The effect of various administration routes of D-cloprostenol at the time of artificial insemination on conception rate in dairy cattle

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ABSTRACT
The aim of the present study was to evaluate the effect of various administration routes of PGF$_2$alpha analogue, D-cloprostenol, at the time of artificial insemination (AI) on conception rate in a large dairy herd. A total number of 659 lactating dairy cows (101 heifers and 558 cows) were enrolled in the study. Following heat detection, the cows were artificially inseminated according to the AM-PM rule. The animals were divided into a control (29 heifers and 178 cows) and three experimental groups; IM (53 heifers and 110 cows), IV (155 cows) and IU (19 heifers and 115 cows) groups that received intramuscular 150 mg, intravenous 150 mg and intrauterine 5 mg of D-cloprostenol, respectively, at the time of AI. The control group did not receive any treatment at the time of AI. To measure serum progesterone concentration, blood samples were obtained from 15 cows in each group at the time of insemination and on days 4 and 15 post-insemination. Pregnancy was diagnosed by rectal ultrasound examination between days 35-40 post-insemination. The effects of treatments, parity, milk production, and days in milk (DIM) on conception rate of adult cows were evaluated using the multivariate logistic regression model. Intramuscular administration of D-cloprostenol at the time of AI significantly increased conception rate in dairy cows compared to the control group (60.1% vs. 40.6%; $p = .005$). However, intravenous and intrauterine administration of D-cloprostenol did not alter the conception rate ($32.3\%$; $p = .29$ and $50\%$; $p = .11$, respectively). D-cloprostenol treatment at AI could not significantly increase the conception rate of heifers following both (IM & IU) administration routes. In conclusion, this study showed that intramuscular administration of D-cloprostenol at the time of insemination might improve the conception rate in adult cows, while its intravenous and intrauterine administration did not induce any positive effects on reproductive efficiency of dairy cows.

HIGHLIGHTS
- Intramuscular administration of D-cloprostenol at the time of insemination might improve the conception rate in adult cows.
- Intravenous administration of D-cloprostenol did not induce any positive effects on reproductive efficiency of dairy cows.
- Intrauterine administration of D-cloprostenol did not induce any positive effects on conception rate of dairy cows.

Introduction
The decline in reproductive performance and fertility in dairy cows has significantly influenced the economic efficiency of dairy industry in recent decades (Lucy 2001). Conception rates to artificial insemination have dramatically dropped since 1950s (Butler 1998; Rajala-Schultz and Frazer 2003). Moreover, reduction in conception rate is more serious during the hot seasons worldwide (De Rensis and Scaramuzzi 2003; Torres-Júnior et al. 2008; Bernabucci et al. 2014; Schüller et al. 2014).
In clinical trials, various reproductive hormones have been prescribed before, during and after AI to increase the conception rate after insemination (Friedman et al. 2012; Friedman et al. 2014). Hormonal treatments at the time of AI are mainly consisted of GnRH and hCG administration, which are mostly used to increase synchronisation between AI and ovulation time. Administration of these two hormones have been reported to result in different outcome and has been sometimes used as a common protocol in dairy farms (Kharache and Srivastava 2007; De Rensis F et al. 2008; Paksoy and Kalkan 2010; Gümén et al. 2011).

Another hormone whose effects at the time of AI has been studied is prostaglandin F\textsubscript{2\alpha}, which has been used as a luteolytic agent in dairy cattle for oestrus synchronisation, regression of corpus luteum, induction of abortion, parturition and treatment of uterine infections (Wenzel 1991; Risco et al. 1994). There is evidence that PGF\textsubscript{2\alpha} plays an important role in ovulation, in addition to its luteolytic properties. The results of previous studies conducted on ovarian follicles of different mammals have indicated that PGF\textsubscript{2\alpha} concentrations increase immediately prior to ovulation at the follicular level (Hedin et al. 1987; Dozier et al. 2008). Moreover, it has been reported that administration of prostaglandin F\textsubscript{2\alpha} inhibitors prevents ovulation (Edelman et al. 2013). As well as, it has been found that prostaglandins play a critical role in follicular rupture (Sirois et al. 2004). On the other hand, several clinical trials have reported that, administration of PGF\textsubscript{2\alpha} during AI, induces a positive effect on the induction and synchronisation of ovulation in cows, which could consequently enhance conception rate (Lopez-Gatius et al. 2004; Leonardi et al. 2012; Pfeifer L et al. 2014). Gallo et al. (Gallo et al. 1992) also reported synchronisation of ovulation improves in super-ovulated heifers treated with cloprostenol immediately before the onset of ovulation. A few studies have examined the effect of PGF\textsubscript{2\alpha} administration at the time of AI on conception rate of dairy and beef cattle, that have reported varying results in different conditions, including season of the year (warm or cold) (Lopez-Gatius et al. 2004), route of administration (Gabriel et al. 2011), parity (Lopez-Gatius et al. 2004), and dose of administered hormone (Ambrose et al. 2015). The aim of this study was to evaluate the effect of administration of PGF\textsubscript{2\alpha} analogue, D-cloprostenol, at the time of AI on the conception rate in dairy cattle in different conditions, including parity, milk yield, days in milk (DIM) and route of administration, in an industrial dairy farm in central regions of Iran.

