



Effect of Exogenous Progesterone or Flunixin Meglumine After AI on Serum Progesterone Concentration and Pregnancy per AI in Lactating Dairy Cows

Saber Barkhori-Mehni¹, Hamed Karami-Shabankareh², Reza Masoumi^{3,9}, Mehdi Kazemi-Bonchenari⁴, Adel Pezeshki⁵, Arya Badiei⁶, Essa Dirandeh^{7,9}, Marcos G. Colazo⁸

¹Department of Health and Food Safety, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

²Department of Animal Science, Faculty of Agriculture, University of Razi, Kermanshah, Iran.

³Department of Animal Science, Faculty of Agriculture, University of Zanjan, Zanjan, Iran.

⁴Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran.

⁵Department of Animal Science, Oklahoma State University, Stillwater, United States of America.

⁶Department of Clinical Sciences, Veterinary Faculty, Islamic Azad University, Karaj, Alborz, Iran.

⁷Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

⁸Livestock Research Section, Alberta Agriculture and Forestry, Edmonton, AB, T6H 5T6, Canada.

Abstract

The objective of this study was to determine the effect of post AI administration of exogenous progesterone (P₄) or a prostaglandin F_{2α} (PGF_{2α}) synthesis inhibitor agent on serum P₄ concentrations and pregnancy per AI (P/AI) in lactating dairy cows. Eighty lactating cows were randomly allocated to one of four treatment groups: 1) CON (control), received 5 mL of saline solution on d 6 and 14 post AI; 2) IP4 (injection of P₄), received 125 mg of P₄ im on d 6 and 14 post AI; 3) CIDR, received a controlled internal drug release insert containing 1.38g of P₄ from d 6 to 20 post AI; and 4) FM (Flunixin Meglumine), received 0.625 g of Flunixin Meglumine, a nonsteroidal anti-inflammatory drug, twice daily on d 15 and 19 post AI. Blood samples were taken on d 0, 6, 14, 17 and 20 post AI to determine P₄ concentrations. Transrectal palpation was performed between 40 and 45 d post AI to determine pregnancy status. All treatment groups (i.e. IP4, CIDR and FM) resulted in greater serum P₄ concentration on d 17 and 20 post AI compared to CON (P < 0.05). Cows given a CIDR insert had greater concentrations of P₄ on d 17 and 20 than IP4 and FM cows (P < 0.05). However, no significant difference was found between IP4 and FM groups for serum P₄ concentrations. The P/AI was greater (P < 0.05) in CIDR-treated cows (55%, 11/20) than CON (25%, 5/20), and intermediate in IP4 (40%, 8/20) and FM (35%, 7/20) cows. In summary, treatment with exogenous P₄ (i.e. CIDR and IP4) or FM increased serum P₄ concentrations in lactating dairy cows. However, results suggest that only CIDR administration would improve P/AI.

Keywords: embryo loss, cattle, progesterone, reproductive performance.

Introduction

Early embryonic development and establishment of pregnancy in cattle depends upon progesterone (P₄) secretion by the corpus luteum (CL; Santos *et al.*, 2004; Leroy *et al.*, 2008; Motavalli *et al.*, 2017). Reduced pregnancy per AI (P/AI) in dairy cattle has been associated with low circulating P₄

concentrations or a delay in the rise of P₄ during the early post ovulatory phase (Mann and Lamming, 1999; Masoumi *et al.*, 2012; Masoumi *et al.*, 2017). Moreover, high producing dairy cows have increased dry matter intake (Holter *et al.*, 1997; Badiei *et al.*, 2014), hepatic blood flow (Sangsritavong *et al.*, 2002), and steroid hormone metabolism resulting in inadequate concentrations of circulating P₄. Therefore, various studies have evaluated the effect of supplementation with exogenous P₄ during metestrus or early diestrus on fertility of dairy cows, but results have been inconsistent (Larson *et al.*, 2007; Arndt *et al.*, 2009; Friedman *et al.*, 2012; Colazo *et al.*, 2013). In this regard, the efficacy of P₄ supplementation post-AI on pregnancy success was recently evaluated in a meta-analysis including 84 treatments involving data from 19,040 cows (Yan *et al.*, 2016). Results showed that P₄ supplementation from d 3 to 7 post-AI was beneficial but supplementation either earlier or later than this period reduced or did not affect P/AI. However, in a recent study, Garcia-Ispierto and López-Gatius (2017) compared pregnancy risk in high-producing lactating dairy cows administered exogenous P₄ during metaestrus (3 to 5 d post AI) or during the time of pregnancy recognition (15 to 17 d post-AI) with that in untreated control cows. Cows treated with P₄ during metaestrus or during the time of pregnancy recognition were 1.71 and 1.4 times, respectively, most likely to become pregnant than untreated control cows. In cattle, the regression of the CL (luteolysis) is initiated by the release of uterine prostaglandin F_{2α} (PGF_{2α}) at the late luteal stage (McCracken *et al.*, 1999; Masoumi *et al.*, 2011). Hence, other studies have attempted to extend the life-span of the CL with the administration of agents that inhibit the synthesis or release of PGF_{2α} to enhance pregnancy in cattle (Elli *et al.*, 2001; Geary *et al.*, 2010). In this regard, greater P/AI was obtained in Italian Friesian cattle treated with ibuprofen lysinate, a Cyclooxygenase 1 and 2 (COX-1 and COX-2) inhibitor on d 17 after AI (Elli *et al.*, 2001). Flunixin Meglumine (FM), a strong non-steroid anti-inflammatory drug (NSAID), also prevents the synthesis of COXs and conversion of arachidonic acid to PGF_{2α} (Purcell *et al.*, 2005; Geary *et al.*, 2010). Although, Anderson *et al.* (1986) reported that FM could potentially extend the life-span of the CL in lactating dairy cows, there is a

⁹Corresponding author: rmasoumi@znu.ac.ir; dirandeh@gmail.com

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lack of publications about the effect of FM on serum concentration of P₄ and P/AI.

