sciendo

Research article

Acta Veterinaria-Beograd 2022, 72 (3), 362-374
UDK: 636.2.082.454
DOI: 10.2478/acve-2022-0029

EFFICIENCY OF TWO PROTOCOLS OF RESYNCHRONIZATION OF ESTRUS AND OVULATION IN HIGH-PRODUCING DAIRY COWS AT PEAK LACTATION

Milan ĐORĐEVIĆ[1], Jovan BLAGOJEVIĆ[2*], Miodrag RADINOVIĆ[3], Marko CINCOVIĆ[3], Jelena MALETIĆ[4], Mario KRESZINGER[5], Milan MALETIĆ[2]

[1]Veterinary station "Mladenovac", Kralja Petra I 347, Mladenovac, Serbia; [2]Department of Reproduction, Fertility and Artificial Insemination, Faculty of Veterinary Medicine, University of Belgrade, Bul. Oslobodjenja 18, Belgrade, Serbia; [3]Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovica 8, 21000 Novi Sad, Serbia; [4]Department of Poultry Science, Scientific Veterinary Institute of Serbia, Vojvode Toze 14, Belgrade, Serbia; [5]Surgery, Orthopedics, and Ophthalmology Clinic, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000, Zagreb

(Received 16 July, Accepted 05 September 2022)

The reproductive efficiency of the cows was monitored after two resynchronization protocols: Ovsynch (OVS) and Double Ovsynch (DOS). The research initially included 70 HF cows who entered the first synchronization protocol – Presynch. Cows that did not conceive after the first synchronization were divided into two groups and introduced to two resynchronization protocols. In the first group of cows (n=35), the DOS protocol began with the application of GnRH on day 22 after the Presynch TAI (Timed Artificial Insemination), and seven days later pregnancy check-up was done and $PGF_{2\alpha}$ was applied only to non-pregnant cows (n=23), which remained in the study. In the second group of cows, the OVS protocol started on day 32 after Presynch TAI only in non-pregnant animals (n=20). Progesterone (P4) concentration was determined at the time of application of GnRH1, $PGF_{2\alpha}$ and GnRH2 in both groups of cows, and then 30 days after Resynch TAI, ultrasound pregnancy diagnosis was done. A higher percentage of pregnant cows were recorded in the OVS group compared to the DOS group (45% and 35%, respectively). The concentration of P4 in the serum of cows in the DOS group during the first measurement (GnRH1) was significantly higher than the value in cows that did not conceive ($p<0.05$), while in the third measurement (GnRH2) the average concentration of P4 in conceiving cows was significantly lower ($p<0.001$) compared to cows that did not coincive. The open days period was significantly longer in pregnant cows that were resynchronized using the DOS protocol compared to cows from the OVS protocol. In conclusion, the OVS protocol of estrus resynchronization in dairy cows proved to be more successful than the DOS protocol. However, considering the advantages the OVS, it is needed to determine which day of the sexual cycle is the best to start resynchronization.

Key words: Holstein-Friesian cow, GnRH, Prostaglandin $F_{2\alpha}$, Ovsynch, Double-Ovsynch, Progesterone

*Corresponding author: e-mail: jovan.blagojevic@vet.bg.ac.rs

INTRODUCTION

In the last years, the trend of research and offerings supplied to farmers has focused on reproductive management programs that go beyond reproductive processes and instead coordinate multiple disciplines such as physiology, animal welfare, nutrition, health management and genetics [1]. Intensive cattle production, where the success largely depends on herd fertility, the manipulation or management of the sexual cycle occupies a significant area. Nowadays, farmers within the dairy industry have diverse approaches to harmonizing the needs of high-producing dairy cows and improving their reproductive efficiency. The aim of sexual cycle control in cows and heifers is synchronization and induction of estrus and ovulation in certain phases of the sexual cycle [2-4]. The two most advanced protocols extensively practiced on large dairy farms are the Ovsynch (OVS; GnRH-7 days- $PGF_{2\alpha}$-56 hours-GnRH-16 hours-TAI [5]) and Double-Ovsynch (DOS; GnRH-7 days- $PGF_{2\alpha}$-56 hours-GnRH-7 days- Ovsynch [6]) timed artificial insemination (TAI) programs [7]. Both programs increase the fertility of subfertile lactating cows, reducing follicle dominance and maintaining progesterone balance between injections of GnRH and $PGF_{2\alpha}$, improving the complete regression of the corpus luteum (CL) before GnRH2 and optimizing the time limit for AI relative to GnRH injection [7].

