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*Original article*

# Second prostaglandin $F_{2\alpha}$ treatment during Ovsynch protocol does not improve fertility outcomes in dairy cows

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## Abstract

The objective of this study was to evaluate the effect of a second prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment during Ovsynch on luteal regression and fertility in dairy cows, compared with standard Ovsynch. The study was conducted on 111 Holstein Friesian multiparous cows on commercial dairy farm. The cows in the experimental group (n=48) received two treatments of $PGF_{2\alpha}$ 24 hours apart during Ovsynch. The cows in the control group (n=63) were synchronized with standard Ovsynch. To assess the progesterone ($P_4$) concentration blood samples were collected at the day of $PGF_{2\alpha}$ treatment and at the 2nd GnRH treatment. Pregnancy was evaluated by ultrasound examination 37-40 days after timed artificial insemination (TAI) by ultrasound. Cows diagnosed pregnant were re-examined between days 70-80 after TAI. The percentage of cows with complete corpus luteum (CL) regression ($P_4$<0.5 ng/ml at the time of the 2nd GnRH treatment) was 89.6 % after two $PGF_{2\alpha}$ treatments and 88.9 % after one $PGF_{2\alpha}$ treatment. There were no statistically significant differences (p>0.05) in the pregnancies per artificial insemination (P/AI) between the experimental and control group (P/AI). However, the pregnancy loss rate was lower in cows receiving two $PGF_{2\alpha}$ treatments than in the control animals (0.0 % vs. 6.4 %; p<0.05). In conclusion, the second $PGF_{2\alpha}$ treatment during Ovsynch protocol had no significant effect on CL regression and P/AI in dairy cows. The pregnancy losses until days 75-80 after TAI were significantly lower after two $PGF_{2\alpha}$ treatments than after one $PGF_{2\alpha}$ treatment.

**Key words:** cows, Ovsynch, second $PGF_{2\alpha}$ treatment, fertility

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## Introduction

Reproductive problems are still a serious issue among dairy cows. Increasing number of animals in herd and lack or weak heat expression are considerable difficulties in heat detection (Lucy 2001, Roelofs et al. 2005, Zduńczyk et al. 2005). To overcome the problems and limitations associated with estrus detection protocols to ovulation synchronization were developed. One of that protocols is Ovsynch (Pursley et al. 1995). Ovulation is synchronized by administration of GnRH, followed 7 days later with $PGF_{2\alpha}$, followed 2 days later with a second treatment with GnRH. The cows receive a timed artificial insemination (TAI) 16-24 h later.

Unfortunately, some cows fail to ovulate. One of the reasons is lack or incomplete luteolysis. In various studies the percentage of cows with incomplete luteolysis after $PGF_{2\alpha}$ injection during Ovsynch protocol ranges between 5 to 25% (Souza et al. 2007, Brusveen et al. 2009, Wiltbank and Pursley 2014). Inadequate corpus luteum (CL) regression in response to a single $PGF_{2\alpha}$ treatment is observed more frequently in younger CL. The reason is defined as lack of luteolytic capacity. The mechanisms involved in the lack of luteolytic capacity are not well characterized. It seems that PGF-induced intra-cellular signaling pathways are altered in CL without luteolytic capacity (Diaz et al. 2002, Mondal et al. 2011).

In order to enhance the percentage of cows with complete luteolysis during Ovsynch two administrations of $PGF_{2\alpha}$ 24 hours apart were proposed (Brusveen et al. 2009). However, the results of studies using such modified Ovsynch protocol were inconsistent (Carvalho et al. 2015, Wiltbank et al. 2015, Heidari et al. 2017).

Thus, the objective of this study was to evaluate the effect of a second injection of $PGF_{2\alpha}$ during Ovsynch on luteal regression and fertility in dairy cows, compared with standard Ovsynch.

## Materials and Methods

### Animals

The study was conducted on 111 Holstein Friesian multiparous cows on a commercial dairy farm located near Olsztyn, Poland. Cows were in the 2[nd]-4[th] lactation, housed in a free stall barn bedded with straw. Average milk yield per year for a cow was 9000 kg. The cows were fed twice daily a total mixed ration that consisted of corn and grass silage and concentrates adjusted to their milk yield. The ration of macro- and microelements was balanced to meet nutritional requirements for dairy cattle. The cows had access to fresh water ad libitum. Cows were milked twice daily. Estrus was observed three times a day for twenty – thirty minutes by the herdsmen during the day time and in the night time by the care takers working on the night shifts. Cows detected in estrus were inseminated by artificial insemination technicians. The animals were inseminated at the first estrus occurring after 60 days post-partum. The cows in the control and experimental group were clinically healthy and without any lameness or endometritis.