The primary objective of this study was to evaluate the effect of post AI administration of exogenous P₄ or a PGF₂α synthesis inhibitor agent on serum P₄ concentrations in lactating dairy cows. A secondary objective was to determine whether post AI administration of exogenous P₄ or a PGF₂α synthesis inhibitor agent would improve P/AI. Our hypothesis was that post AI administration of exogenous P₄ or a PGF₂α synthesis inhibitor agent would increase serum P₄ concentrations and the proportion of cows becoming pregnant after AI.

Material and methods

This study was carried out on a commercial dairy farm located near Kermanshah, Iran. All animal experimental procedures were reviewed and approved by the Iranian Ministry of Agriculture (experimental permission No. 3548). Eighty multiparous Holstein dairy cows (Mean ± SEM; body weight: 652 ± 22 Kg; days in milk: 83 ± 15, milk production 29.8 ± 1.6 L/d; parity: 3.34 ± 0.58, body condition score: 2.84 ± 0.26) were used in the present study. Cows were fed a TMR (Total Mixed Ration) formulated for a lactating dairy cow of 650 kg body weight, producing 35 kg of 3.5% fat milk per day, according to NRC (2001). The TMR was offered thrice daily and cows had free access to water. Estrus detection was performed by two technicians every 3 h for at least 20 min during each observation. Estrus was confirmed by transrectal palpation before AI as previously described (López-Gatius and Camón-Urgel, 1991; Masoumi *et al.*, 2018). Cows confirmed in estrus were inseminated, according to the am/pm rule, with semen from sires available commercially. All cows were inseminated by the same technician. Pregnancy status was determined by transrectal palpation performed between 40 and 45 d post AI.

The day of AI was considered as day 0 of the study and cows (n = 20/group) were randomly allocated to one of four treatment groups (i.e. CON, IP4, CIDR and FM). Cows in the CON group received 5 mL of saline solution on d 6 and 14 post AI. Cows in IP4 group received 125 mg of P₄ (25 mg/mL P₄, Aburaihan Pharmaceutical Co., Tehran, Iran) on d 6 and 14 post AI. Cows in the CIDR group received a controlled internal drug release insert containing 1.38g of P₄ (EAZI-BREED CIDR, Zoetis Inc., Dublin, Ireland) from d 6 to 20 post AI. Cows in the FM group received 0.625 g of Flunixin Meglumine (50 mg/mL, Banamine, Merck Animal Health, Darmstadt, Germany) twice daily on d 15 and 19 post AI. All treatments were carried out at approximately 0007 h.

Blood samples (~10 mL) were collected from coccygeal vein into evacuated tubes (Vacutainer®; Becton Dickinson and Company, New Jersey, USA) on d 0, 6, 14, 17 and 20 post AI in all cows approximately at 1100 h. Blood samples were left undisturbed for

approximately 2 h at room temperature to allow clot formation and then centrifuged at 2000 × g for 10 min, serum harvested and frozen at -20°C until assayed for P₄. The serum P₄ concentration was determined using a commercial ELISA kit (DRG, Marburg, Germany; EIA-1292) as described previously (Heidari *et al.*, 2017; Masoumi *et al.*, 2017). The sensitivity of assay was 0.2 ng/mL and the inter- and intra-assay coefficients of variation for samples were 10.2 and 8.3%, respectively. The inter- and intra-assay coefficients of variation for control values were 2.2 and 3.0%, respectively.

Illustration of experimental procedures and treatments during the study are shown in Figure 1.

Data were analyzed using SAS software (SAS 9.3; 2003; SAS Institute Inc., Cary, NC, USA). A probability of 0.05 or less was considered statistically significant, and a probability between 0.051 and 0.1 was considered a tendency.

Serum P₄ data was analyzed with the MIXED procedure of SAS using the following model:

$$Y_{ijk} = \mu + C_i + T_j + D_k + TD_{jk} + \varepsilon_{ij}$$

Where Y_{ij} is the dependent variable, μ is the overall mean, C_i is the effect of cow i , T_j is the effect of treatment j , D_k is the effect of sampling day k , TD_{jk} is the interaction between treatment j and sampling day k and ε_{ij} is the residual error. Two orthogonal contrasts were planned *a priori* to determine the differences between treatment groups (Contrast 1; FM vs. IP4 and CIDR and Contrast 2; IP4 vs. CIDR). Pregnancy per AI (P/AI) was compared among all experimental groups by using logistic procedure of SAS.

Results

The serum P₄ concentrations in all cows during the entire study are presented and displayed in Table 1 and Figure 2. Serum P₄ concentrations at d 0 and 6 post AI did not differ ($P > 0.05$) among treatment groups. However, cows in the IP4, CIDR and FM groups tended to have greater ($P = 0.06$) serum P₄ concentration at d 14 post AI and had greater ($P < 0.01$) serum P₄ concentration at d 17 and 20 post AI compared to cows in the CON group. In addition, cows given a CIDR had greater ($P < 0.01$) concentrations of P₄ in serum at d 17 and 20 post AI than IP4- or FM-treated animals. There was no significant difference for serum P₄ concentration between IP4 and FM during the entire study.