### Material and methods

#### Farm and reproductive management

This study was conducted in a well-managed herd of 4000 dairy cattle in Shahrekord of Chaharmahal and Bakhtiari province in central region of Iran (32° 19′ 32” N, 50° 51′ 52” E; height above sea level: 2060 m). The average of daily minimum, maximum and mean air temperature during the study period was 10.5, 32.9 and 21.9 °C, respectively, and the average of daily minimum, maximum and mean relative humidity was %10.41 and % 50.8 and 26, respectively. All dairy cows were of the Holstein breed. Animals were fed with a Total Mixed Ration (TMR) based on their milk yield levels. The cows were milked three times a day. The average daily milk production was 40 kg per cow. The cows were kept in a free stall barn on concrete flooring. To reduce the effects of high temperature on cow’s performance in summer season, misting fans were used in the barns. Also, the lack of side walls in the barns led to better air flow.

A veterinarian examined ovarian and uterine health of the post-parturient cows through rectal palpation and ultrasonography, 30-35 days after parturition. The cows were assigned to a voluntary waiting period (VWP) of 50 days. The herd average days open was 120 over the period study.

#### Animals and treatments

A total of 558 cows and 101 heifers that became artificially inseminated over a 30-day period from 1 August 2014 to 6 September 2014 were included in this study. All the animals were clinically healthy at the time of artificial insemination. The cows were in different parity, milk yield level and days in milk (DIM). Therefore, they were different in the number of insemination. The mean days in milk at the time of AI was 143 and 121 for primiparous and multiparous, respectively. The cows were artificially inseminated after observation of heat synchronised induced oestrus (day 0: 10 µg GnRH analogue, Gonadoreline, Aboureihan company, Iran; day 7: 150 µg D-cloprostenol, Rooyan Darou, Iran; day 8: 1 mg oestradiol benzoate, Aboureihan, Iran) based on AM/PM rule by 4 skilled technicians while artificial insemination of heifers was done following spontaneous oestrus in the same way from 13 months onwards. No significant difference was detected in conception rates obtained by each technician. At the time of AI, the heifers and Cows were randomly divided into one control and three experimental groups; namely IM group (n = 163: 53 heifers, 29 primiparous
and 81 multiparous) that received 150 µg D-cloprostenol intramuscularly, IV group (n = 155: 45 primiparous and 110 multiparous) that received 150 µg D-cloprostenol intravenously through milk vein, and IU group (n = 134: 19 heifers, 29 primiparous and 86 multiparous) that received intrauterine infusion of 5 µg D-cloprostenol. The control group (n = 207: 29 heifers, 56 primiparous and 122 multiparous) did not receive any treatment at the time of AI. Due to the invisibility of the milk vein in heifers they were not enrolled in the IV group. In the IU group, 5 µg of D-Cloprostenol was diluted in 0.5 mL normal saline followed by being packaged inside a straw for intrauterine infusion by an insemination pipette and similar to AI process infused into uterine body immediately after insemination. Based on the herd reproductive management program in summer season, a Controlled Internal Drug Release CIDR (Cue-Mate, Bioniche Animal Health, Belleville, New Zealand) was placed inside all the cow between days 4 to 11 post-insemination.

Pregnancy was diagnosed 35-40 days after AI by transrectal ultrasonography (CTS-3300V, SIUI, Guangdong, China). Moreover, ovaries of the pregnant cows were simultaneously scanned for existence of two corpus luteums which are indicator of double ovulation.

Statistical analyses

The effects of D-Cloprostenol treatment, parity, milk yield level, days in milk (DIM) and their interactions on conception rate and double ovulation rate were investigated using the multivariate logistic regression model in SPSS software. The effects of treatments on conception rate of heifers were analysed separately using univariate logistic regression. An overall analysis of serum progesterone concentrations was repeatedly measured by ANOVA using generalised linear procedure (GLM) in SAS.

Results

The results of logistic model for the effect of treatment on conception rate of heifers are shown in Table 1. Neither intramuscular nor intrauterine administration of D-cloprostenol could significantly increases the conception rate of heifers at the time of AI. However, no significant differences were detected in conception rate in the groups treated with intravenous and intrauterine administration of D-cloprostenol (p > 0.05).