Programming the estrogen cycle phase to early diestrus (e.g., days 5-9 of the estrous cycle) when modified protocols are implemented, integrate multiple effects [8]. The first injection of GnRH may cause luteinization of follicles from the first wave and consequently the beginning of a new wave of follicular growth. Adequate luteal progesterone concentration during the Ovsynch protocol gives information about the presence of CL and the response to $PGF_{2\alpha}$ injection aimed to luteolysis [8]. The process of luteolysis is completed at the time of GnRH2 and/or TAI and the quality of the oocyte is programmed for the appropriate period required for fertilization, and there is a subsequent development of a robust corpus luteum to maintain pregnancy [9]. Certainly, GnRH-induced ovulation follicles from the first wave results in the presence of both the original CL and the accessory CL at the time of $PGF_{2\alpha}$ administration [10]. When $PGF_{2\alpha}$ is applied during the growth of the dominant follicle of the first follicular wave, ovulation can be expected in 2-3 days. However, if the $PGF_{2\alpha}$ is administered in a plateau phase when the follicle has lost its dominance, ovulation can be expected only after 4-5 days when the dominant follicle separates from the second follicular wave and ovulates. Therefore, the application of GnRH 7 days before $PGF_{2\alpha}$ allows adequate synchronization [5,8].

Having in mind that better results have been achieved when OVS started on days 5 to 12 of the estrus cycle, both in dairy cows and heifers [10-12], researchers introduced the DOS protocol [6]. Primarily, DOS is a pre-synchronization-Ovsynch protocol [13] developed to improve reproductive performance in anovulatory dairy cows [6]. It was hypothesized that two consecutive Ovsynch will stimulate cyclicity in non-cycling cows [6]. Therefore, the phase of the cycle at the time of synchronization significantly affects

the performance of the Ovsynch protocols [10,11]. The presence of a functional CL at the beginning of the Ovsynch (GnRH1) reduces fertility performance [14]. High levels of P4 inhibit GnRH secretion, while its low levels indicate the absence of the CL and possible ovulation disorders [15], which is significant for the choice of resynchronization protocol over previous AI.

However, even such advanced protocols have limitations that lead to unsuccessful inseminations, prolongation of the service interval, and, consequently, lactation, which inevitably leads to an increase in the costs on a farm [16]. To shorten the time until the new insemination of non-conceiving cows that were previously inseminated through a fixed ovulation and insemination program, estrus and ovulation resynchronization protocols are introduced [17,18]. Moreover, numerous factors affect the pregnancy rate after the resynchronization protocol such as body condition score (BCS), pre- and postpartum disorders, days after calving, and heat stress [19]. Resynchronization programs continue with the original synchronization programs, thus avoiding estrus detection or insemination of anovulatory cows [20].

The aim of this paper was a comparison of two resynchronization protocols – Ovsynch and Double Ovsynch, and an examination of the reproductive efficiency of cows in these precisely controlled resynchronization protocols.

MATERIAL AND METHODS

Animals

Animals used in this experiment were Holsten Frisian cows kept on a dairy farm, in the vicinity of Belgrade, Serbia. The investigation initially included 70 HF cows, at 2nd and 3rd lactations, divided into two equal groups. They were kept under the same conditions, in a free-stall housing system and fed two times a day with the same diet with a total mixed ration (TMR; 5 kg of hay, 23 kg of corn silage, 7 kg of alfalfa haystack and 11 kg of concentrate with 18% of proteins). The practice on the farm is to monitor the general health status every day. All cows were well-conditioned and without significant health problems during the experiment.

At beginning of the study, the cows were in approximately the same stage of lactation (45th – 60th days in milk – DIM). They were milked twice daily at approximately 12-h intervals. Milk yield data were collected from the farm database, using the Blue Diamond milking system (DeLaval, USA), which measures the amount of milk for each cow individually. The groups of cows were uniform in milk production (c_v<30%).

Protocols

The initial estrus was inducted in all of the 70 cows using the Presynch protocol ($PGF_{2\alpha}$ -14 days-$PGF_{2\alpha}$ -12 days-Ovsynch [12]).

The first group of cows (n=35) entered the Double-Ovsynch (DOS) resynchronization protocol starting with the application of GnRH on day 22 after the first Presynch TAI. On day 29 control ultrasound scan was done, and non-pregnant cows (n=23) were given $PGF_{2\alpha}$. On day 32 GnRH was administered, on day 39 GnRH1, on day 46 $PGF_{2\alpha}$, after 56 hours (day 48) GnRH2 was injected and TAI was done 16 h after the GnRH injection (Figure 1). From all the 23 cows blood samples were taken to determine the P4 concentrations at three-time points (GnRH1, $PGF_{2\alpha}$, GnRH2): on day 39 (to determine the presence of CL, when GnRH1 was administered), day 46 (to determine the ovulatory response to GnRH1, when $PGF_{2\alpha}$ was given), and day 48 (to determine the regression of CL, when GnRH2 was administered) after failed TAI.

