IJAS Iranian Journal of Applied Animal Science

Effect of Treatment with Additional Doses of Prostaglandin F_2 Alpha during the Middle Stage of the Oestrus Cycle on Luteolysis Process and Reproduction Performance in Holstein Dairy Cows

Research Article

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Received on: 26 Nov 2021
Revised on: 19 Mar 2022
Accepted on: 14 Apr 2022
Online Published on: Sep 2022

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Online version is available on: www.ijas.ir

ABSTRACT

For the manipulation of the oestrus cycle, the prostaglandin F_2 alpha ($PGF_2\alpha$) is a commonly used strategy in dairy cows. The objective of the study was to evaluate the luteolysis response and reproductive performance after treatment with one or two injections of $PGF_2\alpha$. A total of 84 Holstein dairy cows, being in the middle stage of the oestrus cycle (between days 7 and 12) were enrolled in a completely randomized design and divided into three groups: group 1 (n=30), received a single dose of $PGF_2\alpha$ injection, whereas, group 2 (n=24) and group 3 (n=30), were treated with two doses of $PGF_2\alpha$ injections at 12 and 24 hours intervals, respectively. The corpus luteum diameter was measured using the ultrasound at the beginning, 24, 48 and 72 hours after $PGF_2\alpha$ injection. Blood samples were collected to assess progesterone (P_4) on day 0 and 72 hours after treatment. Oestrus expression, $PGF_2\alpha$-oestrus interval and first service conception rate were evaluated. The results showed that the luteolysis process, the mean of $PGF_2\alpha$-oestrus interval and progesterone concentration at the beginning (day 0) and 72 hours after treatment were similar for all the groups. Oestrus expression ($PGF_2\alpha$+$PGF_2\alpha$-24 h=76.7% *vs.* $PGF_2\alpha$+$PGF_2\alpha$-12 h=66.7% and control=56.7%, P>0.05) and the first service conception rate ($PGF_2\alpha$+$PGF_2\alpha$-24 h=41.2% *vs.* $PGF_2\alpha$+$PGF_2\alpha$-12 h=40.0% and control=33.3%, P>0.05) were relatively higher in cows receiving two doses of $PGF_2\alpha$ injections at 24-hour-interval (P>0.05). In conclusion, treatment with an additional dose of $PGF_2\alpha$ during the middle stages of the oestrus cycle has no significant effect on luteolysis or reproductive performance.

KEY WORDS dairy cows, luteolysis, PGF2α, reproduction performance.

INTRODUCTION

Because of the increased milk production, the duration of oestrus decreases (Lopez *et al.* 2004; Wiltbank *et al.* 2006) and the current detection rate of oestrus is generally not optimal for efficient reproductive management programs in dairy farms (Senger, 1994; Peralta *et al.* 2005). In this regard, conception rates to the first insemination have been decreasing (Washburn *et al.* 2002). Hormonal treatment using prostaglandin F_2 alpha ($PGF_2\alpha$) to manipulate the oestrus cycle is a commonly used strategy in dairy farms (Ahuja *et al.* 2005). It is a well-known concept that if cows do not become pregnant, $PGF_2\alpha$ is produced by uterine glands at the end of the luteal phase, which triggers luteolysis and initiates a new oestrus cycle (Schams and Berisha, 2004; Ginther *et al.* 2009). Luteolysis can be induced phar-

