

*Original Scientific Article***MODIFICATION OF THE STANDARD 7-DAY OVSYNCH PROTOCOL TO INCREASE THE LUTEOLYTIC AND SYNCHRONIZATION RISKS IN DAIRY COWS**

Branko Atanasov<sup>1</sup>, Nikola Adamov<sup>1</sup>, Irena Celeska<sup>1</sup>, Ksenija Ilievska<sup>1</sup>,  
Branko Angjelovski<sup>1</sup>, Zvonko Trbogazov<sup>2</sup>, Filip Davkov<sup>3</sup>, Toni Dovenski<sup>1</sup>,  
Geert Opsomer<sup>4</sup>, Jeffrey Stevenson<sup>5</sup>

<sup>1</sup>Faculty of Veterinary Medicine–Skopje, Ss. Cyril and Methodius University in Skopje,  
Lazar Pop Trajkov 5-7, 1000 Skopje, R. of N. Macedonia

<sup>2</sup>Dairy Farm ZSK “Strumica”, Sushica, Strumica, R. of N. Macedonia

<sup>3</sup>Dairy Farm ZK “Pelagonija”, Bitola, R. of N. Macedonia

<sup>4</sup>Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary  
Medicine, Ghent University, Ghent, Belgium

<sup>5</sup>Department of Animal Sciences and Industry, Kansas State University Manhattan,  
KS, USA

Received 16 June 2020; Received in revised form 13 August 2020; Accepted 28 August 2020

**ABSTRACT**

We hypothesized that a single dose of PGF<sub>2α</sub> belatedly injected on day 8 after GnRH-1 in cows receiving a 7-day Ovsynch-56 protocol (GnRH – 7 days – PGF<sub>2α</sub> – 56h – GnRH – 16h – timed AI) will increase the proportion of cows with complete luteolysis. At day 35±3 postpartum, 70 lactating Holstein cows from one herd were scored for body condition and pre-synchronized with PGF<sub>2α</sub> and GnRH (3 days apart) and 7 days later submitted to an Ovsynch-56 protocol for first AI after random assignment to two treatments: (1) OV-7 (n=35) with an injection of PGF<sub>2α</sub> either on day 7; or (2) OV-8 (n=35) on day 8 after G1, respectively. Blood was collected before the first PGF<sub>2α</sub> at day 7 and day 8 in OV-7 and OV-8, respectively, at AI and at 7 days after AI to assess progesterone concentration. Ten cows were classified as acyclic and were excluded from the analysis resulting in 60 cows (OV-8, n=27; OV-7, n=33). In total, more (P=0.01) OV-8 cows and more (P=0.04) primiparous OV-8 cows had complete luteolysis compared with their OV-7 herd mates. In addition, more (P=0.008) OV-8 cows with BCS<2.75 had complete luteolysis compared with their OV-7 herd mates, whereas no difference was observed between treatments among cows with BCS ≥2.75. In conclusion, delaying the application of PGF<sub>2α</sub> by 1 day reduced the percentage of primiparous cows and cows with poorer BCS having incomplete luteal regression at the time of AI.

**Key words:** luteolysis, synchronization risk, Ovsynch, dairy cows, BCS

**INTRODUCTION**

Currently, many dairy operators have adopted a reproductive strategy known as the

Ovsynch protocol that facilitates 100% artificial insemination (AI) submission risk with a predetermined fixed-time AI without the necessity of estrus detection (1). The Ovsynch protocol consists of an injection of GnRH (G1) at a random stage of the estrous cycle followed 7 days later with a prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) injection and a final GnRH (G2) injection 56 h after the PGF<sub>2α</sub>. For maximal pregnancy per AI, timed AI (TAI) should be performed approximately 16 h after G2 administration (2). Nevertheless, multiple studies have reported that the maximal P/AI that can be achieved with the Ovsynch protocol is limited by at least 3 factors: (a) failure of G1 to induce

*Corresponding author:* Assist. Prof. Branko Atanasov, PhD  
*E-mail address:* batanasov@fvm.ukim.edu.mk  
*Present address:* Faculty of Veterinary Medicine–Skopje, Ss. Cyril and Methodius University in Skopje, Lazar Pop Trajkov 5-7, 1000 Skopje, R. of N. Macedonia  
*Phone:* +38923240731; *Fax:* +3892114619

**Copyright:** © 2020 Atanasov B. This is an open-access article published under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Competing Interests:** The authors have declared that no competing interests exist.