The second group of cows (n=20) was composed of cows that remained non-pregnant after Presynch TAI, which was detected by ultrasound examination on day 32. On the same day, Ovsynch (OVS) resynchronization protocol started with GnRH1. On day 39 $PGF_{2\alpha}$ was administered, 56 h later GnRH2 and TAI was done 16 h later (Figure 1). P4 concentrations were measured in these 20 cows at three-time points (GnRH1, $PGF_{2\alpha}$, GnRH2): on day 32 (to determine the presence of CL, when GnRH1 was administered), day 39 (to determine the ovulatory response to GnRH1, when $PGF_{2\alpha}$ was given), and day 41 (to determine the regression of CL, when GnRH2 was administered) after failed TAI.

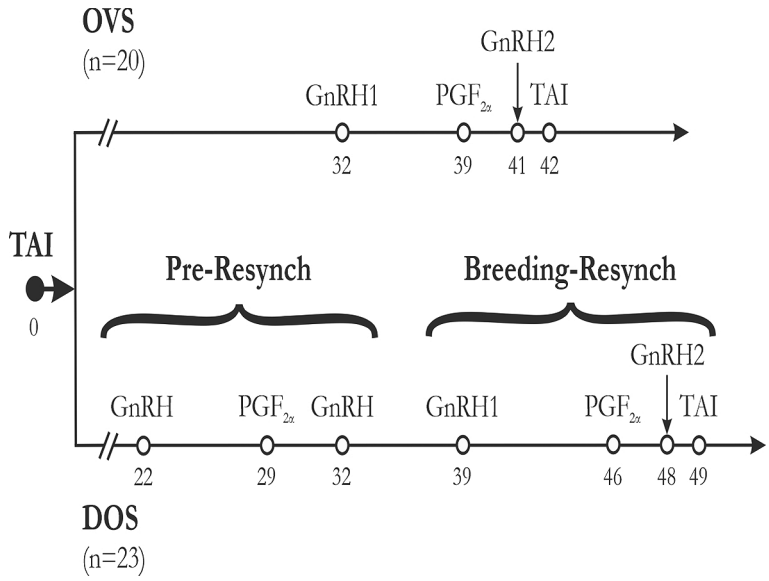

Figure 1. Diagram of Double Ovsynch (DOS) and Ovsynch (OVS) resynchronization protocols used at second postpartum TAI services

In both groups of cows, pregnancy was confirmed by ultrasound examination on day 30 after Resynch TAI. For transrectal ultrasound pregnancy diagnosis, a portable

scanner with a multi-frequency (4.5 – 8.5 MHz) linear probe (Easy-Scan, BCF Technology Ltd., Livingston, UK) was used.

Blood Collection and P4 - analysis

Blood samples were collected by venepuncture of the coccygeal vein with sterile needles in tubes with no anticoagulant (BD Vacutainer®). From each animal, at least 10 ml of blood was obtained. The samples were transported in a portable refrigerator to the laboratory. The blood was centrifuged at 2,000 g for 15 min for serum isolation. Blood serum samples were stored at −20°C until analysis. The concentration of progesterone (P4) was determined with an automatic immunoassay analyser TOSOH AIA360 (Japan). Principle of the assay: competitive enzyme immunoassay performed in TOSOH test cup. Progesterone in the test sample competes with enzyme-labeled progesterone for a limited number of binding sites on a progesterone-specific antibody immobilized on magnetic beads. The lowest measurable concentration in a specimen is 0.1 ng/mL (assay sensitivity), and the highest measurable concentration without dilution is 40 ng/mL. Accuracy in the recovery test is about 97-103%, in the dilution test it is about 99-105%. Specificity and cross-reactivity (%) were between 0.001 to 0.8% for substances such as cortisol, corticosterone, pregnenolone, 17a-hydroxyprogesterone, 11-deoxycorticosterone, cortisol and testosterone.