macologically in cows during the diestrus phase (Wenzinger and Bleul, 2012). But, the newly formed corpus luteum (CL) is resistant to induction of luteolysis by exogenous $PGF_2\alpha$ until day 5 of the oestrus cycle (Levy *et al.* 2000). Devising new synchronisation procedures to promote reproductive efficiency in dairy industry has aroused considerable research interest within the past decade (Wiltbank and Pursley, 2014). Artificial shortening of the oestrus cycle after a $PGF_2\alpha$ treatment is likely to influence follicle selection, subsequent ovulation, or the development of a functional CL in the dairy cows (Schams and Berisha, 2004). $PGF_2\alpha$ has been used in many synchronisation protocols, such as OV-Synch, CO-Synch, Heat-Synch, CO-Synch-controlled internal drug release (CIDR), etc. Previous studies have indicated that incomplete luteal regression occurs in some cows are treated with synchronisation protocols (Moreira *et al.* 2000). CL regression rates induced by $PGF_2\alpha$ vary among different studies ranging from 81% to 95% (Pursley *et al.* 1997; Moreira *et al.* 2000; Gümen *et al.* 2003). Additionally, other studies have reported various percentages of cows with low progesterone (P_4) ranging from 84% to 94%, 48 h following $PGF_2\alpha$ administration (Cartmill *et al.* 2001; El-Zarkouny *et al.* 2004; Souza *et al.* 2007).

Therefore, synchronisation protocols can be undermined due to the absence of a full luteal regression of CL after the administration of a single $PGF_2\alpha$ dose. Several research groups are working on devising practical procedures to induce luteolysis of CL during timed AI protocols, including: the injection of a single large dose $PGF_2\alpha$ (Ribeiro *et al.* 2012; Giordano *et al.* 2013) or two fractioned doses of $PGF_2\alpha$ administered at varying intervals (Brusveen *et al.* 2009; Ribeiro *et al.* 2012). Brusveen *et al.* (2009) observed that cows treated with two doses of $PGF_2\alpha$ during an OV-Synch protocol, had low P4 at the timed artificial insemination (TAI) compared to cows treated with a single $PGF_2\alpha$ dose (97.5 *vs.* 86.1%). These results were consistent with those of Wiltbank *et al.* (2015), who employed an additional dose of $PGF_2\alpha$ in the original OV-Synch and double OV-Synch.

Moreover, they observed a trend towards increasing pregnancies per AI (P/AI) in cows treated with two doses of $PGF_2\alpha$. The second dose of $PGF_2\alpha$ administered 24 h after the first one could reduce the P_4 at TAI, and thus increase P/AI in cows re-synchronised with G6G-Ovsynch protocol (Carvalho *et al.* 2015). Santos *et al.* (2010) and Ribeiro *et al.* (2012) used the same idea to shift back the $PGF_2\alpha$ from day 7 to day 5 and add a further dose on day 6 in the CO-Synch 72 h protocol to completely regress CL, particularly in pre-synchronised cows which have a high ovulation rate in response to the first GnRH and interestingly, both groups could improve fertility parameters.

Previous reports described the ovulation time in cows treated with a single or two consecutive standard doses of $PGF_2\alpha$ followed by various synchronisation protocols (Santos *et al.* 2010; Ribeiro *et al.* 2012). However, details of the luteolysis process following the administration of single or two doses of $PGF_2\alpha$ at 12 and 24-hour intervals have not yet unravelled in such circumstances. Therefore, the present study was designed to evaluate the luteolysis response of mature CL during the middle stage of the unravel cycle after the administration of a single or two doses of $PGF_2\alpha$ at 12 and 24-hour intervals. Additionally, the pregnancy per AI was compared between different administration strategies.

MATERIALS AND METHODS

All procedures used in the present study were licensed by the Research Committee of the Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran (Research Committee No: 10210501942028).

Animals

The current study was carried out on a dairy farm under the standard management in Ardabil province, located in the North-Western of Iran (1351 m above sea level; 38.2 N˚ 48.3 E˚). The herd consisted of 2000 Holstein dairy cows, housed in open shed barns and fed formulated diets. The diets were a mixture of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals. The cows were milked by a pipeline milking machine, three times a day. The voluntary waiting period of the herd was 45 days. Experienced personnel performed heat detection during the day. Estrus detection was performed by visual observation (6 times daily (during the night and day) for at least 20-30 minutes each) and inseminations were conducted based on the am/pm rule.

