



SYNCHRONIZATION OF OVULATION AND TIMED INSEMINATION IN LACTATING DAIRY CATTLE

Moskáľová, L.¹, Pošivák, J.²

¹Department of Microbiology and Immunology; ²Clinic of Ruminants, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 04181, Košice Slovakia

lenka.moskalova@student.uvlf.sk

ABSTRACT

Successful reproduction of lactating dairy cattle, or in other words its reproductive performance is a limiting factor in the profit ratio of cattle breeding regarding market production. The aim of this research was to evaluate the success rate of synchronization protocols Ovsynch, Presynch/Ovsynch, and Double Ovsynch within the period of 24 months. The success rate of the 1st insemination, 2nd insemination, and after more than 2 inseminations was evaluated. The cattle were of Holstein Friesian breed with a milk yield of 10 200 kg. The animals were sorted into two categories – heifers (protocol Ovsynch) and primiparous, multiparous cows (protocols Presynch/Double Ovsynch). The research proved a 54 % success rate in the category of heifers after the 1st insemination. In the category of primiparous and multiparous cows was the success rate of 41 % after the 1st insemination, 39 % after the 2nd insemination, and 52 % after more than 2 inseminations for the Presynch/Ovsynch protocol. The success rate for Double Ovsynch protocol was 45 % after the 1st insemination, 42 % after the 2nd insemination and 51 % after more than 2 inseminations. The results for the given geographic region of Slovakia exceeded the average.

Key words: dairy cow; Double Ovsynch; heifers; Ovsynch; Presynch; reproduction; synchronization protocols; timed insemination

INTRODUCTION

Nowadays, cattle breeding concerns nearly every country in the world and the issue of reproduction belongs to its inseparable parts. Reproduction is considered as the mainstay of production in this area. If there is no reproduction, there is no production either. Cattle farms are influenced by the constant pressure of economic production and the dairy industry. This is one of the reasons why it is important to present the production results; to ensure continuous reproduction. Since the reproductive performance in lactating dairy cows is not optimal, it led to the development of various strategies in the reproductive management industry aimed at improving the use of artificial insemination (AI) and pregnancy rates in dairy herds [14].

Synchronization programmes have become a standard part of current management of dairy cows in the dairy industry. Many of the synchronization protocols also allow timed insemination (TAI) to avoid the practical difficulties associated with oestrus detection [8].

Other factors, such as weather, season, nutrition, temperature, climate, and the management of the farm itself, along with reproductive indicators, affect the reproduction and functioning of cattle farms. The use of timed insemination after synchronization of ovulation has become one of the most widely used reproductive technologies developed in the last 40 years. Nowadays, different variants of this technology are in use all over the world, especially in milk production [16]. Artificial insemination is also important for the genetic improvement, especially in dairy cattle breeds [18].

According to global statistics, the fertility of Holstein cattle has been gradually decreasing each year by 0.5 % in the last four to five decades with a current increase in productivity due to breeding by 1 % or more [3].

At present many synchronization protocols and their variations, as well as modifications with subsequent artificial insemination are very popular. Many of them are based on the use of gonadotropin hormone and prostaglandin and their functions. The oldest used synchronization protocol is the Ovsynch protocol and its subsequent variations. Ovsynch allows artificial insemination (AI) to be performed at the exact optimal time without checking the ovaries and uterus. The use of these protocols in reproduction allows efficient synchronization of oestrus cycles and subsequent insemination of dairy cows without oestrus detection, which is time-consuming and difficult to perform for farms with many cattle. It is for this reason that Ovsynch became the first tool for the application of AI and an alternative to the detection of oestrus in dairy cattle [10].