**Available Online First:** 14 September 2020  
<https://doi.org/10.2478/macvetrev-2020-0028>

ovulation of the dominant follicle to initiate a new follicular wave; (b) accomplishment of complete luteolysis after single  $\text{PGF}_{2\alpha}$  treatment and (c) inability to induce ovulation after G2 (2). Induction of ovulation in response to G1 at random stages of the estrous cycle occurs in only 50 to 66% of cows (3). Increased ovulatory response to GnRH is achieved when the Ovsynch protocol was initiated between days 5 and 12 of the estrous cycle (4). In order to synchronize cows to meet this ideal stage, presynchronization protocols (Presynch, PG-3-G, Double Ovsynch, and GGPG) have been developed (5, 6). Cows with estrous cycles that were pre-synchronized and that had ovulated after G1 generally have greater P/AI than cows that did not ovulate (7). Although it was assumed that the latter responses might be a result of an increased circulating P4 concentration from the newly formed corpus luteum (CL) during the development of the preovulatory follicle (8), the P4 concentration at  $\text{PGF}_{2\alpha}$  did not differ between cows with one single older CL (one) versus cows bearing an older and an induced CL (two CLs) (9). Nevertheless, cows that ovulated after G1 and formed a new CL are more likely to have an incomplete luteolysis than cows that did not ovulate because a CL less than 7 days old might still be resistant to regression after a single standard dose of  $\text{PGF}_{2\alpha}$  (10). Indeed, incomplete luteolysis after a single dose of  $\text{PGF}_{2\alpha}$  has been observed in 5 to 30% of cows submitted either to an Ovsynch protocol (5, 11) or to a single  $\text{PGF}_{2\alpha}$  estrus-synchronization protocol (25%) (12). Small elevations in circulating P4 near AI ( $>0.4$  ng/mL) resulting from incomplete or delayed luteolysis, have been shown to reduce fertility (8). Failure of complete CL regression or luteolysis is a greater limitation to achieving maximal P/AI than ovulation failure after G1. Indeed, cows that had a complete luteolysis and ovulated after the G2 have the greatest fertility independent of whether ovulation from the G1 injection occurred (13).

Therefore, attempts have been made to overcome the problem of incomplete CL regression, by either increasing the dose of  $\text{PGF}_{2\alpha}$  (14) or the frequency of  $\text{PGF}_{2\alpha}$  treatments (15). Increasing the dose of  $\text{PGF}_{2\alpha}$  (cloprostenol) from 500 to 750  $\mu\text{g}$  for cows submitted to a 7-day Ovsynch protocol increased the luteolytic risk, but this effect was only observed in multiparous cows (13). Inclusion of a second  $\text{PGF}_{2\alpha}$  treatment 24 h after the first  $\text{PGF}_{2\alpha}$  (days 7 and 8 after G1), increased the percentage of cows with complete luteolysis from 80% to 97% (16, 15). Although the aforementioned treatments (either increasing the dose or frequency

of  $\text{PGF}_{2\alpha}$  treatments) have enhanced luteolysis and reduced P4 concentrations at TAI compared with a single standard dose of  $\text{PGF}_{2\alpha}$ , both increase the cost of the program. In addition, from a practical standpoint, inclusion of a second  $\text{PGF}_{2\alpha}$  treatment is time consuming and increases the number of times the cows must be handled during the protocol and thus the risk for compliance.

We hypothesized that a single standard dose of  $\text{PGF}_{2\alpha}$  injected on day 8 instead of day 7 after G1 will increase the proportion of cows with complete luteolysis and increase the synchronization risk compared with control cows receiving a standard 7-day Ovsynch protocol. Therefore, our objective was to evaluate the effect of a single standard dose of  $\text{PGF}_{2\alpha}$  treatment on day 8 on the luteolytic and synchronization rates in lactating Holstein cows.

## MATERIAL AND METHODS

### *Animals*

Lactating Holstein cows ( $n=70$ ) in one commercial herd were enrolled in the study. Cows were housed in free-stall barns, fed a total mixed ration once a day to meet or exceed requirements for lactating cows producing 30 L of milk per day. Cows were milked twice daily and had free access to water.

