VETERINARSKI ARHIV 87 (4), 397-408, 2017 doi: 10.24099/vet.arhiv.160413

Progesterone concentration and conception rates after three different synchronization protocols in dairy cows

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ŠPOLJARIĆ, B., S. VINCE, J. GRIZELJ, G. ŠTIBRIĆ, M. SAMARDŽIJA, A. UNIĆ, Ž. ROMIĆ, T. DOBRANIĆ, D. GEREŠ: Progesterone concentration and conception rates after three different synchronization protocols in dairy cows Vet. arhiv 87, 397-408, 2017.

ABSTRACT

Ovsynch is a protocol routinely used for oestrus synchronization with the best results obtained when it is started in the midluteal phase of the oestrous cycle. The best way to achieve this phase is to apply presynchronization protocols. The aim of this study was to compare the success of two combined presynchronization protocols with Ovsynch, according to conception rates and progesterone concentration, in synchronization and resynchronization. The research was conducted on a dairy farm in Croatia, on 60 cows divided into three equal groups. Group A was presynchronized with GnRH and $PGF_{2\alpha}$ nine and two days before starting Ovsynch. Group B was presynchronized with just one application of $PGF_{2\alpha}$ two days before Ovsynch. The third group C served as a control, and was subjected to Ovsynch only. All cows were artificially inseminated at a fixed time. Twenty-three days after TAI, GnRH was applied to all cows, as the beginning of Ovsynch in resynchronization. Seven days later all the cows were examined for pregnancy with ultrasound. Those diagnosed as non-pregnant, continued with Ovsynch and TAI. Blood samples were collected for progesterone analysis. The results indicate that cows subjected to presynchronization had a better pregnancy rate compared to cows subjected to Ovsynch only (A 29.41%, B 47.37% and C 21.05%). In resynchronization, the conception rate did not differ between the groups, and was around 30%. Progesterone concentrations differed significantly between group C and groups A and B ($P<0.01$) at the start of Ovsynch in synchronization (Day 0), indicating the more effective homogenization of folliculogenesis and synchronization of ovulation. Both the combined synchronization protocols used in this research are original. Due to the results obtained, their application should be evaluated further.

Key words: dairy cows, presynchronization, resynchronization, Ovsynch, conception rate, progesterone

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ISSN 0372-5480
Printed in Croatia

Introduction

Protocols with synchronization of follicular growth, CL regression, ovulation and timed artificial insemination (TAI) are being developed due to the increasing number of cows with poorly detectable oestrus (COLAZO and AMBROSE, 2013). Reproductive efficiency is a key to success in the dairy sector (EL-TARABANY et al., 2016). The extended period for renewal of postpartum cyclic activity, poor oestrus detection, wrong insemination time, together with post parturient problems, definitely decrease reproductive efficiency (LUCY, 2001). Given that Ovsynch is limited, new strategic approaches have been investigated. Presynchronization protocols control ovarian activity before applying Ovsynch itself (PETERS and PURSLEY, 2003; NAVANUKRAW et al., 2004). The most commonly used protocols are based on prostaglandin application, but they have wide diversity in ovulation time, and can only be effective when there is an active CL on the ovary. The other protocols, based on application of GnRH before Ovsynch, have undergone many modifications.

To increase the conception rate (CR) and to shorten the period before subsequent inseminations, resynchronization is undertaken of cows that did not conceive on the previous synchronization (PURSLEY et al., 1997). Since the average conception rate after Ovsynch is 35 to 40% (CORDOBA and FRICKE, 2002; EL-ZARKOUNY et al., 2004; EL-TARABANY et al., 2016), it is necessary to identify and re-inseminate non pregnant cows as soon as possible. According to BARTOLOME et al. (2005b), the period between TAI and the next oestrus is 22-23 days in most cows that fail to conceive. Therefore, it is recommended to apply GnRH 22-23 days after TAI, that is, 7 days before ultrasound examination for pregnancy. This way, the new follicular way will be synchronized and an active CL created. The CL created in this way should be responsive to prostaglandin application 30 days after TAI. As a result, the period between two subsequent inseminations is shortened.

The aim of this study is to compare the success of two combined presynchronization protocols with Ovsynch, according to conception rates and the hormonal environment created, that is, progesterone concentration. Furthermore, the success of resynchronization will also be studied. Our intention is to prove that combined protocols are more effective, and that their use is needed and justified.