The mean serum progesterone concentrations of various therapeutic groups on days 0, 4, and 15 post-insemination are presented in Table 3. No significant differences in serum progesterone level were detected between different treatment groups. Progesterone concentration on day 4 post-insemination was not significantly enhanced compared to that on day 0, however, a significant increase in progesterone concentration was detected on day 15 compared to those on days 0 and 4 post-insemination.

None of the treatments, parity, milk production level and DIM variables induced a significant effect on double ovulation; therefore, they were removed from the model.

Discussion

The effects of administration of PGF<sub>2α</sub> at the time of AI on conception rate in beef and dairy cattle have
been one of the interesting research subjects in recent years. Although several studies have repeatedly reported that fertility is enhanced following PGF$_{2\alpha}$ administration at the time of insemination, mainly due to an increased ovulation rate (Lopez-Gatius et al. 2004; Leonardi et al. 2012; Pfeifer L et al. 2014; Pfeifer LFM et al. 2016), to the best of our knowledge, only four studies have reported significant statistical increases in conception rates of dairy cattle, namely in repeat breeders (Lopez-Gatius et al. 2004), normal cows (Prinzen et al. 1991; Ambrose et al. 2015) and buffalos (Neglia et al. 2008).

In the present study, the probability of conception was 1.911 times ($p = .005$) higher in the cows that received intramuscular administration of D-cloprostenol at the time of AI. This increased probability in the studied herd, which received proper reproductive management and presented a conception rate of 40.6% in the control group is noteworthy. This result is supported by the findings of Ambrose et al. (Ambrose et al. 2015), who have reported an increase in conception rate in lactating dairy cows following intramuscular administration of 10 mg dinoprost tromethamine at the time of AI. Moreover, in a study performed by Neglia et al. (Neglia et al. 2008) to investigated the effect of intramuscular administration of cloprostenol on buffaloes, similar results have been obtained. On the other hand, some studies have reported that intramuscular administration of PGF$_{2\alpha}$ failed to affect conception rate in dairy cattle (Archbald et al. 1992; Kauffold et al. 2009; Gabriel et al. 2011; Sauls et al. 2018).

Administration of D-cloprostenol at the time of AI in the two tested routes could not significantly improve the conception rate of heifers. In the heifers, administration of D-cloprostenol was inconclusive that may be due to small sample size of the heifers group. PGF$_{2\alpha}$-triggered ovulation induction, which has been demonstrated by numerous studies, might be the underlying mechanism involved in the enhanced conception rate. The results of a clinical trial have indicated that PGF$_{2\alpha}$ can induce ovulation in prepubertal heifers through a luteolysis-independent mechanism (Leonardi et al. 2012). Some studies have reported that PGF$_{2\alpha}$ administration at the time of insemination can accelerate ovulation or increase ovulation rate in cattle (Pfeifer L et al. 2014; Pfeifer LFM et al. 2016), or it can increase ovulation rate in dairy cattle inseminated in warm season (Lopez-Gatius et al. 2004). Furthermore, some studies have explained the effect of PGF$_{2\alpha}$ administration at the time AI through post-insemination increased serum progesterone concentration (Neglia et al. 2008), which could not be confirmed by the present study. It has been hypothesised that administration of PGF$_{2\alpha}$ during AI may induce its effect via stimulating uterine myometrial contractions and transferring spermatozoids to the uterine tube (Rodriguez-Martinez et al. 1987; Willenburg et al. 2003). Some studies have proved this hypothesis, while it has been rejected by others (Morrison et al. 1988). The significant positive results obtained from the IM group of this study may be due to the fact that the experiment was conducted during the summer months. In a study conducted by lopez-Gatius et al. (Lopez-Gatius et al. 2004), administration of cloprostenol at the time of AI led to an increase in ovulation rate in cows inseminated during the warm season, while it could not affect ovulation during the cold season. On the farm where the study was conducted, the adverse effects of hot season on reproduction were evident so that, the difference between reproductive performance during the cold and hot seasons of the

Table 2. Odds ratios of the included variables in the final multivariate logistic regression models (treatments, parity and DIM) for conception rate of adult cows.

| Treatment | Conception rate % | Odds ratio | 95% confidence interval | p Value |
|-----------|-------------------|------------|------------------------|--------|
| Control   | 207               | 40.6       | 1                      |        |
| IM        | 163               | 60.1       | 1.911                  | .005   |
| IV        | 155               | 32.3       | 0.722                  | .294   |
| IU        | 134               | 50         | 1.265                  | .110   |
| Multiparous | 159           | 44.3       | 1                      |        |
| DIM ≤ 100 | 399               | 39.1       | 0.688                  | .014   |
| DIM > 100 | 293               | 38.23      | 0.689                  | .015   |