### *Experimental design*

Starting at day  $35\pm 3$  postpartum, all cows were scored for body condition (BCS) on a five-point scale (1 = emaciated and 5 = obese) (17). Estrous cycles were pre-synchronized using PG-3-G protocol (9). The protocol consisted of a 25 mg i.m. injection of  $\text{PGF}_{2\alpha}$  (Pre-PG; 5 mL of Dinolytic, Zoetis Animal Health, Madison, NJ) followed by an i.m. injection of GnRH (8  $\mu\text{g}$  Buserelin – a GnRH agonist, Pre-GnRH; 2 mL of Receptal, MSD Animal Health, Intervet, International, GMBH, Germany) 3 days later. Ten days after the Pre-PG injection, an Ovsynch TAI program was initiated and cows were allocated randomly to two treatments: OV-7 ( $n=35$ , G1–7 days –  $\text{PGF}_{2\alpha}$  – 56 h – G2 – 16 h – TAI) with an injection of  $\text{PGF}_{2\alpha}$  on day 7 and OV-8 ( $n=35$ , G1–8 days –  $\text{PGF}_{2\alpha}$  – 56 h – G2 – 16 h – TAI) with an injection of  $\text{PGF}_{2\alpha}$  on day 8 after the G1, respectively. Transrectal ultrasonography was conducted at G1 to map ovarian structures, at  $\text{PGF}_{2\alpha}$  to assess ovulation in response to G1, and at 7 days after TAI to assess ovulation in response to G2.

Blood samples to assess concentrations of P4 were collected at Pre-PG, G1, PGF<sub>2α</sub>, at 72 h after PGF<sub>2α</sub> (at TAI) and at 7 days after TAI from the coccygeal vein or artery into evacuated tubes (BD Vacutainer® Plymouth, UK). The samples were refrigerated immediately and transported in a portable refrigerator to the lab by 3 h after collection. Tubes were centrifuged (1,000 x g for 15 min), and serum was separated and stored at -20 °C until P4 analysis. The P4 analysis was done at the Faculty of Veterinary Medicine – Skopje, North Macedonia using a commercially available kit (HUMAN, Progesterone ELISA Test – GMBH, Germany) on an Immuno-scan BDLS reader. The intra- and inter-assay CV were 7.3% and 8.4%, respectively.

Cyclic status of the cows (cyclic or acyclic) was determined by serum concentrations of P4 collected at Pre-PG and at G1. When concentrations of both samples were <1 ng/mL, the cows were classified as acyclic and data were not included for further analyses (8 OV-8 cow and 2 OV-7 cows) (9). In contrast, when either of the paired samples at Pre-PG and G1 contained concentrations of progesterone ≥1 ng/mL, cows were classified as cyclic (9). In cyclic cows in which progesterone concentration was ≥1 ng/mL at PGF<sub>2α</sub> or at 7 days after TAI, it was furthermore assumed that ovulation was induced by injections of G1 or G2. Complete luteolysis was defined to occur when P4 was >1 ng/mL at PGF<sub>2α</sub> and <0.4 ng/mL 72 h later at TAI (9). Synchronization risk (SR) was defined as the number of cows with complete luteolysis and ovulation success after G2, divided by the number of cows with progesterone >1 ng/mL at PGF<sub>2α</sub> (9).

#### *Statistical analyses*

All statistical analyses were performed with SPSS (version 22.0, IBM SPSS Statistics for Windows, Armonk, NY). Continuous variables such as progesterone concentrations were analyzed using GLM Univariate procedure in SPSS. Because these data were not normally distributed, a two-step transformation to normality was conducted before the analysis. Firstly, a fractional ranking of the data was performed followed by application of the inverse distribution function (IDF, NORMAL) of SPSS.