Materials and methods

Animals. The research was conducted on the "Zdenačka farma d.o.o." dairy farm, Veliki Zdenci, Croatia, and involved 60 primiparous cows of the Holstein-Frisian breed. The cows were randomly divided into three groups of 20 animals. During the procedure four cows were removed (inseminated before planned), so group A comprised 18 animals, and groups B and C 19 animals each. All the cows had a very similar body condition (mean BCS 2.77 ± 0.21) and were approximately 65 days in milk on Day 0, when synchronization started (65 ± 5 days). All procedures used in this research were in compliance with the European guidelines for the care and use of animals in research (Directive 2010/63/EC) and approved by the Ethics Committee for Animal Experimentation, Faculty of Veterinary Medicine, University of Zagreb, Croatia (records No.: 640-01/12-17/75; file No.: 251/61-01/139-12-2) .

Presynchronization. Groups A and B were presynchronized, while group C served as control, and was synchronized with Ovsynch only (PURSLEY et al., 1995). Standard Ovsynch consisted of application of a synthetic GnRH analogue (Gonavet Veyx®, Veyx-Pharma GmbH, Schwarzenborn, Germany, 1 mL i/m per cow) on Day 0, followed by application of a synthetic analogue of $PGF_{2α}$ (PGF Veyx®, 0.5 mg per cow i/m) 7 days later, then application of the second GnRH analogue 48 h after $PGF_{2α}$ and finally TAI, 6 and 24 h after the last GnRH. Since the majority of protocols using TAI result in low CR, the cows were inseminated twice (FRICKE et al., 2003; ŠPOLJARIĆ et al., 2013). Group A was presynchronized with modification of the Double Ovsynch protocol, in which the cows received an injection of the synthetic GnRH analogue 9 days before the start of Ovsynch (Day -9) and the synthetic analogue of $PGF_{2α}$ two days before Ovsynch (Day -2). On day 0, group A continued with Ovsynch as described above. Group B was presynchronized with a single synthetic $PGF_{2α}$ analogue application 2 days (Day -2) before synchronization with Ovsynch, as described above (Fig. 1). After Day 0, the treatment was the same for all animals enrolled in the study.

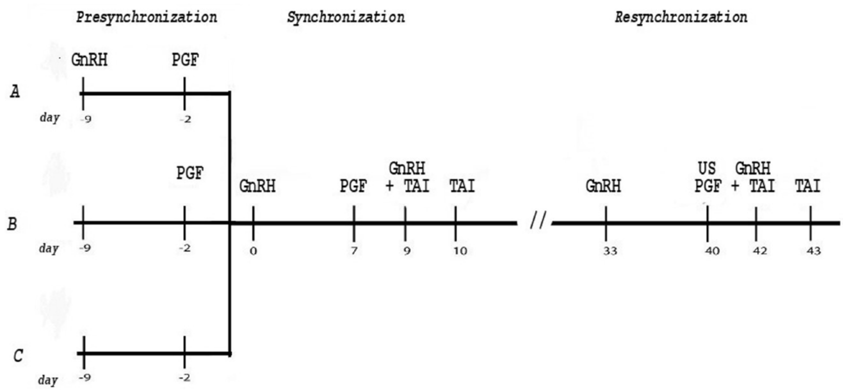

Fig. 1. Scheme of protocols used: Group A was presynchronized with GnRH 9 days before the start of Ovsynch (Day -9) and $PGF_{2α}$ two days before Ovsynch (Day -2); group B was presynchronized with a single $PGF_{2α}$ application 2 days (Day -2) before synchronization with Ovsynch; group C served as control; from day 0 all cows received the same procedure: application of GnRH on Day 0, followed by application of $PGF_{2α}$ 7 days later, then application of the second GnRH 48 h after $PGF_{2α}$ (Day 9) and TAI 6 and 24 h after last GnRH in synchronization; 23 days (Day 33) after TAI all animals received GnRH, as the start of possible resynchronization; 7 days later (Day 40) all cows were checked for pregnancy by ultrasound (US); those found non-pregnant received $PGF_{2α}$ and, two days later, GnRH (Day 42) and were inseminated 6 and 24 h after the last GnRH.

