and pregnancy survival rates under no heat stress condition, while receiving d-cloprostenol alone at the time of artificial insemination increased the probability of a twin pregnancy.

## Acknowledgements

## Conflict of interest

The authors declare that there is no conflict of interest.

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