Variables with a binomial distribution (complete luteolysis, SR, and BCS [ $<2.75$  or  $\geq 2.75$ ]) were analyzed by binary logistic regression. For these variables, data were analyzed for all combinations of parities and treatments. The same

method was also used to analyze the relationship between circulating P4 concentrations and the binomial traits. The relationship between the P4 concentrations, treatment, and BCS, was assessed by two multiple linear regression analyses using the GLM Univariate procedure in SPSS. Two models were constructed which included P4 concentration at G1 and at PGF<sub>2α</sub> as independent variables in each model separately, treatment as fixed effect, and BCS as covariate in both models. Statistical differences were considered significant at  $P \leq 0.05$  and as a tendency for  $P \leq 0.10$ .

## RESULTS

Of 70 cows, 10 cows (14.2% [ $n=8$ ] in OV-8 and OV-7 [ $n=2$ ]) in both treatments were classified as acyclic and were therefore excluded from further analyses leaving a total of 60 cows (OV-8,  $n=27$ , OV-7,  $n=33$ ).

The concentrations of P4 at PGF<sub>2α</sub> treatment did not differ ( $P=0.62$ ) between the OV-7 and OV-8 ( $6.1 \pm 0.57$  and  $6.6 \pm 0.59$  ng/mL, mean  $\pm$  SEM), respectively. Similarly, when P4 concentrations at PGF<sub>2α</sub> were compared between parity groups within treatment, no differences were detected between primiparous ( $P=0.82$ ) nor between multiparous cows ( $P=0.65$ ).

When the P4 concentration at G1 was compared between OV-8 and OV-7 ( $5.1 \pm 0.41$  and  $4.6 \pm 0.39$  ng/mL), respectively, no difference ( $P=0.32$ ) was detected. In addition, the P4 concentrations at G1 did not differ between parity groups within treatments for primiparous ( $P=0.90$ ) or multiparous cows ( $P=0.25$ ).

Nevertheless, a significant difference was observed in the percentage of cows with complete luteolysis between treatments, with more cows having a complete luteolysis in OV-8 compared with OV-7 (Table 1). In addition, a tendency was observed for increased CL regression in multiparous cows in the OV-8 versus the OV-7 treatment. Within the OV-7 group, multiparous cows tended ( $P=0.09$ ) to have a greater proportion of animals with complete luteolysis. When only primiparous cows from both treatments were analyzed, a greater ( $P=0.04$ ) percentage of OV-8 cows had complete luteal regression compared with OV-7 cows, whereas no differences were observed between parities among the OV-8 cows (Table 1).

No differences in SR were detected between treatments or parity groups (Table 2).

**Table 1.** Percentage of cows with complete luteolysis by 72 h after PGF<sub>2α</sub> (time of AI)\*

Item	Treatment†		P-value
	OV-7	OV-8	
<b>Primiparous</b>	33.3% (3/9)	85.7% (6/7)	0.04
<b>Multiparous</b>	66.7% (16/24)	90.0% (18/20)	0.06
<b>P-value</b>	0.09	0.75	
<b>Overall</b>	57.6% (19/33)	88.9% (24/27)	0.01

\*Complete luteolysis was defined to occur when P4 was >1 ng/mL at PGF<sub>2α</sub> and <0.4 ng/mL 72 h later at TAI

†Estrous cycles were presynchronized using PG-3-G protocol (Pre-PGF<sub>2α</sub> and 3 days later a Pre-GnRH). Ten days after the Pre-PGF<sub>2α</sub> injection, an Ovsynch protocol (GnRH1 – 7 days – PGF<sub>2α</sub> – 56 h – GnRH2 – 16 h – TAI) was initiated with the PGF<sub>2α</sub> injection either on day 7 (OV-7) or on day 8 (OV-8)

**Table 2.** Synchronization risk in cyclic cows\*

Item	Treatment†		P-value
	OV-7	OV-8	
<b>Primiparous</b>	44.4% (4/9)	57.1% (4/7)	0.61
<b>Multiparous</b>	70.8% (17/24)	85.0% (17/20)	0.27
<b>P-value</b>	0.17	0.14	
<b>Overall</b>	63.6% (21/33)	77.8% (21/27)	0.23

\*Synchronization risk was defined as the number of cows with complete luteolysis and ovulation success after G2 divided by the number of cows with P4 > 1 ng/mL at PGF<sub>2α</sub>