Statistical analyses

The results of the experiment are presented in graphs showing descriptive statistics: means, median, extreme values, first and third quarters and standard deviation. Statistical methods were chosen following the complex research tasks and the statistical hypotheses were formulated based on them. To compare the average values, the choice between alternative methods was made by the importance of the coefficients of variation and the results of Levene's test for variance homogeneity. Due to the size of the samples, the coefficient of variation was used as an approximate criterion for checking the assumption of data distribution according to the normal distribution model, on which the parametric statistics are based. The t-test was used to compare the means of two samples with homogeneous data (C_v<30%) and homogeneous variances, and in the case of heterogeneous variances and/or heterogeneous data (C_v>30%), a nonparametric Mann-Whitney U-test based on medians. Given the established homogeneity of the data, the correlation of the two variables was analysed based on the Pearson correlation coefficient. The conclusion on the significance of the relationship was made based on the t-test for the correlation coefficient. For all data sets, the two standard significance levels of 0.05 and 0.01 were taken into consideration. Statistical analysis was done with Statistica software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

In this study, the response to two different resynchronization protocols was examined in a population of 43 HF cows. A higher percentage of pregnant cows per AI (P/AI) was observed in OVS than in DOS cows, but the difference was not significant (χ^2=0.104; p=0.746; Figure 2). The percentage of pregnant cows was not significantly lower compared to non-pregnant cows in both groups, in the DOS (χ^2=1.800; p=0.180) and in the OVS group (χ^2=0.200; p=0.655).

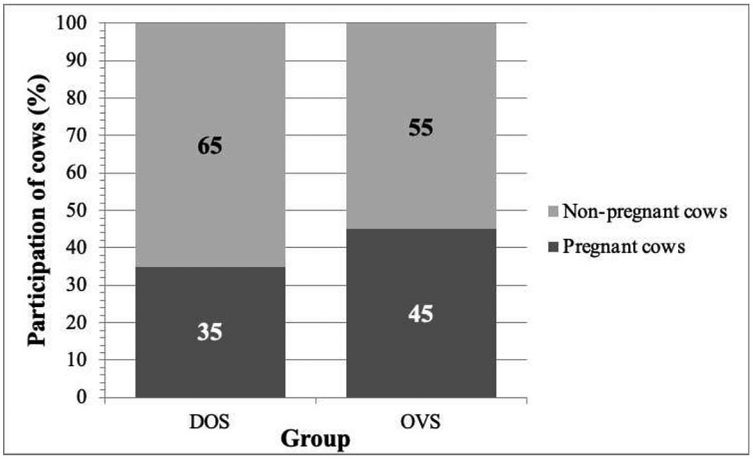

Figure 2. Pregnancy per AI after resynchronization in DOS and OVS group of cows

Given that progesterone was measured during the resynchronization protocols, after which conception was checked, pregnant cows were noted as conceiving, and those non-pregnant as non-conceiving. The results of the Mann-Whitney U-test indicate that the serum P4 concentration in the first measurement (at GnRH1 application) in conceiving DOS cows was significantly higher than the average P4 value in non-conceiving cows (z=2.101; p=0.036). In the second P4 measurement (at $PGF_{2\alpha}$ application), the average values did not differ significantly (z=0.753; p=0.451), but in the third measurement (at GnRH2 application), the average serum progesterone concentration in conceiving cows was significantly lower (z=-3.493; p<0,001) than that in non-conceiving cows. Also, the results of the Mann-Whitney U-test indicated that in conceiving cows from both groups, the average P4 values did not differ in any of the three-blood sampling time points (GnRH1, z=-0.582; p=0.560; $PGF_{2\alpha}$, z=1.536; p=0.125; GnRH2, z=-1.710; p=0.087). There was a significant difference between non-conceiving cows in the DOS group and in the OVS group at the first and third measurements of serum progesterone concentration, (Figure 3). At GnRH1, the average P4 level was significantly lower (p=0.030), and at GnRH2 was higher (p=0.026) in DOS non-conceiving cows. At the $PGF_{2\alpha}$ measurement, the difference was not significant (p=0.977).

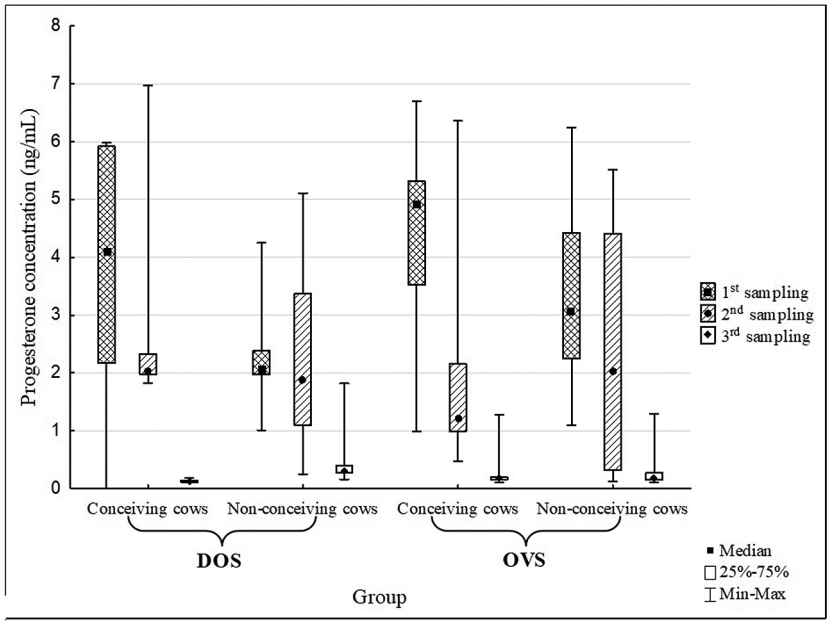

Figure 3. Serum progesterone concentrations during protocol in conceiving and non-conceiving cows in group DOS and OVS