We also compared the serum P₄ concentrations in pregnant (Figure 3) and non-pregnant (Figure 4) cows among CON, IP4, CIDR and FM groups. Serum P₄ concentrations on d 0 and 6 post AI did not differ ($P > 0.05$) among treatment groups in pregnant and non-pregnant cows. Similarly, serum P₄ concentrations on d 14, 17 and 20 post AI were not significantly different ($P > 0.05$) among treatment groups in non-pregnant cows. However, in pregnant cows, serum P₄ concentrations on d 14 post AI were greatest ($P < 0.05$)

in CIDR group compared to CON, IP4 or FM. Moreover, pregnant cows in IP4 and FM groups had greater ($P < 0.05$) serum P_4 concentrations than pregnant cows in CON group. However, P_4 concentrations did not differ between IP4 and FM groups ($P < 0.05$). On d 17 post AI, serum P_4 concentrations were significantly greater in CIDR group ($P < 0.01$) compared to CON, IP4 or FM. Interestingly P_4 concentrations did not differ between CON and FM groups. However, pregnant cows in the IP4 group had greater ($P < 0.01$) P_4 concentrations than pregnant cows in the CON group. Cows in the CIDR group also had the greatest serum P_4

concentrations on d 20 post AI ($P < 0.01$). In addition, cows in FM and IP4 groups had greater P_4 concentrations than cows in CON group ($P < 0.01$). However, serum P_4 concentrations did not differ between IP4 and FM groups ($P > 0.05$).

The P/AI for CON, IP4, CIDR and FM groups were 25% (5/20), 40% (8/20), 55% (11/20) and 35% (7/20), respectively. There was a significant difference for P/AI between CIDR and CON groups ($P = 0.03$). However, P/AI did not differ between IP4 and FM groups ($P = 0.58$) or when both groups were compared with CON ($P = 0.17$).

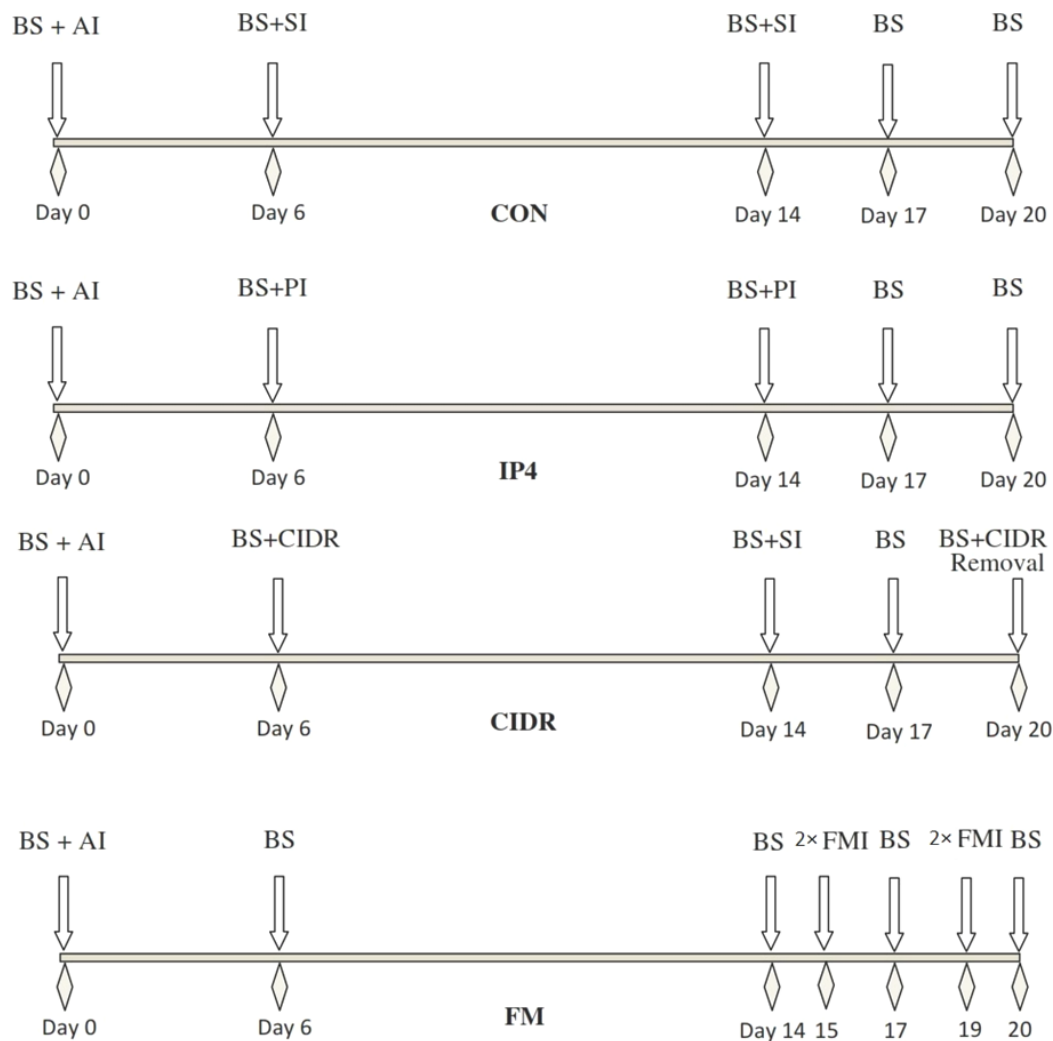


Figure 1. Illustration of experimental procedures and treatments during the study. Lactating dairy cows ($n = 80$) were randomly allocated to one of the following treatments groups: **CON** (control group: injection of saline on d 6 and 14 post AI); **IP4** (injection of 125 mg of progesterone im on d 6 and 14 post AI); **CIDR** (insertion of a controlled internal drug-release device containing 1.38g of P_4 from d 6 to 20 post AI) or **FM** (injection of 0.625 g of Flunixin Meglumine im twice daily on d 15 and 19 post AI). AI (Artificial Insemination); BS (Blood Sampling); SI (Saline Injection); PI (Progesterone Injection); FM (Flunixin Meglumine).