†Estrous cycles were presynchronized using PG-3-G protocol (Pre-PGF<sub>2α</sub> and 3 days later a Pre-GnRH). Ten days after the Pre-PGF<sub>2α</sub> injection, an Ovsynch protocol (GnRH1 – 7 days – PGF<sub>2α</sub> – 56 h – GnRH2 – 16 h – TAI) was initiated with the PGF<sub>2α</sub> injection either on day 7 (OV-7) or on day 8 (OV-8)

When the cows from both treatments were categorized based on their BCS (<2.75 and ≥2.75), overall no differences in complete luteolysis were observed (Table 3). Cows in OV-8 having BCS <2.75 were more likely to have a complete luteal regression compared with their OV-7 herd mates, whereas no difference in complete luteolysis was observed between treatments when BCS ≥2.75. In the OV-7 treatment, cows with BCS ≥2.75 were more likely to have a complete luteolysis compared with their leaner group mates.

In addition, a tendency (P=0.08) was observed for increased SR in OV-8 cows compared with OV-7

cows (Table 4). In addition, in the OV-7 treatment group, there was a tendency (P=0.09) for a greater percentage of synchronized cows in the BCS ≥2.75 group in comparison to their group mates with a lower BCS (Table 4).

Progesterone concentrations also were associated with the BCS at G1 and at PGF<sub>2α</sub>. At G1, for each unit increase in BCS (in the range of 2.00 to 3.75), serum concentration of P4 increased (P=0.03) by 1.68±0.77 ng/mL. At PGF<sub>2α</sub> for each unit increase in BCS (in the range 2.00 to 3.75), serum concentrations of P4 increased (P=0.01) by 3.1±1.16 ng/mL.

**Table 3.** Percentage of cows with complete luteolysis based on body condition score\*

Item	Treatment†		P-value
	OV-7	OV-8	
<b>BCS &lt;2.75</b>	25.00% (2/8)	91.76% (11/12)	0.008
<b>BCS ≥2.75</b>	68.00% (17/25)	86.76% (13/15)	0.20
<b>P-value</b>	0.04	0.68	
<b>Overall</b>	57.67% (19/33)	88.98% (24/27)	0.42

\*Cows were scored for body condition on a five-point scale (1 = emaciated and 5 = obese)

†Estrous cycles were presynchronized using the PG-3-G protocol (Pre-PGF<sub>2α</sub> and 3 days later a Pre-GnRH). Ten days after the Pre-PGF<sub>2α</sub> injection, an Ovsynch protocol (GnRH1 – 7 days – PGF<sub>2α</sub> – 56 h – GnRH2 – 16 h – TAI) was initiated with the PGF<sub>2α</sub> injection either on day 7 (OV-7) or on day 8 (OV-8)

**Table 4.** Synchronization risk based on body condition scores and treatment\*

Body condition score†	Treatment‡		P-value
	OV-7	OV-8	
<2.75	37.5% (3/8)	66.7% (8/12)	0.20
≥2.75	72.0% (18/25)	86.7% (13/15)	0.29
P-value	0.09	0.22	
Overall	63.6% (21/33)	77.8% (21/27)	0.08

\*Synchronization risk was defined as the number of cows with complete luteolysis and ovulation success after G2 divided by the number of cows with P4 >1 ng/mL before PGF<sub>2α</sub>.

†Cows were scored for body condition on a five-point scale (1 = emaciated and 5 = obese)

‡Estrous cycles were presynchronized using PG-3-G protocol (Pre-PGF<sub>2α</sub> and 3 days later a Pre-GnRH). Ten days after the Pre-PGF<sub>2α</sub> injection, an Ovsynch protocol (GnRH-1 -7 days - PGF<sub>2α</sub> - 56 h - GnRH2 - 16 h - TAI) was initiated with the PGF<sub>2α</sub> injection either on day 7 (OV-7) or on day 8 (OV-8)

## DISCUSSION

The present study aimed to determine the efficacy of injecting PGF<sub>2α</sub> on day 8 instead of day 7 to reach a greater percentage of complete luteal regression in the standard Ovsynch protocol in lactating dairy cows. Overall, a greater percentage of OV-8 treated cows had complete luteal regression compared with OV-7 cows. The percentage of OV-8 cows having complete luteal regression observed in our study was similar to that observed in other studies (16, 18); in which 80 to 90% of the enrolled cows had complete luteal regression using a 7-day protocol. The latter implies that PGF<sub>2α</sub> injection on day 8 could be more effective than injection on day 7 in the standard Ovsynch protocol to induce complete luteal regression.

