

Állatorvostudományi Egyetem – University of Veterinary Medicine

Some factors affecting synchronization results in dairy cattle

**Az ovulációsinkronizálások eredményét befolyásoló faktorok vizsgálata tejlő
szarvasmarhákban**

Department of Obstetrics and Food Animal Medicine Clinic
University of Veterinary Medicine Budapest

Julie Messel Larsen

Supervisor: Dr. Zoltán Szelényi, Senior Lecturer

2024

Abstract

Estrus synchronisation in high yielding dairy cows is becoming an increasingly important aspect of reproductive management in the dairy industry, showing results of improved fertility and reproductive efficiency. Therefore, we wanted to study these effects and changes in reproduction of the Double-Ovsynch protocol in a large number of animals over three years. Our research took place at two large-scale dairy farms in Hungary with a total of more than 2,542 Holstein-Friesian dairy cows included in the study, recording the data of both reproduction and milk production parameters, before analysing the results. We discovered during our study that the fertility parameters showed significant improvement, such as a shorter calving interval and a significantly high pregnancy rate per AI. Also noteworthy differences were found in the parity of the cows, suggesting a similar fertility in the older multiparous cows when compared with the younger primiparous individuals. This fact is in contradiction to previous studies. However, even though the results of this study showed promising results for dairy operators, this is also likely linked to the high level of close management seen in large farms as well as the cost of treatments. It may therefore not show the same results in smaller operations. Further research and evaluation of such data is needed in the future in order to improve herd level reproduction.

Abstract

Az ivarzás szinkronizálása a magas hozamú tejelő teheneknél egyre fontosabbá válik a tejiparban a reprodukciós menedzsmentben, valamint a termékenység és a reprodukciós hatékonyság javulásának eredményei mutatkoznak. Ezért ezeket a hatásokat és a Double-Ovsynch protokoll reprodukcióra gyakorolt hatásait kívántuk tanulmányozni nagyszámú állatnál három éven keresztül. Vizsgálatunk két magyarországi nagylétszámú tejtermelő gazdaságban zajlott, összesen 2542 holstein-fríz tejelő tehenet vontunk be a vizsgálatba, mind a szaporodási, mind a tejtermelési paraméterek adatait rögzítve, majd az eredményeket elemezve. Vizsgálatunk során megállapítottuk, hogy a termékenységi paraméterek jelentős javulást mutattak, mint például rövidebb két ellés közötti idő és szignifikánsan magasabb vemhesülési arány egy mesterséges termékenyítésre vetítve. A tehenek életkora szerint is említésre méltó különbségeket találtunk, ami arra utal, hogy az idősebb, többpetű tehenek termékenysége hasonló volt a fiatalabb, elsőpetű egyedekhez képest. Ez a tény ellentmond a korábbi vizsgálatoknak. A vizsgálat eredményei ígéretesek a termelők számára, ez azonban valószínűleg összefügg a gazdaságokban tapasztalható magas szintű tejtermeléssel. Ezért előfordulhat, hogy a modell a kisebb üzemekben nem mutat ugyanilyen eredményeket. Az ilyen adatok további kutatására és értékelésére van szükség a jövőben az állomány szintű reprodukció javítása érdekében.

Table of Contents

Abstract	2
Abbreviations	6
1. Introduction and Aim	7
1.1 Introduction	7
1.2 Aim of the study.....	7
2. Literature review	8
2.1 History of estrus synchronization in dairy cattle	8
2.2 Compounds used for the synchronization of cattle	9
2.2.1 Progesterone/progestogens	9
2.2.2. PGF2-alpha	10
2.2.3. GnRH.....	11
2.3 How to improve reproductive efficacy with estrus synchronization.....	12
2.3.1 Reduces the need of manual estrus detection	12
2.3.2 Improves pregnancy and calving rates.....	14
2.3.3 Improves the success of timed AI	15
2.4 Financial benefits of synchronization	16
2.5 Synchronization used in embryo transfer.....	17
2.6 Current synchronization protocols	18
2.6.1 Ovsynch.....	19
2.6.2 Presynch-Ovsynch.....	20
2.6.3 Double-Ovsynch	21
2.6.4 Double PGF2-alpha during Ovsynch.....	22
2.6.5 Progesterone-based synchronization protocols.....	23
3. Materials and methods.....	25
3.1 Description of Experimental Farms.....	25
3.2. Synchronization methods	25
3.2.1 Experimental Animals	25
3.2.2 Protocol details	26
3.3. Pregnancy Diagnosis.....	26
3.4 Data Collection	27
3.5. Data Analysis	27
4. Results.....	28
4.1 Number and description of animals in the farms	28
4.2 Results of Double-OvSynch protocols in the different farms	29
4.3 Some factors providing effect on pregnancy rates in the Double-Ovsynch protocol	30