Table 1. Serum progesterone concentrations (ng/mL) in lactating dairy cows treated with saline (CON), progesterone (IP4), CIDR, or flunixin meglumine (FM).

Treatments ¹					Contrasts ²		
	CON	IP4	CIDR	FM	P	C1	C2
d 0³							
Non-Pregnant	0.221 ± 0.08	0.238 ± 0.07	0.263 ± 0.01	0.213 ± 0.07	0.93	0.71	0.80
Pregnant	0.245 ± 0.09	0.273 ± 0.09	0.297 ± 0.06	0.249 ± 0.05	0.75	0.67	0.73
Overall	0.234 ± 0.05	0.261 ± 0.04	0.285 ± 0.08	0.230 ± 0.09	0.81	0.74	0.84
d 6							
Non-Pregnant	2.11 ± 0.23	3.09 ± 0.43	3.78 ± 0.30	3.19 ± 0.31	0.36	0.27	0.23
Pregnant	2.40 ± 0.12	3.40 ± 0.18	4.12 ± 0.22	3.56 ± 0.23	0.67	0.56	0.17
Overall	2.28 ± 0.16	3.23 ± 0.14	3.97 ± 0.20	3.36 ± 0.15	0.42	0.38	0.11
d 14							
Non-Pregnant	3.91 ± 0.95	5.01 ± 1.09	5.29 ± 0.83	4.18 ± 0.79	0.12	0.23	0.13
Pregnant	4.42 ± 0.78	5.41 ± 0.96	8.23 ± 75	5.12 ± 0.74	0.05	0.06	0.03
Overall	4.23 ± 0.56	5.23 ± 0.75	7.12 ± 0.47	4.85 ± 0.86	0.06	0.08	0.09
d 17							
Non-Pregnant	3.23 ± 1.09	4.49 ± 0.45	5.37 ± 0.62	3.98 ± 0.57	0.34	0.18	0.21
Pregnant	6.42 ± 95	8.78 ± 0.73	10.19 ± 0.48	8.23 ± 0.97	<0.01	0.02	<0.01
Overall	5.35 ± 0.48	7.12 ± 0.87	9.46 ± 0.92	6.38 ± 0.57	<0.01	<0.01	<0.01
d 20							
Non-Pregnant	2.34 ± 0.35	4.98 ± 0.67	5.19 ± 0.96	4.79 ± 0.65	0.24	0.36	0.42
Pregnant	7.78 ± 0.95	11.87 ± 0.86	13.23 ± 1.25	9.21 ± 1.08	<0.01	<0.01	<0.01
Overall	5.75 ± 0.56	8.73 ± 0.67	9.86 ± 0.86	7.05 ± 0.71	<0.01	<0.01	0.02

¹CON: injection of saline on d 6 and 14 post AI; IP4: injection of 125 mg of P4 im on d 6 and 14 post AI; CIDR: administration of a controlled internal drug-release insert containing 1.38 g of P4 from d 6 to 20 post AI; FM: 1.25 g of Flunixin Meglumine, a non-steroidal anti-inflammatory drug, im twice daily on d 15 and 19 post AI. ²Orthogonal contrasts; contrast 1 (C1), FM vs. IP4 and CIDR; and contrast 2 (C2), IP4 vs. CIDR. ³ day 0 = day of AI.

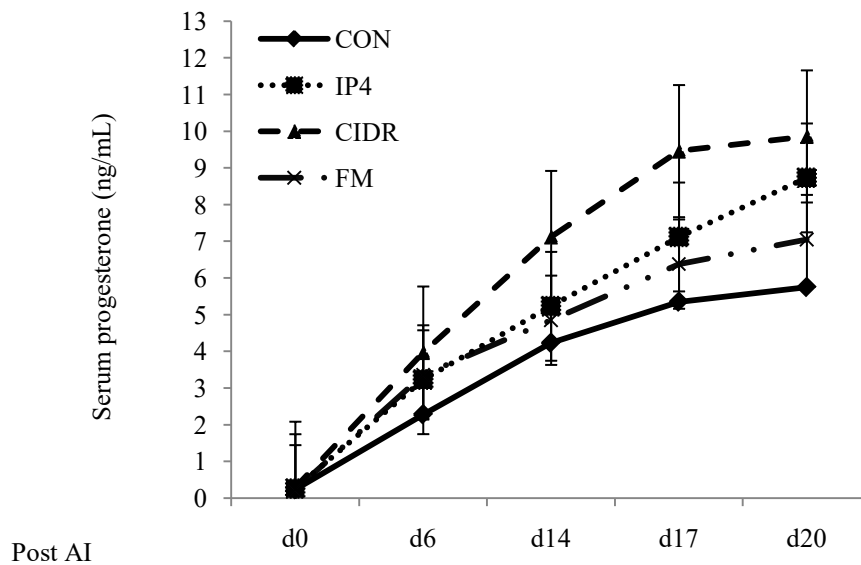


Figure 2. Serum progesterone (P₄) concentrations (ng/mL) in all lactating dairy cows during the study. **CON** (◆): received saline solution im on d 6 and 14 post AI; **IP4** (■): received 125 mg of P₄ im on d 6 and 14 post AI; **CIDR** (▲): given a controlled internal drug-release device containing 1.38 g of P₄ from d 6 to 20 post AI; **FM** (×): received 0.625 g of Flunixin Meglumine (FM), a non-steroidal anti-inflammatory drug, im twice daily on d 15 and 19 post AI.