Treatments

A total of 84 registered Holstein dairy cows were enrolled. The animals had a mean of 75 days in milk (DIM) without a history of peripartum diseases (dystocia, retained placenta, clinical hypocalcemia and ketosis), mastitis, lameness and systemic diseases in the previous lactation. Body condition score (BCS) was recorded at the treatment course (based on a 1 to 5 score), which ranged between 2.75 and 3.5 (average=2.97±0.37) for the enrolled cows. The average milk production and parity of the cows were 31 kg per day and 2.01, respectively. The animals were randomly divided into three groups: group 1 (n=30), cows were intramuscularly (i.m) injected a single dose of 225 µg d-Cloprostenol (Gestavet Prost®, Laboratorios, Hipra); Group 2 (n=24), cows were injected two doses of 225 µg d-Cloprostenol

with 12–hour interval; group 3 (n=30), cows were injected two doses of 225 µg d-Cloprostenol with 24-hour interval. There was no significant difference among the groups in terms of the DIM.

Ovarian transrectal ultrasonography

Transrectal ultrasonography (US) examinations (Hitachi®, Universal Medical System, Japan with a 7.5 MHz linear array transducer) of the ovaries were performed to assure that all the cows were within the middle stage of the estrous cycle (between days 7 and 12) and had a mature and functional CL (>17 mm) before the $PGF_2\alpha$ injection (Pieterse *et al.* 1990; Kayacik *et al.* 2006; Ginther, 2007). The CL diameter was evaluated using the US at the beginning, 24, 48 and 72 hours after the treatment. Following the observation of the maximum CL diameter in the images, the longitudinal and transversal diameters of the CL were measured at right angles with electronic callipers (Pierson and Ginther, 1984).

Progesterone assay

Blood samples were taken on day 0 and at 72 hours after the treatment via puncture of the median caudal vein with 8-mL evacuated tubes. Serum samples were separated from the blood specimens by centrifugation (750 *g*) at 4 ˚C for 15 minutes and stored at -20 ˚C until analysis. Progesterone concentrations were determined in duplicate using a commercial solid-phase, no-extraction radioimmunoassay (Coat-a-Count®; Diagnostic Products Corp., Los Angeles, CA, USA). All the samples were evaluated in a single assay with quality control samples of high (18 ng/mL), medium (4 ng/mL), and low (0.5 ng/mL) concentrations of P_4 evaluated. The calculated sensitivity was 0.005 ng/mL with an intra-assay coefficients of variation (CV) of 5.9%.

Evaluation of reproductive performance

Heat detection was performed 4 times a day for 6 days after the treatment, and then inseminations were performed. The proportion of animals expressing oestrus and $PGF_2\alpha$-oestrus interval was evaluated in all the groups. The first service conception rate was diagnosed and confirmed 30 days after the insemination by the US, based on the visualisation of an embryonic vesicle with a heartbeat, as described previously (Romano *et al.* 2006).

Statistical analysis

The SPSS software (Version 20.0, SPSS Inc., Chicago, Illinois) was employed for data analysis (SPSS, 2011). One-way ANOVA was used for comparison of different variables between the groups and post-hoc Tukey's test was used for significance. Oestrus rate and first service conception rate of different groups were analysed using the Chi-squared test. Effects of parity, milk production level and DIM on the first service conception rate were analysed using the multivariate logistic regression. Data were presented as the percentage or mean (±SD) and values of $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The mean percentage of CL regression was 85.4%, 3 days after treatment. The mean percentage of CL regression was 86.7, 87.0 and 82.8%, 3 days after $PGF_2\alpha$ treatment for cows received single $PGF_2\alpha$ treatment and cows received two $PGF_2\alpha$ doses at 12 and 24 hours intervals, respectively. The luteolysis process following $PGF_2\alpha$ administration was similar for the cows treated with single dose of $PGF_2\alpha$ and the cows received two doses at 12 and 24-hour intervals (P>0.05). No significant difference was observed among the groups regarding the CL size at the 24, 48 and 72 hours after the treatment (P>0.05) (Figure 1). A lack of new ovulation was also observed.