### Study design

Synchronization was started 61 to 67 days postpartum. Cows were randomly divided into two groups: experimental group (n=48) in which cows were synchronized with modified Ovsynch [day 0, buserelin (0,021 mg, Receptal®, MSD Animal Health, Poland) → day 7, dinoprost (25 mg, Dinolytic®, Zoetis, Poland) → day 8, dinoprost (25 mg) → day 9, buserelin (0.021 mg/ml) → day 10, TAI], and control group (n=63) in which cows were synchronized according to standard Ovsynch protocol [day 0, buserelin (0.021 mg) → day 7, dinoprost (25 mg) → day 9, buserelin (0.021 mg) → day 10, TAI] as presented in Fig. 1.

Blood samples were collected on the day of $PGF_{2\alpha}$ treatment (day 7) and the second GnRH injection (day 9) of Ovsynch protocol and were assayed for progesterone (P4) concentrations to determine ovulatory response and CL regression. Ovulatory response to the first GnRH treatment was defined as $P_4$ concentration higher than 1 ng/ml at the day of $PGF_{2\alpha}$ treatment, complete luteolysis was defined as $P_4$ concentration lower than 0.5 ng/ml at the day the of second GnRH treatment.

Pregnancy was evaluated by ultrasound examination 37-40 days after TAI using a portable ultrasound scanner Honda 1500 equipped with a 5 MHz linear-array transducer. Cows diagnosed pregnant were re-examined between 70 and 80 days after TAI.

Pregnancies per artificial insemination (P/AI) at 37 to 40 days and at 70 to 80 days after TAI, and pregnancy loss rate were calculated. Pregnancy loss was defined as the absence of pregnancy at day 70-80 after TAI in cows previously diagnosed as pregnant.

### Samples collection

Blood samples were collected from the coccygeal vessels into vacuum tubes. The samples were immediately centrifuged and stored at -25°C until assayed. The progesterone concentrations were determined by RIA according to the method described by Hoffmann (1977). The intra- and interassay coefficients of variation were 8.1% and 13.2%, respectively. Detection limit of the assay was 50 pg/ml.

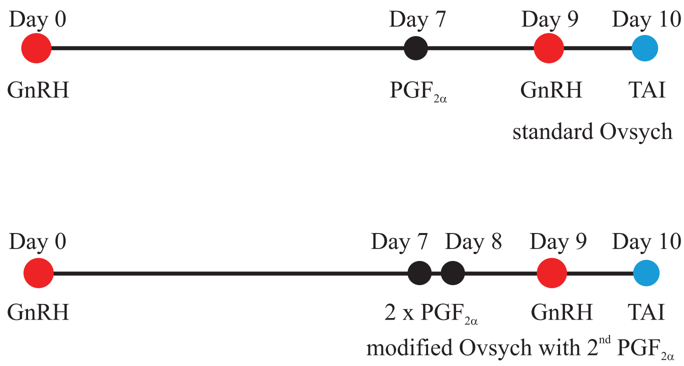


Fig 1. Illustrative diagram of Ovsynch protocols used in the study.

Table 1. Pregnancies pro artificial insemination (P/AI) and pregnancy loss rate in the control (1 x $PGF_{2\alpha}$) and experimental (2 x $PGF_{2\alpha}$) groups.

| Item | 1 x $PGF_{2\alpha}$ % (n/n) | 2 x $PGF_{2\alpha}$ % (n/n) | P-value |
|---|---|---|---|
| P/AI 37-40 d | 33.3 (21/63) | 31.3 (15/48) | > 0.05 |
| P/AI 70-80 d | 27.0 (17/63) | 31.3 (15/48) | > 0.05 |
| Pregnancy loss rate | 6.4 (4/63) | 0.0 (0/48) | < 0.05 |