According to the results of Fisher's test of exact probability (Table 1), the participation of cows with progesterone concentrations above/below a critical value at different time points [12] did not differ significantly between the two groups of cows at the same time point ($p \geq 0.273$). Based on the results of the Mann-Whitney U-test, it follows that conceiving cows from group DOS had significantly longer open days period compared to those from group OVS ($z=2.597$; $p=0.009$; Figure 4).

Table 1. Distribution of cows depending on P4 concentrations in each DOS and OVS sampling and level of significance of Fisher's test of exact probability

Sampling	P4 value	DOS	OVS	p-value
I (GnRH1 applied)	P4 < 1.0 ng/mL	1/23(4.34%)	0/20(0.00%)	>0.999
II (PGF2α applied)	P4 > 1.0 ng/mL	18/23(78.26%)	13/20(65.00%)	0.273
III (GnRH2 applied)	P4 < 0.4 ng/mL	17/23(73.91%)	16/20(80.00%)	>0.999

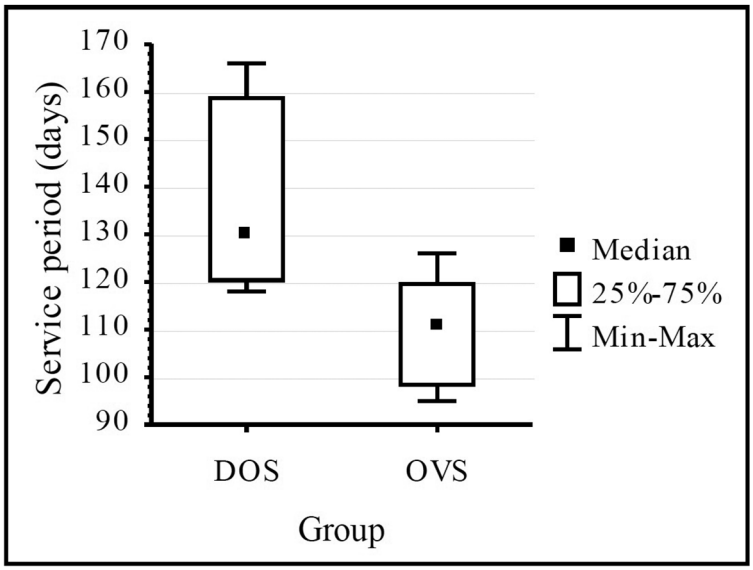

Figure 4. Open days period between DOS and OVS group

DISCUSSION

Resynchronization aims to reduce the AI interval and improve P/AI in dairy cow herds. The first postpartum AI may be unsuccessful in more than 60% of cases [21,22], and most non-pregnant cows do not show any signs of estrus at regular intervals [17]. Therefore, early detection of non-pregnant cows and their introduction of the next TAI protocol is crucial in the resynchronization of non-conceiving cows. In our investigation, OVS and DOS resynchronization protocols were examined. Unexpectedly**,** P/AI was higher in OVS compared with DOS-induced cows (45% and 35%, respectively). These results are in contrast to Giordano et al. [14], who detected significantly higher P/AI in cows resynchronized using DOS. Moreover, Herlihy et al. [23] noted that DOS was more successful compared with Presynch in improving fertility in dairy cows. Presynchronization with a single application of GnRH 7 days before resynchronization with the CO-Synch protocol increases P4 concentration at beginning of resynchronization but does not improve the P/AI in lactating dairy cows [24]. However, Souza et al. [6] achieved higher pregnancy rate after DOS synchronization only in primiparous cows, but not in multiparous ones. In our study, all the cows were multiparous, and only those with first-service TAI were included.