The female's natural cycle is physiologically influenced by sex hormones and their secretion is controlled by the central nervous system. The main role in controlling the sex hormones is the hypothalamus – pituitary axis. The hypothalamus sends the GnRH hormone to the pituitary gland to release FSH and LH. These subsequently act on the ovaries, which produce their own hormones, such as oestrogen and progesterone. These hormones have a feedback effect on the hypothalamus. In this way, the regular sexual cycle of females is ensured. The length of oestrous cycle in female cattle usually lasts 17–24 days (on average 21 days). Day 0 in the oestrous cycle is the day when oestrus and other specific behaviour such as vocalization, displeasure, and mounting herd mates begin to manifest itself [15]. Heifers often have a shorter oestrus than dairy

cows. Irregular sexual cycles can occur due to: improper postpartum care, the occurrence of endometriosis and pyometra, functional disorder of ovarian activity, such as atrophy, acycilia, ovarian cysts and others.

It can be concluded that the number of cattle in the territory of the Slovak Republic is decreasing every year. In 2020, according to DATAcube there were 442,289 cattle of which 122,049 were dairy cows, compared to 2010, when the number of individuals was 467,125 of which 159,260 were dairy cows. Despite the decreasing trends in cattle breeding, the overall percentage of pregnancy after the first insemination hardly changes over the years. According to Burdych, Kocmánek et al. [1], pregnancy after the 1st insemination in Holstein cattle is around 37.7 % by 2020 compared to 2010, when it was 35.3 %. There was also a significant improvement in milk production in Holstein cattle. According to Burdych, Kocmánek et al. [1] the average presents 10,254 kg of milk in 305 days, while in 2010 it was 8,912 kg of milk in 305 days.

The purpose of this study was to evaluate the success of chosen protocols in a period of 24 months. The Ovsynch, Double Ovsynch and Presynch protocols were used in certain categories of farm animals.

MATERIALS AND METHODS

Farm and animals

In this study Holstein cattle and their crossbreeds were evaluated. The herd is currently undergoing Holsteinization and in 2020 consisted of 482 dairy cows and 173 heifers. In 2021 the number of dairy cows was 479 and the number of heifers represented 162 animals.

A farm chosen for study was located in central Slovakia, housed animals of the Holstein cattle breed and their crossbreeds with the Montbéliarde breed. In 2020, there were 65.5 % crossbreeds on the farm, but their share of the Holstein breed ranged from 93.74 to 50 %. In 2021, the percentage of crossbreeds on the farm, with a share of 93.74 % to 50 % Holstein breed, was 61.6 %. The animals were divided into two groups: heifers after the first insemination and dairy cows after the first or more pregnancies. The results were evaluated by the Breeding Service of the Slovak Republic and collected through the breeding information system PLIS. Artificial insemination was carried

out with insemination doses with a minimum concentration of 10 million viable spermatozoa. These insemination doses were subjects of andrological examinations. Peyteted insemination doses were supplied by Slovak Biological Services and Insemas a.s. In 2020, insemination doses were used from bulls with the following codes, BKM – 7, BKM – 23, DIN – 5, DIN – 2, FDE – 1, HGE – 1, MGL – 11, MGL – 14, MGL – 48, SRE – 6, SRE – 10, SRE – 11. In 2021, the following codes were used: BKM – 28, BKM – 29, MGL – 11, MGL – 48, MGL – 50, MGL – 83, SRE – 10, SRE – 31, SRE – 57. Insemination doses came from black Holstein bulls and only certain doses came from sex-sorted semen.

Synchronization protocols

Heifers

The Ovsynch protocol was performed on a group of heifers. This protocol consisted of administration of GnRH (gonadotrophin releasing hormone) on day 0, followed by administration of PGF2 α (prostaglandin F2 alpha) 7 days later. GnRH was again administered after 48 hours, and timed artificial insemination took place after 12–18 hours (Fig. 1). Pregnancy control was carried out on the 32nd day after insemination by USG examination.