Our results indicate a 34.3 percentage unit increase in luteal regression (88.9% vs. 57.6%, Table 1) after the OV-8 treatment compared with OV-7 treatment, respectively. Increased luteal regression in response to the OV-8 treatment could be attributed to the presence of an original CL in combination with a newly formed CL in response to G1 and the maturity of both CL. One study (14) reported that the presence of an older and a newly formed CL has a major effect on whether cows will have complete luteal regression. In that study, only 79.4% of the cows having a new G1-induced CL had a complete CL regression, compared with 93% complete CL regression in cows without a new CL. In contrast, Stevenson et al. (9) reported that the newly formed CL regresses more successfully in the presence of a more mature CL. Regardless of the pathways involved, regression of all luteal structures primarily depends on their maturity at the time of the PGF<sub>2α</sub> injection (10). Because we did not include the data regarding the presence of one or more CL on the ovaries at the time of PGF<sub>2α</sub> treatment, we are not able to confirm these observations. Nevertheless, in the present study,

when ovulation occurred in response to G1 in the presence of an original CL, the newly formed CL and the older CL would be approximately 7 and 14 days old, respectively at PGF<sub>2α</sub> in the OV-8 treatment. In this scenario, both CL should be fully responsive to PGF<sub>2α</sub> resulting in an increased likelihood for complete luteal regression. In the same scenario for the OV-7 treatment, the older and newly formed CL would be approximately 6 and 13 days old respectively, at PGF<sub>2α</sub> (19). Because CL less than 7 days are more resistant to regression after PGF<sub>2α</sub> (10), fewer cows with complete regression would be expected.

When parity was included in the analysis, the results indicated a greater proportion of multiparous cows had complete luteal regression compared with primiparous cows in the OV-7 treatment (Table 1). In contrast, in one report, a greater proportion of primiparous cows had a complete luteolysis in response to a standard dose of PGF<sub>2α</sub> compared with multiparous cows (94 vs 81%, respectively) (18), whereas other studies reported no effects of parity (9, 15). Although, the reasons are still unclear, we hypothesize that some follicles that eventually formed a new CL during a PG-3-G + OV-7 protocol in primiparous cows may be smaller, resulting in a smaller and less functionally mature CL (20, 21) and therefore represent a CL with a lower probability of complete regression.

Particularly intriguing in our analysis was that a greater proportion of primiparous cows (66.7%) in OV-7 treatment that did not have complete luteal regression compared with only 14.3% of the OV-8 cows. We were unable to find any other studies reporting similar results, thus these intriguing findings warrant further investigation. Nevertheless, as mentioned previously, ovulation of small follicles resulting in a less mature CL could be a possible explanation that contributes to inadequate luteolysis.



Although complete luteolysis was less successful in the OV-7 cows, the greatest failure was among thinner cows (BCS<2.75). These results do not corroborate the results reported by Stevenson and Phatak (22), in which thinner cows were more likely to have complete luteal regression than cows with greater BCS. It is unclear how body condition might influence luteolysis; however, it is possible that a less functional follicle ovulated in thinner cows and formed a CL that was 6-days old at the time of PGF<sub>2α</sub> treatment and less sensitive to the luteolytic effects of PGF<sub>2α</sub>. The SR tended to be greater for OV-8 than OV-7 cows. We found no report in the literature corroborating the effect of synchronization risk on luteolysis. Nevertheless, from a practical point of view, these findings should be taken into consideration, when thinner cows are submitted to the standard Ovsynch protocol.

## CONCLUSION

Treatment with PGF<sub>2α</sub> on day 8 rather than day 7 during the PG-3-G Ovsynch protocol reduced the percentage of cows with incomplete CL regression at the time of AI and tended to increase synchronization risk. The treatment was also successful in primiparous cows and in cows with lesser BCS. Based on our results, we suggest that this treatment could be of practical importance for many dairy farms. Further studies are warranted, however, to confirm these demonstrated improvements in CL regression and evaluate potentially concomitant increases in pregnancy per AI in response to this novel OV-8 treatment.