GnRH1 application aims to induce ovulation of the dominant follicle of the current follicular wave in about two days, thus enabling the establishment of a new follicular wave [2]. Medium P4 concentration (1-3.5 ng/mL) at the beginning of OVS (when GnRH1 is administered) are related to better fertility [25]. Giordano et al. [14]

considered that higher P4 in cows with functional CL at the initiation of the OVS protocol can result in lower P/AI in the presynchronized group. High levels of P4 (>3.50 ng/mL) have an inhibitory effect on GnRH secretion, which affects LH secretion and oocyte maturation [26]. In contrast, low levels of P4 (<1.0 ng/mL) indicate the absence of CL and possible ovulatory disorders [15]. The assessment of P4 blood serum concentrations it is possible to decide whether the cows are synchronized at the beginning of resynchronization. The presence of a functional CL at the time when GnRH1 is administered improves P/AI by approximately 10% [27]. In our work, only one in 23 cows (1/23) in the DOS group, and none in OVS had P4 below 1 ng/mL, which means that almost all animals had ovarian activity, but fertility was better by 10% in the non-presynchronized OVS group.

High P4 (>2 ng/mL) concentrations in dairy cows immediately before $PGF_{2\alpha}$ is administered [28] and its low levels at AI [29] are crucial for maximizing the following P/AI in TAI programs [30]. In both groups, cows with a good ovulatory response to $PGF_{2\alpha}$, which was reflected in low P4 values at the time of GnRH2 application (Table 1), had higher P/AI. The most consistent response to $PGF_{2\alpha}$ use was observed in conceiving DOS cows, in which P4 did not exceed 1 ng/mL at the time of GnRH2 application. This means that Pre-resynch contributed to better estrus synchronization and ovarian response to Breeding-Resynch, portion of Double-Ovsynch. Some authors suggest that $PGF_{2\alpha}$ given once, on day 7 after GnRH, does not lead to complete luteal regression in all cows and that an additional dose, after 24 h, would give a better ovulatory response [31,32]. Also, the application of $PGF_{2\alpha}$ on day 8 rather than day 7 in OVS protocol may improve luteolysis [33]. In our experiment, it is possible that in certain cows in both groups incomplete luteolysis after $PGF_{2\alpha}$ resulted in moderate P/AI. However, only marginal effects in fertility were noted when an additional $PGF_{2\alpha}$ dose was administered 24 h after its first application [34].

DOS is a pre-synchronization protocol [13] used within resynchronization. However, Jeong et al. [35] tested the effects of selective presynchronization before Ovsynch, depending on the findings on the ovaries. They used DOS only in the absence of CL at the beginning of the program, while in cows with CL they used $PGF_{2\alpha}$ instead of the first injection of GnRH, which proved to be justified. The open days period was significantly longer in DOS-conceiving cows, in comparison with the OVS group. The potential benefits of the DOS protocol may be the extended time for uterine recovery [36], but also hormonal synchronization owing to the repeated hormonal application. However, in our study, DOS did not improve P/AI regardless of the extended open days period. The best fertility results are achieved when Ovsynch protocols start on days 5-12 of the cycle [37]. The cows in this study underwent resynchronization after the Presynch protocol. DOS started on day 22 after the previous Presynch TAI, i.e., at the beginning of the second estral cycle. In contrast, OVS began on day 32 after TAI, which was approximately day 12 of the following estral cycle. Thus, the day of the estrus cycle at initiation of resynchronization protocols could be the reason for better P/AI in OVS cows, although DOS was expected to improve cyclicity.

CONCLUSIONS

The results of our research pointed out that the OVS protocol was more successful in estrous resynchronization in dairy cows and resulted in higher pregnancy rates in comparison with the DOS protocol. Moreover, resynchronization with the OVS protocol contributed to a lower open days period in pregnant cows. It is necessary to assess these two protocols on larger numbers of cows, bearing in mind that the costs of OVS are lower and is less labour-intensive. In addition, it should also be established on which day of the cycle it is best to start the resynchronization protocol.

Acknowledgements

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2022-14/200143). We appreciate to the company FM Pharm, which provided free of charge the products Neorelin and Neoprost, for supporting the research.

Authors' contributions

MĐ, JB and MM carried out the experimental work, made substantial contributions to the acquisition, analysis and interpretation of data and participated in manuscript writing. MĐ and JB have been involved in drafting the manuscript. MM supervised the entire work. MĐ, MC and MR took samples at the farm, prepared the samples and analyzed them in the laboratory. MR provided all data from the farm including health status and nutrition. JM and MK participated in the design of the study and performed the statistical analysis. All authors provided critical feedback and helped to shape the research and final paper.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Thatcher WW, Staples CR, Santos JE: The Future of Reproductive Management. In Dairy Cattle Reproduction Conference Indianapolis, EE. UU 2013.
2. Stevenson JS, Kobayashi Y, Thomson KE: Reproductive performance of dairy cows in various programmed breeding systems including OvSynch and combinations of gonadotropin-releasing hormone and prostaglandin F2α. J Dairy Sci 1999, 82:506–515
3. Pursley JR, Silcox RW, Wiltbank MC: Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratios after synchronization of ovulation in lactating dairy cows. J Dairy Sci 1998, 81:2139–2144.
4. Carvalho PD, Santos VG, Giordano JO, Wiltbank MC, Fricke PM: Development of fertility programs to achieve high 21-day pregnancy rates in high-producing dairy cows. Theriogenology 2018,114:165-72.