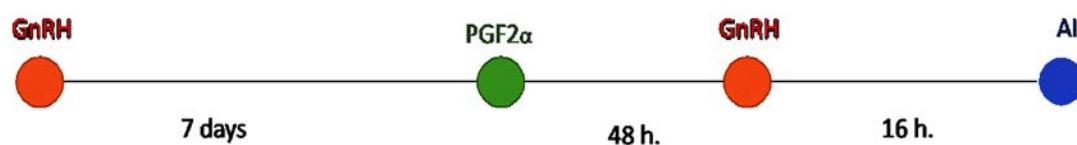


Fig. 1. Timeline for the Ovsynch synchronization protocol
Source: custom image

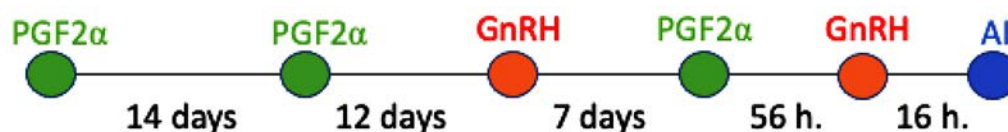


Fig. 2. Timeline for the Presynch/Ovsynch protocol
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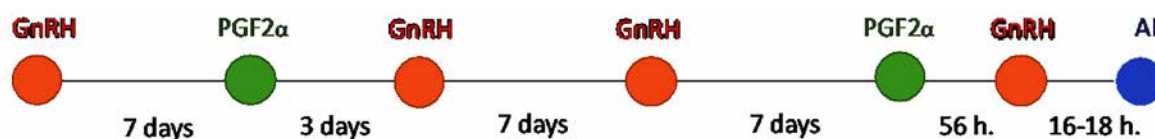


Fig. 3. Timeline for Double Ovsynch protocol
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Primiparous and multiparous cows

For the second category of primiparous and multiparous cows Presynch/Ovsynch (PO) protocol was used. The Presynch/Ovsynch synchronization protocol consisted of synchronization that started on day 0. Preparation based on PGF2 α was administered and its application was repeated after 14 days. After 12 days, a preparation based on GnRH was applied and after 7 days of its administration, the preparation with PGF2 α was administered again and after 56 hours GnRH was applied (Fig. 2). At the end, a timed artificial insemination took place 16 hours after the last application of the hormonal preparation.

When the farm transitioned to the Double Ovsynch (DO) (October 2021) synchronization protocol, the preparations were applied as follows: on day 0, the GnRH-based preparation was applied to the dairy cows and after 7 days followed the application of the PGF2 α preparation. GnRH preparation was applied 3 days later, and the process was repeated in 7 days. After the following 7 days, the PGF2 α preparation was administered. The next application of GnRH was in 56 hours and timed artificial insemination took place approximately 16 hours later (Fig. 3)

The PGF2 α -based preparations with the trade name Cyclicx 250 micrograms/ml inj. (Virbac) were adminis-

tered to the animals without distinction with the active substance *cloprostenolum natricum* administered in the amount of 2 ml per animal and Enzaprost T (Ceva) with the active substance dinoprostum, administered in the amount of 5 ml per animal. Preparations based on GnRH with the commercial name Ovarelin 50 µg.ml⁻¹ (Ceva), with the active substance gonadorelin was administered in the amount of 2 ml per animal, Acegon 50 50 µg.ml⁻¹ (Zoetis) with the active substance gonadorelin administered in the amount of 2 ml per animal, injection solution for cattle and Gonavet inj. (Veyx) administered in the amount of 2 ml per animal with the active substance gonadorelinum. These preparations were chosen based on availability. In both categories, after the pregnancy confirmation on the 32nd day, a re-check pregnancy test was held in the period between the 60th and 90th day after insemination.

ETHICAL STATEMENT

The results and protocols evaluated in this study were obtained from the investigated farm therefore ethical approval was not required.

STATISTICAL ANALYSIS

Statistical evaluation was performed in the statistical program GraphPad Prism version 3.00 using Fisher's exact test. The results were considered significant if the probability value was higher than 95 % ($P < 0.05$).