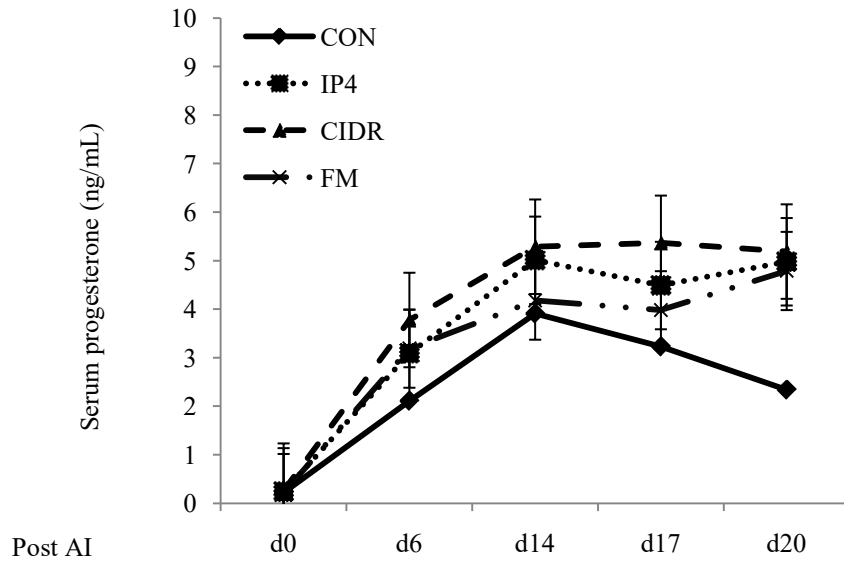


Figure 3. Serum progesterone (P₄) concentrations (ng/mL) in non-pregnant lactating dairy cows during the study. **CON** (◆): received saline solution im on d 6 and 14 post AI; **IP4** (■): received 125 mg of P₄ im on d 6 and 14 post AI; **CIDR** (▲): given a controlled internal drug-release device containing 1.38 g of P₄ from d 6 to 20 post AI; **FM** (×): received 0.625 g of Flunixin Meglumine (FM), a non-steroidal anti-inflammatory drug, im twice daily on d 15 and 19 post AI.

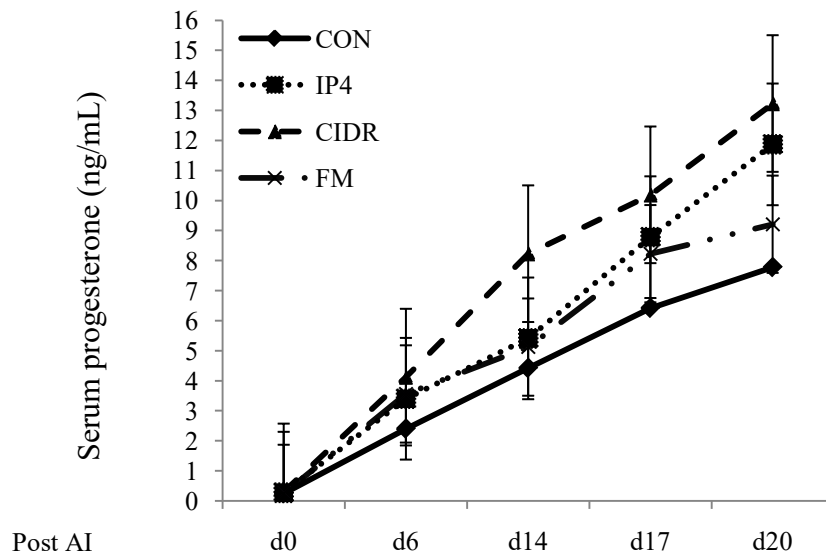


Figure 4. Serum progesterone (P₄) concentrations (ng/mL) in pregnant lactating dairy cows during the study. **CON** (◆): received saline solution im on d 6 and 14 post AI; **IP4** (■): received 125 mg of P₄ im on d 6 and 14 post AI; **CIDR** (▲): given a controlled internal drug-release device containing 1.38 g of P₄ from d 6 to 20 post AI; **FM** (×): received 0.625 g of Flunixin Meglumine (FM), a non-steroidal anti-inflammatory drug, im twice daily on d 15 and 19 post AI.

Discussion

Progesterone is essential to successful establishment of pregnancy in cattle and inadequate circulating P₄ is one of the main reasons of low fertility in lactating dairy cows (Inskeep, 2004; Dirandeh *et al.*, 2018). The present study evaluated the effect of post AI administration of exogenous P₄ or a PGF₂α synthesis inhibitor agent on serum P₄ concentrations in lactating dairy cows. There were no significant differences for serum P₄ concentrations among treatment groups on d 0

and 6 post AI in pregnant and non-pregnant cows. This was expected as treatments were initiated on d 6 or after d 6 post AI. However, serum P₄ concentrations were affected by treatments on d 14, 17 and 20 post AI. Cows given exogenous P₄, in particular those receiving a CIDR insert, tended to have greater P₄ concentrations on d 14 post AI compared to those receiving FM or control group. On d 17 and 20, the average serum P₄ concentration remained greater in cows given a CIDR insert but it was not statistically different between cows in IP₄ and FM groups. Control cows had lower serum P₄



concentrations compared to all three treatment groups. The differences in serum P₄ concentrations on d 20 post AI between treatment groups and control might have also been associated to the pregnancy status of the cows, rather than merely to the treatment per se, as numerically more cows were pregnant in all three treatment groups compared to control.