5. Pursley JR, Mee MO, Wiltbank MC: Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 1995, 44:915–923.
6. Souza AH, Ayres H, Ferreira RM, Wiltbank MC: A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 2008, 70:208–215.
7. Nowicki A, Barański W, Baryczka A, Janowski T: OvSynch protocol and its modifications in the reproduction management of dairy cattle herds–an update. J Vet Res 2017, 61:329-336.
8. Wiltbank MC, Pursley JR. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology 2014, 81:170-185.
9. Stevenson JS, Kobayashi Y, Shipka MP, Rauchholz KC: Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin F2α. J Dairy Sci 1996, 79:402-10.
10. Ahmed N, Kathiresan D, Ahmed FA, Lalrintluanga K, Mayengbam P, Gali JM: Pattern of induced estrus and conception rate following Ovsynch and Ovsynch based gonadotropin-releasing hormone treatments initiated on day 6 of estrous cycle in repeat breeding crossbred cows. Vet World 2016, 9:342-345.
11. Vasconcelos JL, Silcox RW, Rosa GJ, Pursley JR, Wiltbank MC: 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 1999, 52:1067–1078.
12. Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW: Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 2001, 84:1646-1659.
13. Stangaferro ML, Wijma R, Masello M, Giordano JO: Reproductive performance and herd exit dynamics of lactating dairy cows managed for first service with the Presynch-Ovsynch or Double-Ovsynch protocol and different duration of the voluntary waiting period. J Dairy Sci 2018, 101:1673-1686.
14. Giordano JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP, Fricke PM: Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J Dairy Sci 2012, 95:639-53.
15. Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC: Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J Dairy Sci 2004, 87:905-20.
16. Cabrera VE: Economics of fertility in high-yielding dairy cows on confined TMR systems. Animal 2014, (s1):211-21.
17. Dewey ST, Mendonca LG, Lopes GJ, Rivera FA, Guagnini F, Chebel RC, Bilby TR: Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. J Dairy Sci 2010, 93, 4086–4095
18. Thompson IM, Cerri RL, Kim IH, Green JA, Santos JE, Thatcher WW: Effects of resynchronization programs on pregnancy per artificial insemination, progesterone, and pregnancy-associated glycoproteins in plasma of lactating dairy cows. J Dairy Sci 2010, 93:4006-18.
19. Kim IH, Jeong JK, Kang HG: Factors affecting reproductive outcomes in lactating dairy cows that undergo presynchronization-Ovsynch and successive resynchronization programs. Theriogenology 2022, 187:9-18.

20. Carvalho PD, Guenther JN, Fuenzalida MJ, Amundson MC, Wiltbank MC, Fricke PM: Presynchronization using a modified Ovsynch protocol or a single gonadotropin-releasing hormone injection 7 d before an Ovsynch-56 protocol for submission of lactating dairy cows to first timed artificial insemination. J Dairy Sci 2014, 97:6305-15.
21. Galvão KN, Santos JEP, Cerri RL, Chebel RC, Rutigliano HM, Bruno RG, Bicalho RC: Evaluation of methods of resynchronization for insemination in cows of unknown pregnancy status. J Dairy Sci 2007, 90:4240–4252
22. Ayres H, Ferreira RM, Cunha AP, Araujo RR, Wiltbank MC. Double-Ovsynch in high-producing dairy cows: Effects on progesterone concentrations and ovulation to GnRH treatments. Theriogenology 2013, 79:159-64.
23. Herlihy MM, Giordano JO, Souza AH, Ayres H, Ferreira RM, Keskin A: Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J Dairy Sci 2012, 95:7003-7014.
24. Alkar A, Tibary A, Wenz JR, Nebel RL, Kasimanickam R: Presynchronization with GnRH 7 days prior to resynchronization with CO-Synch did not improve pregnancy rate in lactating dairy cows. Theriogenology 2011, 76:1036-1041.
25. Bisinotto RS, Chebel RC, Santos JE: Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J Dairy Sci 2010, 93:3578-87.
26. Revah I, Butler WR: Prolonged dominance of follicles and reduced viability of bovine oocytes. Reproduction 1996, 106:39-47.
27. Borchardt S, Pohl A, Heuwieser W: Luteal presence and ovarian response at the beginning of a timed artificial insemination protocol for lactating dairy cows affect fertility: a meta-analysis. Animals 2020, 10:1551.
28. Stojanov B, Atanasov B, Grizelj J, Vince S, Nikolovski M, Kjosevski M, Dovenski T, Adamov N, Ilievska K, Samardzija M: Induction and formation of accessory corpus luteum after artificial insemination (AI) might increase pregnancy rate per AI in heat stressed dairy cows. Mac Vet Rev 2020, 43: 37-43.
29. Bajagić B, Mrkun J, Kirovski D, Savić Đ, Budimir D, Maletić J, Maletić M. Assessment of different diagnostic methods for the identification of subclinical endometritis in dairy cows with pathological puerperium and their reliability to conceive. Acta Vet 2021, 71:462-476.
30. Stevenson JS, Lamb GC: Contrasting effects of progesterone on fertility of dairy and beef cows. J Dairy Sci 2016, 99:5951-64.
31. Wiltbank MC, Baez GM, Cochrane F, Barletta RV, Trayford CR, Joseph RT. Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. J Dairy Sci 2015, 98:8644-54.
32. Barletta RV, Carvalho PD, Santos VG, Melo LF, Consentini CE, Netto AS, Fricke PM. Effect of dose and timing of prostaglandin F2α treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows. J Dairy Sci 2018, 101:1730-6.
33. 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, 43:161-167.
34. Giordano JO, Wiltbank MC, Fricke PM, Bas S, Pawlisch R, Guenther JN, Nascimento AB: Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 2013, 80:773-783.