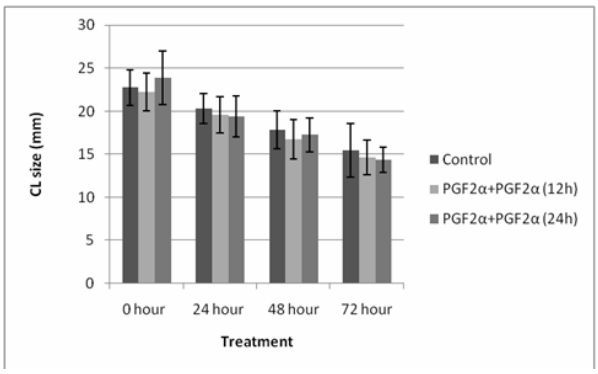

Figure 1 The CL diameter (Mean±SD) of treatment groups at the 0, 24, 48 and 72 hours after the treatment

The mean concentration of progesterone before (day 0) and 3 days after the treatment were 4.82 ± 0.15 and 0.76 ± 0.03 ng mL^{-1}, respectively, for all the cows. The concentration of progesterone was 0.74 ± 0.23, 0.75 ± 0.26 and 0.76 ± 0.39 in the single $PGF_2\alpha$ dose and two doses of $PGF_2\alpha$ administered 12 and 24 h apart, respectively, on day 3. However, the mean concentration of progesterone among treated groups was similar (P>0.05) at different days of sampling (Table 1).

The data revealed that, 56.7% of animals were in oestrus after the injection of a single dose of $PGF_2\alpha$. however, the repeated injections (two doses) at 12 and 24-hour intervals induced estrus in a proportion of 66.7% and 76.7% cows, respectively, with non-significant differences.

The mean $PGF_2\alpha$-oestrus interval was 3.35 ± 0.49, 3.37 ± 0.71 and 3.73 ± 0.61 in the single $PGF_2\alpha$ dose and two doses of $PGF_2\alpha$ administered 12 and 24 h apart, respectively.

Table 1 Concentrations of progesterone (ng mL-1) (Mean±SD) of the treatment groups at the beginning (day 0) and 3 days (day 3) after $PGF_2\alpha$ treatment

Item	Treatment			P-value
	$PGF_2\alpha$	$PGF_2\alpha$ + $PGF_2\alpha$ (12 h)	$PGF_2\alpha$ + $PGF_2\alpha$ (24 h)	
Day 0	5.00±1.50	4.42±1.48	4.89±1.30	0.3
Day 3	0.74±0.23	0.75±0.26	0.76±0.39	0.9

The mean $PGF_2\alpha$-oestrus interval after the treatment was similar (P>0.05) for the cows who received the single dose of $PGF_2\alpha$ treatment or two doses at 12 and 24-hour intervals (Figure 2).

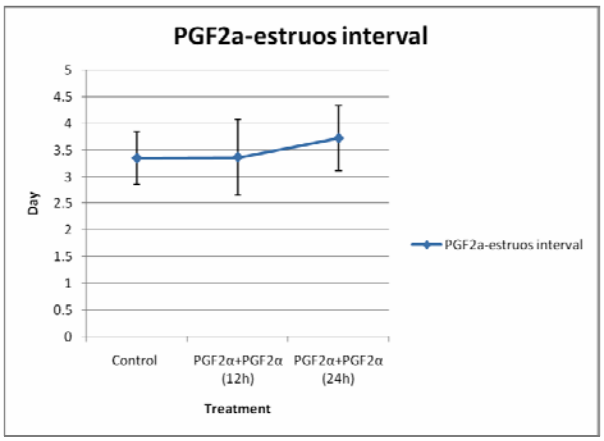

Figure 2 The mean $PGF_2\alpha$-oestrus interval (Mean±SD) of treatment groups

The first service conception rate was presented in Figure 3.