## CONFLICTS OF INTEREST

The authors declared that they have no potential conflict of interest with respect to the authorship and/or publication of this article.

## ACKNOWLEDGEMENTS

We would like to acknowledge ZK Pelagonija Dairy Farm (Bitola, R. M) for allowing us the use of their facilities and Zoetis Animal Health Inc. (Madison, NJ) for donating Dinolytic used in this research.

## AUTHORS' CONTRIBUTION

BA, TD, JS and GO planed the study design. BA and TD performed the ultrasonographic examination of the

cows. GO and JS performed the proofreading. JS and NA have run the statistical analysis and IC has run the laboratory analysis. KI and BA have synchronized the cows. ZT and FD collected the blood samples.

## REFERENCES

1. Pursley, J.R., Kosorok, M.R., Wiltbank, M.C. (1997). Reproductive management of lactating dairy cows using synchronization of ovulation. *J Dairy Sci.* 80(2): 301-306.  
[https://doi.org/10.3168/jds.S0022-0302\(97\)75938-1](https://doi.org/10.3168/jds.S0022-0302(97)75938-1)
2. Stevenson, J.S. (2016). Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. *J Dairy Sci.* 99(12): 10077-10092.  
<https://doi.org/10.3168/jds.2016-11247>  
PMid:27720155
3. Vasconcelos, J.L.M., Silcox, R.W., Rosa, G.J., Pursley, J.R., Wiltbank, M.C. (1999). Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52(6): 1067-1078.  
[https://doi.org/10.1016/S0093-691X\(99\)00195-8](https://doi.org/10.1016/S0093-691X(99)00195-8)
4. Santos, J.E.P. (2011). Reproductive management of lactating dairy cows for first postpartum insemination. In: Carlos A Risco; Pedro R., Retamal (Eds.), *Dairy Production Medicine* (pp. 81-98). John Wiley & Sons, Inc.  
<https://doi.org/10.1002/9780470960554.ch7>
5. Moreira, F., Orlandi, C., Risco, C.A., Mattos, R., Lopes, F., Thatcher, W.W. (2001). Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci.* 84(7): 1646-1659.  
[https://doi.org/10.3168/jds.S0022-0302\(01\)74600-0](https://doi.org/10.3168/jds.S0022-0302(01)74600-0)
6. Stevenson, J.S., Pulley, S.L. (2012). Pregnancy per artificial insemination after presynchronizing estrous cycles with the Presynch-10 protocol or prostaglandin F<sub>2α</sub> injection followed by gonadotropin-releasing hormone before Ovsynch-56 in 4 dairy herds of lactating dairy cows. *J Dairy Sci.* 95(11): 6513-6522.  
<https://doi.org/10.3168/jds.2012-5707>  
PMid:22939782
7. Bello, N.M., Steibel, J.P. Pursley, J.R. (2006). Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J Dairy Sci.* 89(9): 3413-3424.  
[https://doi.org/10.3168/jds.S0022-0302\(06\)72378-5](https://doi.org/10.3168/jds.S0022-0302(06)72378-5)