35. Jeong JK, Kim UH, Kang HG, Kim IH. Selective use of a modified pre-synchronization-Ovsynch and resynchronization reproductive strategy in dairy herds: A field application study. Reprod Domest Anim 2022, 57:45-54.

36. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol Reprod 2009, 81:1025-32.

37. Moreira F, de la Sota RL, Diaz T, Thatcher WW. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J Anim Sci 2000, 78:1568–76.

ISPITIVANJE EFIKASNOSTI DVA PROTOKOLA RESINHRONIZACIJE ESTRUSA I OVULACIJE KOD VISOKOMLEČNIH KRAVA U PIKU LAKTACIJE

Milan ĐORĐEVIĆ, Jovan BLAGOJEVIĆ, Miodrag RADINOVIĆ, Marko CINCOVIĆ, Jelena MALETIĆ, Mario KRESZINGER, Milan MALETIĆ

Reproduktivna efikasnost krava je praćena nakon dva protokola resinhronizacije estrusa mlečnih krava – Ovsynch (OVS) i Double Ovsynch (DOS). U istraživanje je inicijalno uključeno 70 krava HF rase, koje su između 45. i 60. dana laktacije ušle u prvi protokol sinhronizacije estrusa – Presynch. Krave koje nakon prve sinhronizacije nisu koncipirale, podeljene su u dve grupe i uvedene u protokole resinhronizacije. Kod prve grupe krava (n=35) DOS protokol je otpočeo aplikacijom GnRH 22. dana posle VO, da bi nakon sedam dana bila izvršena kontrola steonosti i aplikacija $PGF_{2\alpha}$ samo negravidnim jedinkama (n=23), koje su i dalje ostale u istraživanju. U drugoj grupi krava OVS protokol je započeo 32. dana nakon VO samo kod negravidnih jedinki (n=20). Koncentracija progesterona (P4) je određivana u momentu aplikacije GnRH1, $PGF_{2\alpha}$ i GnRH2 kod obe grupe krava, a zatim je 30 dana nakon VO obavljena ultrazvučna dijagnostika graviditeta. Zabeležen je veći procenat steonih krava u OVS grupi u poređenju sa DOS grupom (45%, odnosno 35%). Koncentracija P4 u serumu krava u DOS grupi prilikom prvog merenja (vreme aplikacije GnRH1) bila je značajno viša od vrednosti kod krava koje nisu koncipirale ($p<0,05$), dok je u trećem merenju (vreme primene GnRH2) prosečna koncentracija P4 kod krava koje su koncipirale bila značajno niža ($p<0,001$) u odnosu na krave koje nisu. Servis period je bio značajno duži kod gravidnih krava čiji je estrus resinhronizovan primenom DOS protokola u odnosu na krave iz OVS protokola. U zaključku, OVS protokol resinhronizacije estrusa mlečnih krava pokazao se uspešnijim od DOS protokola. Potrebna su dalja ispitivanja primene oba protokola na većem broju jedinki, imajući u vidu prednosti OVS i potrebe da se utvrdi koga dana polnog ciklusa je najbolje početi sa resinhronizacijom.