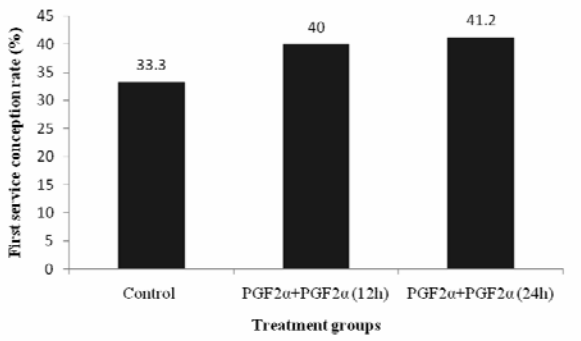

Figure 3 First service conception rate of treatment groups

The P/AI was 33.3, 40.0 and 42.2% in the single $PGF_2\alpha$ dose and two doses of $PGF_2\alpha$ administered 12 and 24 h apart, respectively. The second $PGF_2\alpha$ treatment had no significant effect on P/AI in cows. The first service conception rate among the groups was not significantly different (P>0.05). However, the first service conception rate was numerically higher in cows treated with an additional dose of $PGF_2\alpha$ at 24-hour intervals.

The parity, milk production level and DIM of cows were 2.1 ± 0.99, 1.7 ± 0.92 and 2.2 ± 1.47 and 31.01 ± 7.89, 28.0 ± 6.09 and 34.9 ± 8.50 and 73.63 ± 17.90, 75.41 ± 18.92 and 73.50 ± 17.59 in the single $PGF_2\alpha$ dose and two doses of $PGF_2\alpha$ administered 12 and 24 h apart, respectively. The parity, milk production level and DIM of cows do not affect the first service conception rate (P>0.05). Moreover, milk production on the day of treatment had no relationship with the interval to oestrus after treatment. No possible stress associated with milk yield appeared to influence the luteolysis process, the proportion of animals expressing oestrus and interval to oestrus following $PGF_2\alpha$ administration.

The current study evaluated whether the injection of two consecutive doses of $PGF_2\alpha$ at 12 and 24-hour intervals would promote the luteolysis process of mature and functional CL as well as reproductive performance during the middle stage of the oestrus cycle in dairy cows. The previous studies reported lower fertility in cows that had not complete luteal regression after the $PGF_2\alpha$ treatment in the OV-Synch protocol (Giordano *et al.* 2012b; Giordano *et al.* 2013). An acceptable luteal regression is needed around the AI to decrease P_4 concentrations and adequately optimise fertility (Ribeiro *et al.* 2012). Numerous studies demonstrated that in OV-Synch-type protocols, in which a single dose of $PGF_2\alpha$ is used, 10 to 25% of cows have inadequate regression of the CL (Giordano *et al.* 2012b). Therefore, several studies are currently being conducted to increase the effects of $PGF_2\alpha$ administration during synchronisation protocols for complete regression of CL. The present study showed that a single dose intra-muscular injection of $PGF_2\alpha$ was equally effective to those of 2 injections. The luteolysis process was similar between the cows treated with a single dose of $PGF_2\alpha$ and those treated with two doses of $PGF_2\alpha$ 12 and 24-hour intervals. No difference was observed among the groups regarding the CL size at different sampling days. Moreover the CL regression rate was 97.0% and 83.0% in animals treated with the two doses and a single dose of $PGF_2\alpha$ during the OV-Synch protocol (Wiltbank *et al.* 2015). The experiments revealed that the addition of a second $PGF_2\alpha$ injection could increase the rate of luteal regression and result in lower P_4 concentrations at a final GnRH of the OV-Synch protocol (Carvalho *et al.* 2015; Barletta *et al.* 2018). The second $PGF_2\alpha$ treatment 24 h increased the percentage of cows with low P4 concentration (≤0.5 ng/mL) at the second GnRH treatment of the OV-Synch protocol compared to the control group (73.2% *vs.*