8. Wiltbank, M.C., Souza, A.H., Carvalho, P.D., Cunha, A.P., Giordano, J.O., Fricke, P.M., Baez, G.M., Diskin, M.G. (2014). Physiological and practical effects of progesterone on reproduction in dairy cattle. *Animal* 8 (Suppl. 1), 70-81.  
<https://doi.org/10.1017/S1751731114000585>  
PMid:24703103
9. Stevenson, J.S., Sauls, J.A., Mendonça, L.G.D., Voelz, B.E. (2018). Dose and frequency of PGF<sub>2α</sub> administration to lactating dairy cows exposed to presynchronization and either five- or seven-day Ovsynch protocols: ovulation, luteolysis, and pregnancy rates. *J Dairy Sci.* 101(10): 9575-9590.  
<https://doi.org/10.3168/jds.2018-14653>  
PMid:30100501
10. Momont, H.W., Seguin, B.E. (1984). Treatment of unobserved estrus in lactating dairy cows with prostaglandin F<sub>2α</sub> products. *Proc Symp Comp Cont Edu Pract Vet.* pp. 28-31.
11. Wiltbank, M.C., Pursley, J.R. (2014). The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology* 81(1): 170-185.  
<https://doi.org/10.1016/j.theriogenology.2013.09.017>  
PMid:24274420
12. Atanasov, B., Hostens, M., Celeska, I., Ilieska, K., Opsomer, G., Dovenski, T. (2015). Follicular dynamics following induced luteolysis and transvaginal ultrasound-guided aspiration of the largest follicle in dairy cows. *Veterinarski Arhiv* 85(3): 247-260.
13. Bisinotto, R.S., Ribeiro, E.S., Santos, J.E.P. (2014). Synchronization of ovulation for management of reproduction in dairy cows. *Animal* 8(Suppl. 1): 151-159.  
<https://doi.org/10.1017/S1751731114000858>  
PMid:24709227
14. Giordano, J.O., Wiltbank, M.C., Fricke, P.M., Bas, S., Pawlisch, R., Guenther, J.N., Nascimento, A.B. (2013). Effect of increasing GnRH and PGF<sub>2α</sub> dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. *Theriogenology* 80(7): 773-783.  
<https://doi.org/10.1016/j.theriogenology.2013.07.003>  
PMid:23932174
15. Wiltbank, M.C., Baez, G.M., Cochrane, F., Barletta, R.V., Trayford, C.R., Joseph, R.T. (2015). Effect of a second treatment with prostaglandin F<sub>2α</sub> during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *J Dairy Sci.* 98(12): 8644-8654.  
<https://doi.org/10.3168/jds.2015-9353>  
PMid:26433418
16. Brusveen, D.J., Souza, A.H., Wiltbank, M.C. (2009). Effects of additional prostaglandin F<sub>2</sub> alpha and estradiol-17 beta during Ovsynch in lactating dairy cows. *J Dairy Sci.* 92(4): 1412-1422.  
<https://doi.org/10.3168/jds.2008-1289>  
PMid:19307622
17. Ferguson, J.D., Galligan, D.T., Thomsen, N. (1994). Principal descriptors of body condition score in Holstein cows. *J Dairy Sci.* 77(9): 2695-2703.  
[https://doi.org/10.3168/jds.S0022-0302\(94\)77212-X](https://doi.org/10.3168/jds.S0022-0302(94)77212-X)
18. Martins, J.P.N., Policelli, R.K., Neuder, L.M., Raphael, W., Pursley, J.R. (2011). Effects of cloprostenol sodium at final prostaglandin F<sub>2α</sub> of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. *J Dairy Sci.* 94(6): 2815-2824.  
<https://doi.org/10.3168/jds.2010-3652>  
PMid:21605751
19. Fricke, P.M. (2016). Fertility programs for lactating dairy cows. *Dairy Herd Management*. Accessed Aug. 15, 2016. Available at: [http://www.dairyherd.com/advice-and-tips/reproduction/fertility\\_programs\\_lactating-dairy-cows](http://www.dairyherd.com/advice-and-tips/reproduction/fertility_programs_lactating-dairy-cows)
20. Vasconcelos, J.L.M., Sartori, R., Oliveira, H.N., Guenther, J.N., Wiltbank, M.C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 56(2): 307-314.  
[https://doi.org/10.1016/S0093-691X\(01\)00565-9](https://doi.org/10.1016/S0093-691X(01)00565-9)
21. Echternkamp, S.E., Cushman, R.A., Allan, M.F. (2009). Size of ovulatory follicles in cattle expressing multiple ovulations naturally and its influence on corpus luteum development and fertility. *J Anim Sci.* 87(11): 3556-3568.  
<https://doi.org/10.2527/jas.2009-2049>  
PMid:19684271
22. Stevenson, J.S., Phatak, A.P. (2010). Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost. *Theriogenology* 73(8): 1127-1138.  
<https://doi.org/10.1016/j.theriogenology.2010.01.014>  
PMid:20096923

**Please cite this article in press as:** Atanasov B., Adamov N., Celeska I., Ilievska K., Angjelovski B., Trbogazov Z., Davkov F., Dovenski T., Opsomer G., Stevenson J. Modification of the standard 7-day Ovsynch protocol to increase the luteolytic and synchronization risks in dairy cows. *Mac Vet Rev* 2020; 43 (2): i-vii. <https://doi.org/10.2478/macvetrev-2020-0028>