Resynchronization. Twenty-three days (Day 33) after TAI all animals received a synthetic GnRH analogue, as the start of possible resynchronization treatment. Seven days later (Day 40) all the cows were checked for pregnancy by ultrasound. Those found non-pregnant received a synthetic analogue of $PGF_{2\alpha}$, and, two days later a synthetic GnRH analogue and were inseminated 6 and 24 h after the last GnRH (Fig. 1). Artificial insemination was always performed by the same veterinarian, with deeply frozen semen chosen by the farm manager. Thirty days after TAI the cows were examined for pregnancy by ultrasound, and 30 days later those found pregnant were re-examined. Ultrasound diagnostics were performed using a Medison SonoVet 2000 machine equipped with a transrectal linear probe (5 MHz).

Blood sampling. Blood was drawn from the coccygeal vein of all cows on the days of hormone application, starting with day -9, for analysis of progesterone, into plastic vacuum tubes with a clot activator (Venosafe® plastic tube: Serum VF- 076SP, 7 mL, Terumo Europe N. V., Belgium), always at the same time after milking. After collection, the blood was left at room temperature for 3 hours, then centrifuged (3500 rpm for 10 min). Afterwards, the serum was separated and stored in Eppendorf micro-centrifuge tubes at -20 °C until the tests were performed. The progesterone concentration in the serum was analysed using a standard commercial reagents package (Access Progesterone, Beckman Coulter Inc., Ireland) on a UniCel DxI 600 analyser (Beckman Coulter, Tokyo, Japan) in the Clinical Department for Laboratory Diagnostics at Dubrava Clinical Hospital, Zagreb, Croatia, within the next six months.

Statistical analysis. The statistical analysis of data was performed using SAS 9.3. Software (2002-2010 by SAS Institute Inc., Cary, NC, SAD). Descriptive statistics (mean, SEM) were calculated using PROC MEANS. Normal distribution of data was tested by PROC UNIVARIATE, while the homoscedasticity of variance was tested using the Levene test with a general linear model (PROC GLM) and option HOVTEST. Progesterone concentration was not normally distributed and values were transformed using a logarithm transformation on base 10. After analysis, the transformed data were back-transformed and are, as such, shown in the results. The generalized linear mixed model (PROC GLIMMIX) was used to analyse progesterone concentration depending on conception rates. The statistical model included the fixed effects of group (protocol), day of hormone application (day of blood sampling), and conception rate, and their mutual interactions. The identification number of the cow (ID) was also included in the model with repeated measures during time with the command RANDOM and the RESIDUAL option. The multiple comparison test of least-squares means was performed using the Tukey-Kramer method of correction, with the level of statistical significance at $P<0.05$. When there was a need to specify interactions of some fixed effects, the SLICE option was used. The conception rate (CR) and the conception rate ratio (CRR) between the groups were analysed with the GENMODE procedure with the log link function and binomial distribution.

Results

The conception rates for presynchronized and resynchronized animals diagnosed 30 and 60 days after TAI are shown in Fig. 2.

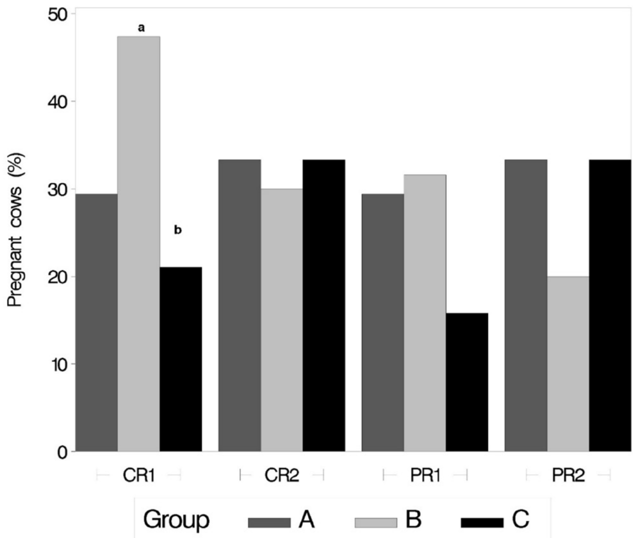

Fig. 2. The percentage of pregnant cows 30 and 60 days after TAI during presynchronization and resynchronization (CR1- conception rate 30 days after TAI in presynchronization; PR1- pregnancy rate 60 days after TAI in presynchronization; CR2- conception rate 30 days after TAI in resynchronization; PR2- pregnancy rate 60 days after TAI in resynchronization; group A was presynchronized with GnRH (day -9) and $PGF_{2\alpha}$ (day -2), group B with $PGF_{2\alpha}$ (day -2), group C served as control, Ovsynch started at Day 0 to all three groups)

[a, b] tendency towards a statistically significant difference (P<0.10)