5. Discussion	32
5.1 Ovulation synchronization results in two Hungarian high yielding dairy farms.....	32
5.2 Factors affecting the successful fertilization of the first synchronized ovulation	33
6. Summary	35
7. References	36
8. Acknowledgements.....	41

Abbreviations

AI: artificial insemination

LH: luteinising hormone

FSH: follicle stimulating hormone

PGF2-alpha: prostaglandin F2-alpha

GnRH: gonadotropin releasing hormone

CL: corpus luteum

ET: embryo transfer

1. Introduction and Aim

1.1 Introduction

The reproduction of high producing dairy cattle and its efficiency is one of the most pressing problems within the dairy industry today. With the modern dairy cows producing such high amounts of milk for the need and consumption of humans, amongst other factors such as the environment etc., their reproductive performance is stagnating. With a medium level fertility and conception rate of high yielding dairy cows, difficulties and delays in birthing calves as well as starting the high milk production is heavily influencing the income and profit of dairy farmers. Estrus synchronization was developed several decades ago to increase reproductive efficiency and to theoretically solve these problems, making dairy cows more fertile and physiologically ready for a successful conception upon insemination. However, the results of these decades of hormonal synchronization has left us with a somewhat unclear definite solution. Despite huge amounts of research in the field of estrus synchronization in dairy cows, we wanted to figure out how such a protocol can affect the fertility and possibly other aspects of the production in such high production farms.

In this paper, we performed a study in two Hungarian high-yielding dairy farms using the Double-Ovsynch protocol in order to figure out how the fertility parameters in such farms would be affected when such an estrus synchronization protocol is being used.

1.2 Aim of the study

The aim of our study was to collect and analyze a large amount of data in two Hungarian large scale dairy farms on reproductive performance. We collected data regarding the application of the Double-Ovsynch protocol as a method for synchronizing the first ovulation and to achieve acceptable numbers in the first service conception rate parameter, which is a basic key performance index in the management of cattle herds.

2. Literature review

2.1 History of estrus synchronization in dairy cattle

A good reproductive performance in cattle can be considered as one of the most important aspects of the cattle industry in order to make profit. This is especially true for dairy cows to produce the optimal quantity of milk and other dairy products, which is the main source of income for dairy farmers. Calves born are also an important income as they can be used as future replacement dairy cows, or sold to other businesses[1]. The history of estrus synchronization in cattle goes back to the very understanding of the hormonal and physiological aspects and effects of the estrus cycle itself. Firstly, the significant discovery and understanding of using artificial insemination (AI), in order to improve the efficiency of breeding as well as the enhanced genetic variability, led to a need for a deeper knowledge of the female estrus cycle in order to find the optimal time for insemination[2, 3]. This eventually led to further research into different aspects of the normal estrus cycle, in addition to the hormonal effects and aspects concerning the function of the corpus luteum during the 1950s to the 1980s. More importantly, detailed research started to focus on progesterone and gonadotropins and their use in synchronizing the estrus cycles of different animals in the 1940s to the 1960s[4]. This laid the groundwork and established the foundation of estrus synchronization protocols, leading the way to further development in producing cost-efficient synchronization products in the future. Such products first became available in 1967 as the predecessor of today's commercially available synchronization products[4].