55.7% P< 0.01) (Tippenhauer *et al.* 2021). The addition of $PGF_2\alpha$ injection during the Ovsynch protocol increased +11.6 percentage units luteal regression (Borchardt *et al.* 2018). On the contrary, other studies have claimed that there is no need for an injection of $PGF_2\alpha$ at two separate times, which is consistent with the results of this study (Santos *et al.* 2010; Giordano *et al.* 2012a; Atanasov *et al.* 2021). For example, it has been reported that the complete CL regression rate was between 73.9 to 95.2% following a single dose treatment with different types of $PGF_2\alpha$ analogues in lactating dairy cows and there was no significant difference between the groups (Santos *et al.* 2010; Giordano *et al.* 2012a; Atanasov *et al.* 2021). In our study, the mean percentage of CL regression was 85.4%, 3 days after treatment. The mean percentage of CL regression was 86.7, 87.0 and 82.8%, 3 days after $PGF_2\alpha$ treatment for cows received single $PGF_2\alpha$ treatment and cows received two $PGF_2\alpha$ doses at 12 and 24 hours intervals, respectively. The percentage of heifers that had regressed CL after 1 or 2 injections of $PGF_2\alpha$ was 86.9 and 92.8%, respectively, when utilising the 5-day CO-Synch + CIDR protocol in dairy heifers. Consistently, no significant difference was observed between various groups regarding the percentages of luteolysis (Rabaglino *et al.* 2010).

Luteal regression in cows with a functional CL was greater for multiparous cows receiving the 750 mg dose of $PGF_2\alpha$ compared to those receiving the 500 mg dose under breeding-OV-Synch. However, it has been reported that the greater dose of $PGF_2\alpha$ did not increase the percentage of primiparous cows with luteal regression during Double-OV-Synch (Giordano *et al.* 2013). Consistently, Say *et al.* (2016) demonstrated that there was no difference in CL regression between a single (25 mg) *vs.* double injection of $PGF_2\alpha$ (50 mg) in dairy heifers. The percentage of cows with complete luteal regression did not differ among double $PGF_2\alpha$ treatment group and control cows at the second GnRH treatment of the OV-Synch protocol (Barletta *et al.* 2018; Tippenhauer *et al.* 2021). It has been claimed that the luteolytic response to a single treatment of $PGF_2\alpha$ (regardless of the dose) is insufficient in young CLs (0 to 20% of cows had complete luteolysis); however, older CLs have a greater luteolytic response (Valldecabres-Torres *et al.* 2012). At the later stage of CL maturity, the luteolytic response depends on the $PGF_2\alpha$ dose (Valldecabres-Torres *et al.* 2012). Cows in different physiological statuses might be responsive to $PGF_2\alpha$ at an earlier stage of CL maturation (Nascimento *et al.* 2014). As evidence, on day 5 of the oestrus cycle, heifers were responsive to a single $PGF_2\alpha$ treatment, but none of the none-lactating or lactating cows were responsive. Furthermore, on day 7 of the cycle, the response rate of none-lactating cows was higher than those of heifers and lactating cows. Therefore, the differences observed in the response rate in various studies can be due to variations in physiological state or breed of cow used in these experiments (Momont and Seguin, 1984). It has been claimed that $PGF_2\alpha$ invokes some but not all of the biological responses in younger compared with older CL (Mondal *et al.* 2011). Receptors of $PGF_2\alpha$ can primarily be found on large luteal cells of CL (Anderson *et al.* 2001), either with or without luteolytic capacity (Diaz *et al.* 2002).

In the present study, the the concentration of progesterone was 0.74 ± 0.23, 0.75 ± 0.26 and 0.76 ± 0.39 in the single $PGF_2\alpha$ dose and two doses of $PGF_2\alpha$ administered 12 and 24 h apart, respectively, on day 3. The concentration of progesterone was not significantly different among various groups. Consistent results have been reported from cows in the final stage of OV-Synch, under treatment with GnRH (Brusveen *et al.* 2009). Contradictory results are also available, in cows during the OV-Synch protocol. The circulating P_4 concentrations at 56 h after $PGF_2\alpha$ treatment were greater for cows receiving a single dose than two doses (Wiltbank *et al.* 2015). Furthermore, Giordano *et al.* (2013) reported an increased number of cows with low P_4 concentrations at final GnRH during an OV-Synch protocol. Another study showed that cows receiving two doses of $PGF_2\alpha$ treatments had lower P_4 at final GnRH compared to cows receiving a single dose of $PGF_2\alpha$ in OV-Synch protocol and pre-synchronisation with GnRH followed by an OV-Synch protocol after 6 days (Nascimento *et al.* 2014). Cows treated with a double dose of $PGF_2\alpha$ (50 mg) and two doses (given at 8-hour intervals) had lower P_4 concentrations 12 and 24 hours after the treatment (0–24 hours) (Nascimento *et al.* 2014).