The pharmacokinetics of P₄ following CIDR insertion has been previously evaluated in cattle (Martinez *et al.*, 2007; Mariano *et al.*, 2010). In a study, ovariectomized beef cows received a previously used CIDR insert for 7 d. Plasma P₄ concentrations increased to ~2 ng/mL by 6 h after CIDR insertion. Thereafter, plasma P₄ concentrations decreased by 84 h after CIDR insertion and remained relatively constant. By 12 h after CIDR removal on day 7, P₄ concentrations declined to <0.2 ng/mL (Martinez *et al.*, 2007). In another study using lactating dairy cows without a functional CL, serum P₄ peaked within 3 h after CIDR insertion followed by a progressive decrease over the next 2 d until reaching sustained concentrations over the remaining 6 d of CIDR treatment. The range of sustained P₄ serum concentrations varied between 2.25 and 4.09 ng/mL (Mariano *et al.*, 2010). In the present study, serum P₄ concentrations increased from ~4 to ~9 ng/mL by 11 d after CIDR insertion and remained relatively constant until CIDR removal (d 20 post AI) in CIDR-treated cows.

Neither the pharmacokinetics of P₄ following intramuscular injections nor their effect on serum P₄ concentrations and PR/AI has been reported in lactating dairy cows. However, Turino *et al.* (2010) examined the pharmacokinetics of P₄ in lactating dairy cows given an intravenous injection of 100 mg P₄. Plasma P₄ reached a concentration of 140 ng/mL 1 min after treatment and dropped to baseline concentrations within 2 h after treatment (Turino *et al.*, 2010). In our study, serum P₄ concentrations increased from ~3 (day 6 post AI) to ~5 ng/mL (day 14 post AI) and from ~5 (day 14 post AI) to ~9 ng/mL (day 20 post AI) in cows given P₄ im.

Multiple factors can affect the very complex process of pregnancy maintenance during the time of recognition of pregnancy. During implantation, appropriate antiluteolytic signals i.e. interferon-tau produced by conceptus is vital to prevent endometrial PGF₂α secretion (Poyser, 1995; Dirandeh *et al.*, 2015). Conversely, during maternal recognition of pregnancy, interferon-tau increases endometrial prostaglandin E₂ (PGE₂), which is considered a potent luteoprotective factor (Arosh *et al.*, 2016). Interestingly, endometrial PGE₂ induces additional PGE₂ biosynthesis from CL which counteracts the luteolytic effect of PGF₂α during maternal recognition of pregnancy or at the time of establishment of pregnancy (Romero *et al.*, 2015). Agents that prevent the synthesis of PGF₂α such as Flunixin Meglumine or Meloxicam are licensed to be used in cattle and can potentially extend the CL life span. These agents are classified as non-steroidal anti-inflammatory drugs (NSAIDs) which may act on different prostaglandin pathways (i.e. COX-1 and COX-2). Flunixin Meglumine is both a COX-1 and COX-2 inhibitor but is more selective for COX-1; it has an

elimination half-life of 3 to 8 h (Odensvik, 1995). In a study, supplementation with 1 g of FM during the first 6 d postpartum decreased the release of PGF₂α as mirrored by decreased plasma concentrations of 13, 14-dihydro-15-keto-PGF₂α in brown Swiss cows (Guilbault *et al.*, 1987).

The current study also evaluated the effect of post AI administration of exogenous P₄ or a PGF₂α synthesis inhibitor agent on P/AI in lactating dairy cows. It has been shown that low circulating P₄ concentrations or a delay in the rise of P₄ during the early post ovulatory phase is associated with reduced conceptus development and fertility in cattle (Mann and Lamming, 1999; Masoumi *et al.*, 2012; Masoumi *et al.*, 2017). Several studies have evaluated the effect of post AI supplementation with exogenous P₄ during metaestrus or early diestrus on fertility of lactating dairy cows. Administration of a previously used CIDR, which originally contained 1.9 g of P₄, from day 3.5 to day 10 post AI resulted in improved P/AI of dairy cows (Larson *et al.*, 2007). However, other researchers (Arndt *et al.*, 2009; Colazo *et al.*, 2013) did not observe any improvement in P/AI in lactating cows treated with a CIDR containing 1.38 g of P₄ or a PRID containing 1.55 g P₄, from d 4 to 18 or 4.5 to 11.5 post AI, respectively.

A research group from Spain has investigated the effect of P₄ supplementation during late diestrus or at the time of maternal recognition of pregnancy on reproductive performance of high producing dairy cows. Circulating concentrations of P₄ and fertility did not increase in dairy cows given an insert containing 1.55 g of P₄ from d 5 to 19 post AI (Garcia-Ispierto and López-Gatius, 2012). In a more recent study, Garcia-Ispierto *et al.* (2016) determined fertility response to P₄ supplementation from day 15 to 17 post-AI in high-producing dairy cows. Cows with no history of early postpartum reproductive disorders (i.e. retained placenta) that were supplemented with P₄ during maternal recognition of pregnancy were 1.6 times more likely to become pregnant than the control (no P₄ treatment) herd mates.

In the present study, P/AI was enhanced in cows given a CIDR insert compared to control cows. Albeit, no statistically significant, P/AI in IP₄ cows was 14% greater than in control cows. It is noteworthy to mention that the effect of P₄ on fertility was not the primary objective of this study, which was under powered to detect a 14% difference in P/AI among treatments. However, our results are in agreement with others and support the notion that P₄ supplementation during early and late diestrus has a positive effect on P/AI in lactating dairy cows. In addition, numerically more FM cows (35%) become pregnant compared to control cows (25%), but the difference in P/AI was not statistically significant. Current research has not investigated the effect of administration of FM on P/AI in lactating dairy cows, but several reports have been published regarding the effect of FM on P/AI in dairy heifers. In agreement with our results, dairy heifers (n = 2325) treated twice with 400 mg of FM im at d 15 and 16 post AI had similar P/AI compared to untreated heifers (59.4 vs 59.5%) (Rabaglino *et al.*, 2010).