The interest of estrus synchronization started in cattle with the introduction of artificial insemination (AI) at the end of the 18th century in Russia. Later in Denmark, the first corporate artificial breeding institution was introduced in 1936[5]. The development and practice of AI quickly spread worldwide as more research was published concerning its benefits regarding genetic variability, reproductive efficiency, prevention of venereal diseases and better control of the quality and quantity of the semen before insemination. This also sparked further interest in improving the estrus detection in female animals in connection with a timed AI to further increase the fertilization and pregnancy rates by inseminating at the correct time of estrus according to the visual detection of estrus signs[3]. This was in addition to other significant breakthroughs in new understanding of the hormonal background involved in the estrus cycle during the same time period. As a result of this, intensive research was put into the hormonal regulation of estrus, which continued during

the next several decades[6]. The discovery and isolation of the gonadal and pituitary hormones in the 1930s and 1940s led to huge strides in the field, including further isolation of hormones such as estrogen[3, 7], progesterone[3, 8], luteinising hormone (LH) and follicle stimulating hormone (FSH)[3, 9]. One of the first examples of early estrus synchronization in cattle was in 1952, where progesterone was administered in dairy cows in order to find out the effect of the ovarian hormone on ovulation, the release of LH and the physiologic role of such ovarian hormones in female animals[10]. Later on, the discoveries and developmental research into further reproductive hormones such as prostaglandin F₂ α (PGF₂-alpha) and the hypothalamic peptide gonadotropin-releasing hormone (GnRH), leading to the introduction of commercial products for synchronization and control of estrus and ovulation in the 1970s[3]. The use of intravaginal progesterone products as a way to inhibit estrus cycle, in combination with GnRH injections to control release of LH and the time of ovulation, was introduced as new technologies in the field of reproduction to control the estrous cycle and ovulation time.[11]. As the cattle and dairy industry has become more and more industrialized and intensified in the modern age, various synchronization protocols have been developed and are in use worldwide today, often combined with timed AI programs[3].

2.2 Compounds used for the synchronization of cattle

Various veterinary products are used in the estrus synchronization programs in dairy cattle to result in a timed estrus in correlation with a timed insemination procedure. In this section of this paper, a brief explanation of the most commonly used compounds for synchronization, mainly in dairy cows, will be presented individually in more detail.

2.2.1 Progesterone/progestogens

As mentioned earlier, progesterone has been used as one of the earliest synchronization methods in cattle, dating all the way back to the 1940s[3, 8]. Early on, its effect in delaying estrus and ovulation was demonstrated during its application. However, estrus would be expressed in the animal again approximately 4-5 days later after the application has ceased. Progesterone is a natural hormone present in the body of both female and male animals, and has various roles mainly in the reproductive system concerning normal estrus cycles. In female animals it is mainly produced in the CL after ovulation, as well as in the placenta during pregnancy[12, 13]. Progestogens on the other hand, are different variations of progesterone with various effects in the body, whilst progestins are the synthetic variants of

progesterone[14]. Progesterone can be used as a hormonal agent to reestablish cyclicity in anestrous animals and, to some degree, be used as a part of a synchronization protocol in combination with other agents[15, 16]. As a synchronization tool, progesterone is generally administered intravaginally or by injection, keeping the body progesterone levels high during this time. After the termination of the administration, a synchronized estrus usually follows after a few days. It has also been proven that following progesterone applications, the fertility rate of the cows would significantly decrease, limited to the following timed AI[3, 12]. It has been suggested that one of the reasons for this temporary drop in fertility can be linked to the formation of early follicular development and abnormal luteal growth seen in the cows who were treated[17]. It is generally believed that estrus synchronization including progesterone will help maintain a normally timed estrus and luteal phase in anestrous animals, as well as improve conception rates if compared to animals in unprovoked cycles or control groups[16]. Today there are several different progestogen products on the market, used for various synchronization protocols. One of the most commonly used today is the CIDR (Controlled Internal Drug Release) devices; an intravaginal device which enables slow, long-term release of progesterone over several days, minimizing the need for continuous application and handling of the animals[16, 18]. Other progesterone products used are the melengesterol acetate (MGA) applied via feed or injection, as well as the Syncro-mate-B ear implant[18].