The results indicate that cows subjected to presynchronization had a better conception rate 30 days after TAI (CR1) compared to cows subjected to Ovsynch only (A 29.41%, B 47.37% and C 21.05%). Although the CR did not differ significantly, group B had a tendency towards a higher CR compared to group C (P<0.10), and, according to CRR, it had a 3.37 times better chance to conceive compared to the control group, and a 2.16 times better chance to conceive than group A. When compared to the control group, group A, however, had a 1.56 times better chance to conceive. The CR after resynchronization (CR2) was fairly equal (33.33% for groups A and C, and 30% for group B). As seen in Fig. 2, cows in group B had the highest embryo mortality rate, followed by group C cows, while in group A there were no such cases. Therefore, final pregnancy rates, established 60 days after TAI in presynchronization (PR1), were 29.41% for group A, 31.58% for group B and 15.79% for group C, while after resynchronization (PR2) the pregnancy rate was 33.33% for groups A and C and 20% for group B. In other words, after presynchronization, group B had a 1.10 times better chance to conceive than group A, and 2.6 times better than group C, while after resynchronization, groups A and C had an equal chance to conceive, and a 2 times better chance to conceive when compared to group B.

Table 1. Progesterone concentration (ng/mL) on all days of the experiment when blood was collected (mean ± SEM)

Group/ day	-9	-2	0	7	9	33	40	42
A	2.77 ± 0.88	2.77 ± 0.71	0.15 ± 0.07[a]	2.38 ± 0.28	0.69 ± 0.37	2.99 ± 0.97	3.84 ± 1.35	0.42 ± 0.24
B	2.10 ± 0.44	3.01 ± 0.76	0.43 ± 0.26[a]	2.99 ± 0.61	0.35 ± 0.10	3.88 ± 0.72	4.46 ± 1.28	0.33 ± 0.09
C	3.15 ± 0.66	2.52 ± 0.68	2.71 ± 0.66[b]	3.27 ± 0.66	0.50 ± 0.22	2.42 ± 0.60	5.17 ± 0.77	1.27 ± 0.65

Group A was presynchronized with GnRH (day -9) and $PGF_{2\alpha}$ (day -2), group B with $PGF_{2\alpha}$ (day -2), group C served as control. Ovsynch started at day 0 in presynchronization and on day 33 in resynchronization to all three groups: GnRH was applied on days 0, 9, 33 and 42, and $PGF_{2\alpha}$ on days 7 and 40. [a, b] values marked with different letters indicate statistically significant difference (P<0.01).

Table 1 shows the average progesterone concentration (ng/mL) on all days when blood was collected. There is a statistically significant difference in progesterone concentrations on day 0 between the presynchronized and the control groups (group A 0.15 ± 0.07; group B 0.43 ± 0.26 and group C 2.71 ± 0.66, respectively; P<0.01).

Tables 2 and 3 show the progesterone concentrations (ng/mL) on days 0, 7 and 9 during presynchronization (Table 2) and on days 33, 40 and 42 in resynchronization (Table 3), shown as least square mean (LSM) and pooled SEM, by protocol used. It can be seen that the progesterone concentrations in presynchronization were mostly higher in animals that failed to conceive in groups A and B, and on day 0 in group C (Table 2).

Table 2. Progesterone concentrations (ng/mL) on days 0, 7 and 9 during synchronization, shown as LSM and pooled SEM

Group	CR	Day			Pooled SEM
		0	7	9	
A	0	0.17[a]	2.65	0.95	0.91
	1	0.09	1.71	0.14	1.40
B	0	0.66	3.92	0.54	0.99
	1	0.18	1.96	0.14	1.05
C	0	2.76[b]	2.89	0.26	0.81
	1	2.54	4.71	1.39	1.57

Group A was presyncronized with GnRH (day -9) and $PGF_{2α}$ (day -2), group B with $PGF_{2α}$ (day -2), group C served as control. All groups started with Ovsynch on day 0 (conception rate, CR; 1- cows that conceived, 0- cows that failed to conceive). [a,b] values marked with different letters indicate statistically significant difference (P<0.05).

In resynchronization, progesterone concentrations were higher in cows that had conceived, with the exception of group B on day 33, group C on day 40, and groups A and C on day 42, when cows that had not conceived had higher progesterone concentrations (Table 3).