RESULTS

In the heifers, the success rate of pregnancy after the 1st insemination was on average 54 % in the experimental period. This period lasted from January 2020 to December 2021, but in some months the synchronization was not performed. The evaluation was in the process for 16 months. In the category of dairy cows after one or more births, we recorded the results of pregnancy success after 1st, 2nd and >2 inseminations. The pregnancy rate success, in the period from January 2020 to September 2021, after the 1st insemination was 41 % for the synchronization protocol Presynch/Ovsynch (PO), after the 2nd insemination on average 39 % and 52 % after 2> inseminations. In the period from October 2021 to December 2021, the success rate after the 1st insemination for the Double Ovsynch (DO) synchronization protocol was 45 %, after the 2nd insemination 42 % on average, and after >2 inseminations on average 51 %. No differences were found between PO and DO synchronization protocols. Nevertheless, better but statistically insignificant results were obtained for the DO synchronization protocol.

The service period for the entire period represented an average of 125 days and an intermediate period of 407 days. The insemination interval in cows after one or more births was on average 70 days. The insemination index for the entire observed period was 1.65 for heifers and 2.0 for cows after the 1st and additional births. The results are summarized in Table 1.

Table 1. Summarization of the results obtained after using different synchronization protocols

	Ovsynch	Presynch/Ovsynch	Double Ovsynch
Pregnancy rate after 1st insemination	54 %	41 %	45 %
Pregnancy rate after 2nd insemination	–	39 %	42 %
Pregnancy rate after > 2 inseminations	–	52 %	51 %
Insemination index	1.65	2	2
Insemination interval	–	71	65

DISCUSSION

The synchronization of ovulation is used almost continuously in milk production today and has become an integral part of daily practice, as well as timed artificial insemination. Synchronization of ovulation includes not only the application of hormonal preparations, but also: a proper and regular management of the herd, control over the overall health of the animals, animal welfare, and the success of the protocols. The administration of hormonal preparations was determined according to the relevant protocol. The administration of the first preparation is referred to as day 0 and it continues according to the composition of the given protocol. However, it should be noted that their application does not solve the causes of low fertility [3].

The purpose of the study was to evaluate the success of chosen protocols in a period of 24 months. The Ovsynch, Double Ovsynch and Presynch protocols were used in certain categories of farm animals. The success rate of conception after the first, second and more than 2 inseminations with simultaneous use of artificial insemination (AI) was evaluated.

Synchronization protocols that allow TAI and minimize visual detection of oestrus must be practical to implement within the daily operation of a dairy farm, otherwise the protocol would fail due to a lack of compliance [4]. The success rate of the Ovsynch protocol applied in the group of heifers was 54 %. However, we must point out that the result during the year could have been affected by heat stress, environment, herd management and many other factors. Thermal stress acts as a negative factor on the health and biological functions of dairy cows and manifests itself in the reduced milk production and reduced reproductive capacity [11]. The Ovsynch protocol is the oldest used synchronization protocol. In addition to its original use for synchronizing ovulation, this protocol is also used as a therapeutic method for many ovarian diseases, silent oestrus and heat stress [10]. According to their results, N a v a n u k r a w et al. [9] reported a pregnancy rate of 37.3 % when using the Ovsynch protocol. P u r s l e y and W i l t b a n k [12] reported that for heifers treated with the Ovsynch protocol, they achieved a pregnancy rate of 35.1 % and Y i l m a z et al. [17] obtained a pregnancy rate of 32.5 %. The use of the Ovsynch protocol seems to be an effective and advantageous method. However, this method has been shown to be more effective for dairy cows

than for heifers [10]. However, the success of the Ovsynch synchronization protocol is closely related to the synchronization of the ovulatory follicle and corpus luteum (CL) [12]. In the herd for the category of heifers, synchronization of ovulation with the following TAI was omitted in some months due to the absence of suitable animals on the farm that could undergo this method. Overall, the pregnancy rate percentage for this category covers a period of 16 months. The Presynch/Ovsynch synchronization protocol was performed in the category of cows after one or more births. The time period for this synchronization protocol was from January 2020 to September 2021. Subsequently, there was a change in the synchronization protocol used by the farm itself. The success rate of the Presynch/Ovsynch protocol in the given period, for a certain category of animals, was 41 % after the 1st insemination. The success rate after the 2nd insemination and the > 2 insemination was 39 % and 52 %, respectively.