2.2.2. PGF2-alpha

Other early methods which are considered as a synchronization protocol in dairy cows is the use of PGF2-alpha injections to induce luteolysis of the CL, in combination with timed AI[19, 20]. When administered to diestrus cows, the luteolytic effect will cause the animal to enter a new estrus phase within 2-5 days post-injection[21]. PGF2-alpha is a prostaglandin which is produced and excreted in the uterus and has a luteolytic effect[22, 23]. The luteolytic effects causes the regression of the CL, which develops after the previous ovulation and the rupture of the dominant follicle at the end of the follicular phase, and this will eventually create the possibility of new follicular growth and a subsequent estrus cycle to commence[23, 24]. Essentially, PGF2-alpha can be administered to induce estrus, and in combination with estrus detection, be inseminated once the signs of estrus were detected. However, the efficacy of the injections are still highly dependent on the manual estrus detection and it did not control the timing of the following AI[25]. Another significant flaw of this method was the significant decrease in conception rates in the cows treated with the

PGF2-alpha injections after a timed AI compared to the cows inseminated after a manually detected estrus[25, 26]. It has also been stated that the effect of the PGF2-alpha injections is variable, depending on where in the estrus cycle the individual cow is when the injection is given. Due to its luteolytic effect, the particular stage of the estrus cycle of the animal at the time of the PGF2-alpha injection will have a big impact on the results of CL regression. This means that the cows in the luteal phase with a present CL will be able to have an effect on the PGF2-alpha and stimulate a new estrus cycle[20, 25, 27]. Cattle in the later stages of the luteal phase will also have a greater response, showing better fertility and stronger estrus after correctly-timed injections compared to the treatment given in the early luteal phase[28]. However, due to this protocol still needing manual estrus detection, although to a less significant degree than before, as well as no controlled AI program and a variable effect, there was still a need for improvement to make the synchronization more reliable and profitable as a generalized synchronization method.

The early synchronization protocols involving the use of PGF2-alpha started to develop after its discovery in the 1970s[21]. Two separate injections would be given to cows in different parts of estrus, as the exact stage of estrus would normally not be known under practical circumstances. The injections would be given 11-14 days apart in order for the highest possible number of cows to undergo CL regression by the time of the second injection. However, as described earlier, the percentage of cows showing estrus signs after the second injection was not satisfactory and therefore not considered an accurate protocol[20, 21, 29]. Today, PGF2-alpha is still partaking in various estrus synchronization protocols in lactating dairy cows, most famously in the Ovsynch protocol, due to its previously mentioned luteolytic effect with the subsequent estrus.

2.2.3. GnRH

GnRH is a peptide produced in the hypothalamus of the brain which causes a pulsatile release of LH and FSH from the anterior pituitary gland during the normal estrus cycle of female animals. It was first linked to the release of LH from the anterior pituitary gland[3, 30, 31]. Later it was demonstrated and documented that the introduction of GnRH will induce the pulsatile secretion of both LH and FSH in the pituitary gland concurrently[3, 21], as well as inducing a high level of LH leading to the ovulation of follicles over a respectable size of more than 10 mm in diameter, a so-called dominant follicle[3, 29]. The stimulated ovulation, caused by the peak of the LH levels, will lead to a new wave of follicular growth produced

by the effects of the FSH. This would result in the development of a new dominant follicle in approximately 7 days[29]. These effects of the GnRH led to the possibilities of a more accurate way of controlling the time of ovulation more precisely than before, as well as the development of new follicles at a more controlled rate. The administration of GnRH would result in ovulation of large dominant follicles present at the time, as well as inducing a new follicular wave to emerge, depending on where in the estrus cycle the individual animal would be at the time of the treatment[20]. When it comes to its use in synchronization protocols, the usage of GnRH together with PGF2-alpha was starting to get thoroughly researched in the 1990s. The combination of these two compounds at strict time intervals proved to be largely efficacious in the successful synchronization as well as in the conception rates, showing promising results[3]. It became evident that the manipulation and stimulation of the follicular growth should be combined with a timed regression of the formed CL to more accurately synchronize ovulation and the time of AI. This discovery in combination with a more profound understanding of the follicular development and its complicated physiology is what led to the current modern synchronization protocols we have today[3].