Table 2. Effect of single or two $PGF_{2\alpha}$ treatments on $P_4$ concentrations.

| Item | 1 x $PGF_{2\alpha}$ | 2 x $PGF_{2\alpha}$ | P-value |
|---|---|---|---|
| $P_4$ at $PGF_{2\alpha}$ ng/ml | 3.01 ± 2.7 | 3.05± 2.8 | > 0.05 |
| Cows with $P_4$ > 1 ng/ml at $PGF_{2\alpha}$ % (n/n) | 74.6 (47/63) | 77.1 (37/48) | > 0.05 |
| Cows with $P_4$ < 1 ng/ml at $PGF_{\alpha}$ % (n/n) | 25.4 (16/63) | 22.9 (11/48) | > 0.05 |
| Cows with $P_4$ > 0.5 ng/ml at 2nd GnRH % (n/n) | 11.1 (7/63) | 10.4 (5/48) | > 0.05 |
| Cows with $P_4$ < 0.5 ng/ml at 2nd GnRH % (n/n) | 88.9 (56/63) | 89.6 (43/48) | > 0.05 |

### Statistical analysis

Statistical analysis of the data was performed by Mann-Whitney U test using GraphPad Prism version 7.00 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $p<0.05$.

## Results

There were no differences between the experimental and control group in P/AI at 37 to 40 and 70 to 80 days after TAI (31.3% vs. 33.3% and 31.3% vs. 27.0%, respectively). In the experimental group there were no pregnancy losses, whereas in the control group pregnancy loss rate was 6.4%. The difference between the groups was statistically significant ($p<0.05$; Table 1).

At day 7 ($PGF_{2\alpha}$ treatment) average $P_4$ concentration was similar in both groups (5.3 vs. 5.9 ng/ml). There were no differences in the proportion of cows with $P_4$ concentration above 1.0 ng/ml at the time of $PGF_{2\alpha}$ treatment (ovulatory response) between cows receiving two or one $PGF_{2\alpha}$ treatments (77.1% vs. 74.6%; $p>0.05$). Complete regression of CL (P4<0.5 ng/ml at final GnRH treatment) was observed in 89.6% of cows after two $PGF_{2\alpha}$ treatments and in 88.9% of cows after one $PGF_{2\alpha}$ treatment ($p>0.05$; Table 2).

Pregnancies per artificial inseminations at day 37 to 40 and 70 to 80 tended to be higher in cows in both groups with high (>1 ng/ml) $P_4$ concentrations at $PGF_{2\alpha}$ treatment and with low (<0.5 ng/ml) $P_4$ concentrations at the 2nd GnRH treatment. Pregnancy losses were observed only in cows with high $P_4$ concentrations (incomplete regression of CL) at the 2nd GnRH treatment (Table 3).

## Discussion

In the present study, the second injection of $PGF_{2\alpha}$ during Ovsynch did not affect significantly the percentage of cows with complete CL regression. In previous studies, an increased percentage of cows with complete CL regression was found after two $PGF_{2\alpha}$ treatments

Table 3. Pregnancies per artificial insemination (P/AI) and pregnancy losses for cows with low and high $P_4$ concentrations at the time of $PGF_{2α}$ and 2nd GnRH treatments.

| Item | 1 x $PGF_{2α}$ % (n/n) | 2 x $PGF_{2α}$ % (n/n) | P-value |
|---|---|---|---|
| P/AI 37-40 d | | | |
| High $P_4$ at $PGF_{2α}$ | 36.1 (17/47) | 32.4 (12/37) | > 0.05 |
| Low $P_4$ at $PGF_{2α}$ | 25.0 (4/16) | 27.2 (3/11) | > 0.05 |
| P/AI 70-80 d | | | |
| High $P_4$ at $PGF_{2α}$ | 31.9 (15/47) | 32.4 (12/37) | > 0.05 |
| Low $P_4$ at $PGF_{2α}$ | 12.5 (2/16) | 27.2 (3/11) | > 0.05 |
| Pregnancy loss | | | |
| High $P_4$ at $PGF_{2α}$ | 4.3 (2/47) | 0.0 (0/37) | > 0.05 |
| Low $P_4$ at $PGF_{2α}$ | 12.5 (2/16) | 0.0 (0/11) | > 0.05 |
| P/AI 37-40 d | | | |
| High $P_4$ at 2nd GnRH | 28.6 (2/7) | 0.0 (0/5) | > 0.05 |
| Low $P_4$ at 2nd GnRH | 33.9 (19/56) | 34.8 (15/43) | > 0.05 |
| P/AI 70-80 d | | | |
| High $P_4$ at 2nd GnRH | 26.8 (15/56) | 0.0 (0/5) | > 0.05 |
| Low $P_4$ at 2nd GnRH | 28.6 (2/7) | 34.8 (15/43) | > 0.05 |
| Pregnancy loss | | | |
| High $P_4$ at 2nd GnRH | 57.1 (4/7) | 0.0 (0/5) | > 0.05 |
| Low P4 at 2nd GnRH | 0.0 (0/56) | 0.0 (0/43) | > 0.05 |