The presynchronization protocol has become one of the most widely used protocols to synchronize the oestrous cycle and ovulation for the first AI postpartum in lactating dairy cows. Synchronization may begin in the period between the 30th and 35th day after parturition [3, 6]. A study by K a r a k a y a - B i l e n et al. [7] pointed out the success of the pregnancy rate on the 31st day of the Presynch synchronization protocol at 59.3 %, when using the conventional type of insemination dose, and the pregnancy rate on the 31st day of 35.5 % when using the sex-sorted type of insemination dose. G i o r d a n o et al. [6] recorded a pregnancy rate after AI using PO at a 12-day interval in primiparous dairy cows on day 39 of 44.7 % and in multiparous dairy cows 32.4 %, for a 14-day interval in primiparous dairy cows 38.7 % and multiparous 34.7 % for TAI. C o l a z o et al. [2] recorded a pregnancy rate after AI on day 60 in primiparous animals of 55.8 % and 32.9 % in multiparous animals [2].

Our results of the period October 2021 to December 2021 for the synchronization protocol Double Ovsynch were as follows: 45 % after 1st insemination, 42 % after 2nd insemination and 50 % after >2 inseminations. However, the evaluation of this period is short and therefore the results may be distorted and incomplete. A study by S o u z a et al. [13] reported a pregnancy rate of 49.7 % using the Double Ovsynch protocol with a pregnancy rate of 65.2 % in primiparous animals and 37.5 % in multiparous animals. Double Ovsynch appears to be a better choice for

the treatment of anovulatory conditions [13]. A study by Giordano et al. [5] reported the effectiveness of DO on the 29th day as 38.7% and on the 39th day as 35.3% pregnancy rate.

However, for an objective evaluation of Double Ovsynch, we would need a longer research period to obtain more accurate results.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

CONCLUSIONS

We monitored the use of synchronization protocols in high-yielding dairy cows of the Holstein Friesian breed and their crossbreeds. The results partially approach the global parameters and the results reported after synchronization when synchronization protocols were used. The use of these synchronization protocols requires precision and discipline of the breeder, as this is the only way to achieve the desired effect of synchronizing the sexual cycle, ensuring the desired pregnancy and reproductive process, since the staff and the management of the company is not able to cover the methods and reproductive procedures of classical reproduction in cattle breeding. The tendency to increase the production of dairy cows suggests, that this method is currently highly effective and recommended for practice.