Table 3. Progesterone concentration (ng/mL) on days 33, 40 and 42 during resynchronization, shown as LSM and pooled SEM

Group	CR	Day			Pooled SEM
		33	40	42	
A	0	0.20	1.81	0.65	1.70
	1	1.74	6.87	0.19	2.11
B	0	3.38	2.56	0.33	1.97
	1	3.01	6.84	0.35	2.37
C	0	0.46	5.85	1.91	1.36
	1	4.05	3.96	0.14	1.84

Group A was presyncronized with GnRH (day -9) and $PGF_{2α}$ (day -2), group B with $PGF_{2α}$ (day -2), group C served as control. All groups started resynhronization with Ovsynch on day 33; received GnRH on days 33 and 42, and $PGF_{2α}$ on day 40, after pregnancy diagnostics. (conception rate, CR; 1- cows that conceived, 0- cows that failed to conceive).

Discussion

The aim of this paper was to prove that combined synchronization protocols are more effective and therefore advisable in modern dairy farming (GEREŠ et al., 2014). Groups A and B were presynchronized, while group C served as control and was subjected to standard synchronization with Ovsynch. Group A received modified Double Ovsynch (DO), with one GnRH application less than in standard DO, while group B received modified Presynch, with only one $PGF_{2\alpha}$ application. Our results differ from data in literature, since various other authors have reported better CR with presynchronization protocols based on GnRH and $PGF_{2\alpha}$ than those based on $PGF_{2\alpha}$ (YOUSSEFI et al., 2013; STEVENSON and PULLEY, 2012; HERLIHY et al., 2012). According to GALVÃO et al. (2007) shorter combined protocols are more effective than longer ones. Our results confirm this thesis, since group B had higher CR (47.3%) than group A (29.41%), with a tendency towards a statistically significant difference ($P<0.10$). Furthermore, both presynchronized groups had a better CR than the control group (21.05%), thus obviously proving the better efficiency of combined protocols. This is only logical, since combined protocols give an extra opportunity, due to additional hormone application, to more successful homogenization of folliculogenesis (CHEBEL et al., 2006). This is supported by the significant difference in progesterone concentrations on day 0 between both presynchronized groups compared to the control group ($P<0.01$). Conception rate ratio (the 1.56 times better chance of conception in group A and the 3.37 times better chance in group B compared to group C, respectively) indicates that synchronization in the control group was started at a different and inadequate time of the oestrous cycle (CHEBEL et al., 2003; SOUZA et al., 2008).

Special attention has been paid to the effect of progesterone level on conception rates during protocols with TAI in dairy cows. A strong increase in progesterone concentration before AI, during presynchronization treatments, positively affects fertility, while low concentrations dramatically decrease fertility, accompanied with inadequate luteolysis in spite of prostaglandin application. In our research, the progesterone concentration was mostly higher in cows that had not conceived during presynchronization. SOUZA et al. (2008) concluded that, in all protocols based on Ovsynch in dairy cows, fertility is conditioned by primary progesterone concentration before synchronized ovulation and TAI, the ovulation percentage after GnRH and higher progesterone concentrations during the protocol. Subluteal progesterone concentrations (less than 2 ng/mL) are the reason for reduced fertility, because they result in the prolonged life of the dominant follicle, thus making it less able to ovulate, presumably due to the hormonal environment that favours its persistency (BRIDGES and FORTUNE, 2003). This results in a prolonged time of increased pulsation but more difficult onset of LH peak (KOJIMA et al., 2003). Therefore, this persistent follicle contains an old oocyte, which results in decreased conception rates and/or higher embryonic mortality rate (MIHM et al., 1999). We presume this is the reason for the initially high CR in group B, followed by the highest percentage of pregnancy loss, which was 33% in the presynchronization part in group B, while groups A and C had either no embryonic mortality (group A) or it was lower (25% in group C). Regarding protocol B, we must emphasize that it was not assumed that it would be successful, and our results differ somewhat from data in the literature, since other authors have reported better CR with presynchronization protocols based on GnRH and $PGF_{2α}$ than those based on $PGF_{2α}$ (YOUSSEFI et al., 2013; STEVENSON and PULLEY, 2012). These PGF based protocols can only be effective when there is a functional CL on the ovaries at the time of prostaglandin application (RIBEIRO et al., 2012). However, a single $PGF_{2α}$ application concurrent with TAI can result in better CR, especially in cows at earlier stages of lactation (AMBROSE et al., 2015).