Table 3. Mean serum progesterone level (s) (ng/mL) of different groups on days 0, 4, and 15 post-insemination.

| Insemination day | After 4 day | After 15 day |
|------------------|------------|-------------|
| Control          | 0.77 ± 0.6a| 1.64 ± 0.62a| 6.5 ± 0.66b |
| IM               | 0.37 ± 0.7a| 1.02 ± 0.72a| 8.94 ± 0.7b |
| IV               | 0.46 ± 0.62a| 0.77 ± 0.66a| 8.23 ± 0.66b |
| IU               | 0.68 ± 0.72a| 1.03 ± 0.85a| 7.82 ± 0.91a |
| Total            | 0.57 ± 0.33a| 1.12 ± 0.36a| 8.09 ± 0.37a |

IM: intramuscular; IV: intravenous; IU: intrauterine.
year was significant. Therefore, administration of D-cloprostenol during pre-ovulatory period might compensate (eliminate) the adverse effects of hot season on ovulation.

Despite the expectation that, compared to intramuscular administration, intravenous administration of D-cloprostenol could affect the ovary or uterus rapidly and to a greater extent, it could not induce a significant difference in conception rate under the conditions of our study.

Considering the results of this study, the question definitely arises "why intravenous administration of D-cloprostenol failed to increase conception rate, unlike its intramuscular administration?"

It is likely that intravenous injection, due to the short half-life of D-cloprostenol (Martin and Liptrap 1981), may not provide effective concentration of the hormone on the verge of ovulation compared to intramuscular administration. This finding contradicts the Prinzen et al. (Prinzen et al. 1991) report that intravenous cloprostenol administration at the time of AI resulted in a 15.2% increase in pregnancy rates.

To date, to the best of our knowledge, the differences between the effects of intramuscular and intravenous administration of PGF2α or its analogues have not been investigated in cattle. These differences have been studied in buffalos (Neglia et al. 2008), indicating that both administration routes equally increased the conception rate. These findings are in contrast with the results of the present study. However, it should be considered that, besides being more practical compared to intravenous administration, intramuscular injection is also more effective.

The effects of intratuterine infusion of PGF2α or its addition to semen have been evaluated in several past studies that have reported different results. Moreover, some older studies on the effect of intratuterine administration of PGF2α in rabbits (Sorgen and Glass 1972; Spilman et al. 1973), sheep (Gustafsson et al. 1975) and horses (Bader et al. 1999) have achieved contradictory results. However, the effect of intratuterine administration of PGF2α in cattle has only been evaluated by Gabriel et al. (Gabriel et al. 2011) who have reported an increase in conception rate ($p = .12$). In the present study intratuterine administration of D-cloprostenol at the time of AI led to a 1.26 fold increase in the conception rate for cows ($p = .11$) which was consistent with those of reported by Gabriel et al., (2011). Perhaps further studies on a larger scale can prove the beneficial effects of intratuterine administration of PGF2α or its analogues at the time of AI on reproductive performance of cattle.

In this study, no difference was observed between the treatment and control groups in terms of serum progesterone concentration on days 4 and 15 post-insemination. Therefore, the hypothesis that D-cloprostenol can increase conception rate by increasing progesterone concentration during the post-insemination days could not be confirmed by the present study. This finding is in agreement with those of Ambrose et al. (Ambrose et al. 2015) and Sauls et al. (Sauls et al. 2018) who have reported that PGF2α treatment at the time of AI failed to induce greater plasma progesterone level and corpus luteum diameter were detected on day 11 post PGF2α administered at the time of AI (Neglia et al. 2008).

Our findings indicated no significant difference in serum progesterone levels between day 0 and 4 post-insemination, while it was significantly increased on day 15 in all the treatment and control groups compared to that on day 4 post-insemination, as expected according to the previous studies (Willard et al. 2003; Stronge et al. 2005; Vadhanakul et al. 2008).

There was no significant difference in double ovulation rate in any of the treatment groups, which is contradictory with the findings of Lopez-Gatius et al. (Lopez-Gatius et al. 2004) who have reported an increase in double ovulation in dairy cows following cloprostenol treatment at the time of insemination. The results of this study indicated that parity, DIM, and milk production variables did not affect double ovulation rates, while the results of a study by Lopez-Gatius et al. (López-Gatius et al. 2005) demonstrated that parity and DIM were correlated with double ovulation.

Conclusion

The results of the present study indicated that under the conditions of this study, Prostaglandin F2α analogue, D-cloprostenol, could effectively increase conception rate in dairy cows when administered intramuscularly at the time of AI, however, intravenous and intratuterine administration of D-cloprostenol showed no favourable effect on conception rate in dairy cattle.

Ethical approval

The ethical Committee of Shahrekord University approved the study.
Disclosure statement

No potential conflict of interest was reported by the author(s).

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