However, Guzeloglu *et al.* (2007) reported that the administration of 1.1 mg/kg FM 12 h apart at 15.5 and 16 d after timed-artificial insemination (TAI) improved P/AI by 23% in dairy heifers (69.2 vs 46.2%). Also, FM has been administered to prevent early regression of the CL and increase pregnancy rate in recipient cattle immediately before embryo transfer (Schrick *et al.*, 2001; Aguiar *et al.*, 2013; Kasimanickam *et al.*, 2018). Amiridis *et al.* (2009) assessed the effectiveness of three different NSAIDs including ketoprofen, Meloxicam and FM on the length of estrous cycle and showed that Meloxicam was the most potent among the three NSAIDs. In addition, Aguiar *et al.* (2013) reported that Meloxicam had a positive effect on the overall pregnancy rate of embryo recipient heifers. However, pregnancy rate was not affected by administration of Meloxicam recipient heifers classified as Grade I (easy passing catheter) but Meloxicam increased the pregnancy rate of heifers classified as Grade II (difficult passing catheter). Therefore, the effect of the administration of FM or Meloxicam on P/AI in lactating dairy cows warrants further investigation.

Conclusions

In summary, treatment with exogenous P₄ (i.e. CIDR insertion from d 6 to 20 post AI or P₄ injection on d 6 and 14 post AI) or Flunixin Meglumine (PGF₂α synthesis inhibitor; twice daily on d 15 and 19 post AI) increased serum P₄ concentrations compared to treatment with saline solution (control). However, results suggest that only CIDR administration would improve P/AI in lactating dairy cows.

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Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

References

Aguiar TS, Araújo CV, Tirloni RR, Martins LR. 2013. Effect of meloxicam on pregnancy rate of recipient heifers following transfer of in vitro produced embryos. *Reprod Dom Anim*, 48:984-988.

Amiridis GS, Tsiligianni TH, Dovolou E, Rekkas C, Vouzaras D, Menegatos I. 2009. Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. *Theriogenology*, 72:542-548.

Anderson K, Kindahl H, Smith A, Davis L, Gustafsson B. 1986. Endotoxin-induced bovine

mastitis: arachidonic acid metabolites in milk and plasma and effect of flunixin meglumine. *Am J Vet Res*, 47:1373-1377.

Arndt WJ, Holle AJ, Bauer ML, Kirsch JD, Schimek DE, Odde KG, Vonnahme KA. 2009. Effect of post-insemination progesterone supplementation on pregnancy rate in dairy cows. *Can J Vet Res*, 73:271.

Arosh JA, Banu SK, McCracken JA. 2016. Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition and establishment of pregnancy in ruminants. *J Dairy Sci*, 99:5926-5940.

Badiei A, Aliverdilou A, Amanlou H, Beheshti M, Dirandeh E, Masoumi R, Moosakhani F, Petit HV. 2014. Postpartum responses of dairy cows supplemented with n-3 fatty acids for different durations during the peripartur period. *J Dairy Sci*, 97:6391-6399.

Colazo MG, Dourey A, Rajamahendran R, Ambrose DJ. 2013. Progesterone supplementation before timed AI increased ovulation synchrony and pregnancy per AI, and supplementation after timed AI reduced pregnancy losses in lactating dairy cows. *Theriogenology*, 79:833-841.

Dirandeh E, Towhidi A, Ansari Z, Saberifar T, Akhlaghi A, Rezaei Roodbari A. 2015. The endometrial expression of prostaglandin cascade components in lactating dairy cows fed different polyunsaturated fatty acids. *Theriogenology*, 83:206-212.

Dirandeh E, Masoumi R, Didarkhah M, Samadian F, Dadashpour Davachi N, Colazo MG. 2018. Effect of presynchronization prior to Ovsynch on ovulatory response to first GnRH, ovulatory follicle diameter and pregnancy per AI in multiparous Holstein cows during summer in Iran. *Ann Anim Sci*, DOI: <https://doi.org/10.2478/aoas-2018-0011>

Elli M, Gaffuri B, Frigerio A, Zanardelli M, Covini D, Candiani M, Vignali M. 2001. Effect of a single dose of ibuprofen lysinate before embryo transfer on pregnancy rates in cows. *Reproduction*, 121:151-154.

Friedman E, Roth Z, Voet H, Lavon Y, Wolfenson D. 2012. Progesterone supplementation post insemination improves fertility of cooled dairy cows during the summer. *J Dairy Sci*, 95:3092-3099.

Garcia-Ispuerto I, López-Gatius F. 2012. Effects of GnRH or progesterone treatment on day 5 post AI on plasma progesterone, luteal blood flow and leucocyte counts during the luteal phase in dairy cows. *Reprod Domest Anim*, 47:224-229.

Garcia-Ispuerto I, López-Helguera I, Serrano-Pérez B, Paso V, Tuono T, Ramon A, Mur-Novales R, Tutusaus J, López-Gatius F. 2016. Progesterone supplementation during the time of pregnancy recognition after artificial insemination improves conception rates in high-producing dairy cows. *Theriogenology*, 85:1343-1347.

Garcia-Ispuerto I, López-Gatius F. 2017. Progesterone supplementation in the early luteal phase after artificial insemination improves conception rates in high-producing dairy cows. *Theriogenology*, 90:20-24.

Geary T, Ansotegui R, MacNeil M, Roberts A, Waterman R. 2010. Effects of flunixin meglumine on pregnancy establishment in beef cattle. *J Anim Sci*,



88:943-949.