Our results in resynchronization show rather uniform success, with CR being around 30%, which is in accordance with CHEBEL et al. (2003) and BARTOLOME et al. (2005a, 2005b, 2005c), and suggests that the presynchronization method has no effect on the pregnancy rate from subsequent insemination in resynchronization (HERLIHY et al., 2012). The negative side of this resynchronization is the blind treatment of all cows with GnRH 23 days after TAI. The other way is to treat only non-pregnant cows after early pregnancy diagnoses 27 - 28 days after TAI. Cows that have failed to conceive receive either prostaglandin (and are artificially inseminated at detected heat) or are subjected to presynchronization with Ovsynch, with TAI ending on day 49. This system is better because only non-pregnant cows are treated, which is a safer and more economical method (BARTOLOME et al., 2005b), although the period between the two inseminations is somewhat longer. GnRH application 23 days after TAI in cows of unknown pregnancy status results in good conception rates. Starting resynchronization with Ovsynch 23 days after the previous TAI and before pregnancy diagnosis does not affect the fertility of cows in lactation, and shortens the interval to the next insemination (FRICKE et al., 2003).

Conclusion

There is no doubt that the protocols based on Ovsynch (simple and combined) represent a major step in understanding and managing reproduction. The two combined synchronization protocols used in this research are original and had not been used on dairy cow farms before. Therefore, they are not found in recent literature. The achieved CRs are higher than in cows synchronized with Ovsynch only, indicating the need for presynchronization. In resynchronization, CRs were rather uniform. Progesterone concentrations tended to be higher in cows that did not conceive, and differed significantly between the presynchronized and the control groups at the start of Ovsynch in synchronization (Day 0), indicating that combined protocols are more effective in homogenization of folliculogenesis and synchronization of ovulation. Due to the results obtained, their application should be evaluated further on a larger sample of cows.

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Received: 13 April 2016
Accepted: 9 December 2016

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SAŽETAK

Ovsynch je sinkronizacijski protokol koji se rutinski koristi u poslijeporođajnim sinkronizacijskim i resinkronizacijskim postupcima u mliječnih krava. S obzirom na to da se najbolji uspjeh koncepcije postiže započinjanjem protokola u lutealnoj fazi, optimalno vrijeme za početak sinkronizacije postiže se primjenom presinkronizacijskih protokola. Cilj ovog rada bio je usporediti dva presinkronizacijska protokola međusobno i s Ovsynchom u sinkronizaciji i resinkronizaciji. Istraživanje je provedeno na farmi mliječnih krava u Hrvatskoj, na 60 primiparih krava holštajnsko-frizijske pasmine, podijeljenih u tri jednake skupine. Skupina A je bila presinkronizirana s GnRH i $PGF_{2\alpha}$ devet (dan -9) i dva dana (dan -2) prije početka Ovsyncha (modificirani Double Ovsynch protokol); skupina B s jednokratnom aplikacijom $PGF_{2\alpha}$ dva dana (dan -2) prije Ovsyncha (modificirani Presynch), dok je skupina C služila kao kontrolna i sinkronizirana je samo primjenom Ovsyncha, koji je u sve tri skupine počeo na dan 0. Sve su krave bile umjetno osjemenjene u određeno vrijeme. Dvadeset tri dana po UO sve su krave primile GnRH, a sedam dana poslije ultrazvučno im je dijagnosticirana gravidnost. Krave koje nisu bile gravidne potom su dobile $PGF_{2\alpha}$ i dva dana kasnije GnRH te su ponovno bile osjemenjene. Svim kravama su na dane primjene hormona uzeti uzorci krvi za analizu progesterona. Rezultati pokazuju kako su krave iz presinkroniziranih skupina imale bolji postotak koncepcije u usporedbi s kontrolnom skupinom (A 29,41 %, B 47,37 % i C 21,05 %). U resinkronizaciji postotak gravidnih životinja bio je ujednačen (oko 30 %). U krava koje nisu koncipirale koncentracija progesterona je bila viša nego kod krava koje su koncipirale. Nadalje, značajno se razlikovala između presinkroniziranih i kontrolne skupine na dan 0 (P<0,01), ukazujući na to da su složeni sinkronizacijski protokoli uspješniji u homogeniziranju folikulogeneze i sinkronizaciji ovulacije. Oba presinkronizacijska protokola opisana u ovom istraživanju originalna su i do sada nisu rabljena na farmama mliječnih krava. S obzirom na postignute rezultate, njihovu učinkovitost bi bilo dobro istražiti na većem broju krava.

Ključne riječi: mliječne krave, presinkronizacija, resinkronizacija, Ovsynch, postotak gravidnosti, progesteron