According to our results, all the animals were in oestrus at the same rate and there was no significant difference between single and multiple injections of $PGF_2\alpha$. Consistently, a recent study showed the same proportion of cows in oestrus in the CO-Synch protocol used (Alnimer *et al.* 2019).

Our study showed that the two $PGF_2\alpha$ treatments had no significant effect on P/AI in cows at the first service. But, treatment with a second dose of $PGF_2\alpha$ 12 and 24 h induced about 7% more pregnancies compared with the control group (40.0% and 41.2% respectively, vs. 33.3%) although, it was not significant. Also, the other observed a 5-7% for greater (not significant) P/AI for cows with a second dose of $PGF_2\alpha$ compared (35.4% and 36.5%) with control cows (30.7% and 29.0%) (Barletta *et al.* 2018; Kuru *et al.* 2020).

In contrast, previous studies showed that heifers treated with two doses of $PGF_2\alpha$ at 6-hours intervals on day 5 had greater P/AI than those treated with two Co- $PGF_2\alpha$ or a single dose of $PGF_2\alpha$ in a 5-day CO-Synch CIDR protocol (Say *et al.* 2016). Treatment with a second dose of $PGF_2\alpha$ during the OV-Synch protocol induced about 10% more

pregnancies (Giordano *et al.* 2011; Tippenhauer *et al.* 2021). In consent with our study, one or two doses of $PGF_2\alpha$ at CIDR removal resulted in similar P/AI. The P/AI was 51.2%, 51.9%, for a single and two $PGF_2\alpha$ injections, respectively, in a 5-day progesterone-based CO-Synch protocol (Kasimanickam *et al.* 2014). Although two doses of $PGF_2\alpha$ have been shown to successfully induce luteolysis of early CL of fewer than 5 days old, the procedure did not always improve fertility compared with a single dose of $PGF_2\alpha$ (Kasimanickam *et al.* 2009; Peterson *et al.* 2011). No differences in P/AI were observed between a single and two doses of $PGF_2\alpha$ in dairy heifers (Rabaglino *et al.* 2010), but multiple injections increased the P/AI in dairy cows (Cruppe *et al.* 2010). Increasing the dose of $PGF_2\alpha$ from 500 mg to 750 mg had no significant effect on cows during Double-Ovsynch (Giordano *et al.* 2013). The double dose of PGF2α (50 mg) don't affect P/AI compared with control cows (25 mg) in a 7-d Ovsynch protocol (30.7 *vs.* 32.4 P=0.30 and 31.8 *vs.* 33.4 P=0.46) (Barletta *et al.* 2018; Tippenhauer *et al.* 2021). However, the observed differences in response rate following a single or multiple injections or increasing the dose of $PGF_2\alpha$ in different studies can be related to differences in the luteinisation process and the age of CL (young *vs.* old). The younger CL does not regress after a single dose of $PGF_2\alpha$ treatment and needs an additional dose of $PGF_2\alpha$ during $PGF_2\alpha$ protocols (Nascimento *et al.* 2014).

CONCLUSION

In conclusion, single or two doses of PGF2α at 12 and 24-hour intervals during the middle stage of the estrous cycle on the equally effective for luteolysis process. In addition, estrus and pregnancy rates did not vary when single or two doses of PGF2α are used for the luteolysis in cows. Of course, using an additional dose of $PGF_2\alpha$ can be of a small benefit, but it is not significant.

ACKNOWLEDGEMENT

The authors thank the owners and staff of the Moghan Agro-industry Company for their cooperation.

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