compared with single $PGF_{2α}$ treatment (Brusveen et al. 2009, Carvalho et al. 2015, Wiltbank et al. 2015, Heidari et al. 2017). The reason for these differences is not clear. It could result from various status of the follicle development at the time of the 1st GnRH treatment and thus different diameters of CL and $P_4$ concentrations. In our study $P_4$ concentrations at the time of $PGF_{2α}$ injection were in both groups similarly high. Corpus luteum responsiveness to exogenous $PGF_{2α}$ seems to be dependent on $P_4$ concentration (Howard and Britt 1990, Wenzinger and Bleul 2012). Martins et al. (2011) reported that cows with greater circulating $P_4$ at time of $PGF_{2α}$ injection during Ovsynch had a greater probability of complete luteal regression.

In our study no statistically improvement in P/AI at 37 to 40 d and 70 to 80 d after TAI due to the second $PGF_{2α}$ administration was detectable. Similarly, in a study by Brusveen et al. (2009) addition of the a second $PGF_{2α}$ treatment had no effect on fertility. Carvalho et al. (2015) and Wiltbank et al. (2015) reported that cows receiving two $PGF_{2α}$ treatments during Ovsynch protocol had a tendency for increased P/AI compared with cows receiving only one $PGF_{2α}$ treatment. However, in these studies the proportion of cows with incomplete luteolysis was lower after the second PGF treatment. Incomplete CL regression, defined as plasma P4 concentrations > 0.5 ng/ml at TAI, results in reduced pregnancy rate (Souza et al. 2007, Brusveen et al. 2009, Martins et al. 2011, Wiltbank and Pursley 2014). In the study of Heidari et al. (2017) P/AI at 32 days after TAI did not differ statistically between cows receiving one or two $PGF_{2α}$ treatments. However, an improvement in fertility was detectable at the 60th day after TAI in cows receiving two $PGF_{2α}$ treatments, because they had fewer pregnancy losses. In our study the second $PGF_{2α}$ treatment reduced also the pregnancy loss rate (0.0% vs. 6.4%; $p<0.05$), but it did not influence statistically the pregnancy rate.

In the present study P/AI tended to be higher in cows with high $P_4$ concentrations at $PGF_{2α}$ treatment and with low $P_4$ concentrations at the 2nd GnRH treatments. Our findings were in agreement with other studies on the association between $P_4$ concentrations during Ovsynch protocol and fertility in cows. Many authors (Bello et al. 2006, Bisinotto et al. 2010, Pursley and Martins 2011, Wiltbank et al. 2015) reported that cows with greater concentrations of $P_4$ at time of induced luteolysis have a greater chance of pregnancy. An inverse relationship between the concentration of $P_4$ at the time of AI and fertility of dairy cows has been found in several studies (Souza et al. 2007, Brusveen et al. 2009, Wiltbank and Pursley 2014, Colazo et al. 2017).

Interestingly, in our study the pregnancy losses were observed only in cows with incomplete regression of CL. The relationship between elevated $P_4$ at AI and pregnancy losses was observed also by Ghanem et al. (2006) and Motavalli et al. (2017). More studies are needed to confirm this relationship.

In conclusion, the second $PGF_{2α}$ treatment during

Ovsynch protocol had no statistically significant effect on CL regression and P/AI. However, the pregnancy losses until days 75-80 after TAI were significantly lower after two $PGF_{2\alpha}$ treatments than after one $PGF_{2\alpha}$ treatment.

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