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REFERENCES

1. Burdych, V., Kocmánek, J., et al., 2021: *Reproduction of Cattle* (In Czech). Cooperative for Performance Control, Czech Republic, 194 pp.
2. Colazo, M. G., Ponce-Barajas, P., Ambrose, D. J., 2013: Pregnancy per artificial insemination in lactating dairy cows subjected to 2 different intervals from presynchronization to initiation of Ovsynch protocol. *J. Dairy Sci.*, 96, 12, 7640–7648. DOI: 10.3168/jds.2013-6750.
3. Coufalík, V., 2013: *Current Issues in Cattle Reproduction* (In Czech). Agripint, Olomouc, CR, 184 pp.
4. Fricke, P. M., Caraviello, D. Z., Weigel, K. A., Welle, M. L., 2003: Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. *J. Dairy Sci.*, 86, 3941–3950. DOI: 10.3168/jds.S0022-0302(03)74003-X.
5. Giordano, J. O., Wiltbank, M. C., Guenther, J. N., Pawlisch, R., Bas, S., Cunha, A. P., Fricke, P. M., 2012: Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. *J. Dairy Sci.*, 95, 2, 639–653. DOI: 10.3168/jds.2011-4418.
6. Giordano, J. O., Thomas, M. J., Catucuamba, G., Curler, M. D., Wijma, R., Stangaferro, M. L., Masello, M., 2016: Effect of extending the interval from Presynch to initiation of Ovsynch in a Presynch-Ovsynch protocol on fertility of timed artificial insemination services in lactating dairy cows. *J. Dairy Sci.*, 99, 1 746–757. DOI: 10.3168/jds.2015-10083.
7. Karakaya-Bilen, E., Yilmazbas-Mecitoglu, G., Keskin, A., Guner, B., Serim, E., Santos, J. E. P., Ahmet Gümen, A., 2019: Fertility of lactating dairy cows inseminated with sex-sorted or conventional semen after Ovsynch, Presynch–Ovsynch and Double-Ovsynch protocols. *Reprod. Dom. Anim.*, 54, 2, 309–316. DOI: 10.1111/rda.13363.
8. Macmillan, K. L., 2010: Recent advances in the synchronization of oestrus and ovulation in dairy cows. *J. Reprod. Devel.*, 56, S, S42–S47. DOI: 10.1262/jrd.1056S42.
9. Navanukraw, C., Redmer, D. A., Reynolds, L. P., Kirsch, J. D., Grazul-Bilska, A. T., Fricke, P. M., 2004: A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *J. Dairy Sci.*, 87, 5, 1551–1557.
10. Nowicki, A., Barański, W., Baryczka, A., Janowski, T., 2017: OvSynch protocol and its modifications in the reproduction management of dairy cattle herds, an update. *J. Vet. Res.*, 61, 3, 329–336. DOI: 10.1515/jvetres-2017-0043.
11. Polsky, L., von Keyserlingk, M. A., 2017: Invited review: Effects of heat stress on dairy cattle welfare. *J. Dairy Sci.*, 100, 11, 8645–8657. DOI: 10.3168/jds.2017-12651.
12. Pursley, J. R., Wiltbank, M. C., Stevenson, J. S., Ottobre, J. S., Garverick, H. A., Anderson, L. L., 1997: Pregnant-

- cy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized oestrus. *J. Dairy Sci.*, 80, 2, 295–300. DOI: 10.3168/jds.S0022-0302(97)75937-X.
13. Souza, A. H., Ayres, H., Ferreira, R. M., Wiltbank, M. C., 2008: A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology*, 70, 2, 208–215. DOI: 10.1016/j.theriogenology.2008.03.014.
 14. Thatcher, W. W., Bilby, T. R., Bartolome, J. A., Silvestre, F., Staples, C. R., Santos, J. E., 2006: Strategies for improving fertility in the modern dairy cow. *Theriogenology*, 65, 30–44. DOI: 10.1016/j.theriogenology.2005.10.004.
 15. Williams, G. L., Cardoso, R. C., 2021: Neuroendocrine control of oestrus and ovulation. *Bovine Reprod.*, 269–291. DOI: 10.1002/9781119602484.ch24.
 16. Wiltbank, M. C., Pursley, J. R., 2014: The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology*, 81, 1, 170–185. DOI: 10.1016/j.theriogenology.2013.09.017.
 17. Yilmaz, C., Yilmaz, O., Ucar, M., 2011: Effect of PGF2 α and GnRH injections applied before Ovsynch on pregnancy rates in cows and heifers. *Kafkas Univ. Vet. Fak. Derg.*, 17, 4, 641–644. DOI: 10.9775/kvfd.2011.4638.
 18. Yousuf, M., 2021: Challenges and opportunities of artificial insemination on dairy cattle in Ethiopia. *Res. Horizon*, 1, 2, 47–54. DOI: 10.54518/rh.1.2.2021.47-54.

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