- Guilbault L, Thatcher W, Drost M, Haibel G.** 1987. Influence of a physiological infusion of prostaglandin F_{2α} into postpartum cows with partially suppressed endogenous production of prostaglandins. 1. Uterine and ovarian morphological responses. *Theriogenology*, 27:931-946.
- Guzeloglu A, Erdem H, Saribay M, Thatcher W, Tekeli T.** 2007. Effect of the administration of flunixin meglumine on pregnancy rates in Holstein heifers. *Papers & Articles. Vet Rec*, 160:404-406.
- Heidari F, Dirandeh E, Pirsaraei ZA, Colazo MG.** 2017. Modifications of the G6G timed-AI protocol improved pregnancy per AI and reduced pregnancy loss in lactating dairy cows. *Animal*, 11:2002-2009.
- Holter J, West J, McGilliard M.** 1997. Predicting *ad libitum* dry matter intake and yield of Holstein cows. *J Dairy Sci*, 80:2188-2199.
- Inskip E.** 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J Anim Sci*, 82 E-Suppl: E24-39.
- Kasimanickam RK, Hall JB, Estill CT, Kastelic JP, Joseph C, Aziz RA, Nak D.** 2018. Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. *Theriogenology*, 107:70-77.
- Larson SF, Butler W, Currie WB.** 2007. Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Anim Reprod Sci*, 102:172-179.
- Leroy J, Opsomer G, Van Soom A, Goovaerts I, Bols P.** 2008. Reduced fertility in high yielding dairy cows: Are the oocyte and embryo in danger? Part I the importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. *Reprod Domest Anim*, 43:612-622.
- López-Gatius F, Camón-Urgel J.** 1991. Confirmation of estrus rates by palpation per rectum of genital organs in normal repeat dairy cows. *Transbound Emerg Dis*, 38:553-556.
- Mann G, Lamming G.** 1999. The influence of progesterone during early pregnancy in cattle. *Reprod Domest Anim*, 34:269-274.
- Mariano RN, Turino LN, Cabrera MI, Scándolo DE, Maciel MG, Grau RJ.** 2010. A simple pharmacokinetic model linking plasma progesterone concentrations with the hormone released from bovine intravaginal inserts. *Res Vet Sci*, 89:250-256.
- Martinez MF, Kastelic JP, Colazo MG, Mapletoft RJ.** 2007. Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. *Domest Anim Endocrinol*, 33:77-90.
- Masoumi R, Towhidi A, Javaremi AN, Nabizadeh H, Zhandi M.** 2011. Influence of PGF₂ alpha on semen quality and libido in Holstein bulls. *Turk J Vet Anim Sci*, 35:1-6.
- Masoumi R, Badieli A, Imam G, Mosakhani F, Curtiss A.** 2012. Evaluation of uterotonic effects of Dinoprost in pyometric and clinically healthy lactating dairy cows. *Reprod Domest Anim*, 47:477.
- Masoumi R, Badieli A, Shahneh A, Kohram H, Dirandeh E, Colazo MG.** 2017. A short presynchronization with PGF_{2α} and GnRH improves ovarian response and fertility in lactating Holstein cows subjected to a Heatsynch protocol. *Ann Anim Sci*, 17:169-177.
- Masoumi R, Badieli A, Mousakhani F, Dirandeh E, Zhandi M, Stear M.** 2018. Quantification of the uterine involution and dimensions, hormonal response and reproductive performance of pyometric and healthy dairy cows treated with Dinoprost. *S Afr J Anim Sci*, 48:222-233.
- McCracken JA, Custer EE, Lamsa JC.** 1999. Luteolysis: a neuroendocrine-mediated event. *Physiol Rev*, 79:263-323.
- Motavalli T, Dirandeh E, Deldar H, Colazo MG.** 2017. Evaluation of shortened timed-AI protocols for resynchronization of ovulation in multiparous Holstein dairy cows. *Theriogenology*, 95:187-192.
- Odensvik K.** 1995. Pharmacokinetics of flunixin and its effect on prostaglandin F_{2α} metabolite concentrations after oral and intravenous administration in heifers. *J Vet Pharmacol Ther*, 18:254-259.
- Poyser N.** 1995. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. *Prostag Leukotr Ess*, 53:147-195.
- Purcell S, Beal W, Gray K.** 2005. Effect of a CIDR insert and flunixin meglumine, administered at the time of embryo transfer, on pregnancy rate and resynchronization of estrus in beef cattle. *Theriogenology*, 64:867-878.
- Rabaglino M, Risco C, Thatcher M-J, Lima F, Santos J, Thatcher W.** 2010. Use of a five-day progesterone-based timed AI protocol to determine if flunixin meglumine improves pregnancy per timed AI in dairy heifers. *Theriogenology*, 73:1311-1318.
- Romero JJ, Antoniazzi AQ, Nett TM, Ashley RL, Webb BT, Smirnova NP, Bott RC, Bruemmer JE, Bazer FW, Anthony RV, Hansen TR.** 2015. Temporal release, paracrine and endocrine actions of ovine conceptus-derived interferon-tau during early pregnancy. *Biol Reprod*, 93:1-10.
- Sangsritavong S, Combs D, Sartori R, Armentano L, Wiltbank M.** 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *J Dairy Sci*, 85:2831-2842.
- Santos J, Thatcher W, Chebel R, Cerri R, Galvao K.** 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci*, 82:513-535.
- Schrick F, Hockett M, Towns T, Saxton A, Wert N, Wehrman M.** 2001. Administration of a prostaglandin inhibitor immediately prior to embryo transfer improves pregnancy rates in cattle. *Theriogenology*, 55:370.
- Turino LN, Mariano RN, Cabrera MI, Scándolo DE, Maciel MG, Grau RJ.** 2010. Pharmacokinetics of progesterone in lactating dairy cows: Gaining some insights into the metabolism from kinetic modeling. *J Dairy Sci*, 93(3):988-999.
- Yan L, Robinson R, Shi Z, Mann G.** 2016. Efficacy of progesterone supplementation during early pregnancy in cows: A meta-analysis. *Theriogenology*, 85:1390-1398.