2.3 How to improve reproductive efficacy with estrus synchronization

The reproductive efficiency in dairy cows is extremely important in order to be able to run an economically successful and profitable business within the dairy industry. For the milk production to be consistent throughout the year and produced in acceptable amounts, the pregnancy and successful calving rates of the herd will need to be relatively high to be able to maintain a profitable production and more or less continuous milk production. In the dairy industry, there are several factors which cause problems and reduce reproductive efficacy, such as poorly expressed estrus, anestrus, low conception rates, poor health status and body condition, early and late embryonic death, heat stress and infectious diseases[32–34]. These factors will all have a negative impact on the cows ability to have a successful pregnancy and deliver a viable calf. Synchronization programmes are useful in the way that it eliminates the need for manual estrus detection before breeding and reduces the labor required by the workers, making the reproduction management more efficient as a whole[35, 36]. Estrus synchronization can therefore be considered as a great alternative to the traditional methods.

2.3.1 Reduces the need of manual estrus detection

Heat detection is one of the most important tasks when it comes to the reproduction of cattle, which can be very challenging and time-consuming using the traditional methods by means

of simple observation[37, 38]. Heat is considered the period of time when female, non-pregnant cows are receptive to being mounted by nearby bulls. Heat takes place in female animals every three weeks, due to their 21 day estrus cycle, and will be accompanied by behavioral and physical signs of heat for approximately 12-18 hours[39]. In cattle, there are several behavioral signs indicating that the female animal is in heat and ready for a fertile estrus. The term “standing heat” indicates the immobilization of the cow during mounting, and accepting the bull’s attempt to mount her without objection for a limited time during the estrus cycle[40]. The “standing heat” sign of estrus is often considered to be the most reliable visual sign in comparison with the rest, which are more variable in accordance with the intensity of the individual hormonal levels of the female animals[41, 42]. Other significant behavior signs of heat in cows include restlessness and increased physical motility, rubbing, the flehmen response and mounting other animals as well as being mounted without immobilization[40]. Changes in and around the vulva can also be observed as reddening and swelling, together with behaviors like sniffing other female animals' vulva and urine[43]. These behavioral signs, in addition to several others depending on the individual cow, should be observed by regular visual checks by the farmers or workers. During the visual inspection as part of the heat detection, these heat signs are what is of importance to be able to tell which animals are ready for insemination or breeding within the next several hours. The quality of the observation in terms of successful heat detection depends mainly on the farmer's experience and motivation to invest the amount of time needed to be reasonably accurate enough for a successful insemination at the correct timing in the estrus cycle[40]. This clearly leaves a lot of room for managerial mistakes and missing estrus signs, as visual observation during all hours of the day and night generally is not possible for the average farmer. In addition to it being a somewhat inaccurate method to detect estrus, simple observation is no longer feasible with the developing intensity of the modern dairy industry with an increasing number of animals per holding, inevitably increasing the inaccurate estrus detection even further.

In order to make the estrus detection more successful and manageable for the farmers, hormonal estrus synchronization protocols can be introduced in combination with timed AI, making the need of such time-consuming observation no longer needed and greatly improving the reproductive efficiency in the production[37]. Estrus synchronization protocols can therefore be used as a great alternative to the manual estrus detection in dairy herds, saving costs of labor and intense observation to detect the signs of heat in many

different animals in various stages of estrus[19]. This is one of the main reasons why synchronization is considered so valuable for the farmers and the dairy industry as a whole. It is also worth mentioning the fact that a considerable number of cows in a dairy herd will not show any signs of heat at all, a so-called “silent heat”, resulting in an increase in days open and economical losses[19, 44]. It would be near impossible to estimate the time of insemination in such cows, but by means of estrus synchronization this would be a much less significant problem.

2.3.2 Improves pregnancy and calving rates

Another big issue regarding the reproductive efficacy in cattle, especially considering dairy cattle, is the generally low reproductive efficiency of the cows. In the dairy industry, the production is highly dependent on the cows ability to become pregnant relatively fast after the previous calving to maintain a more or less stable milk production throughout the year[45]. If a cow repeatedly fails to become pregnant after repeated inseminations, fails to conceive, maintain the pregnancy or deliver viable calves, it will be considered of little production value and be culled based on such reproductive performance, which is a huge loss to the farmer as well[46]. Therefore, optimizing the conditions for a successful pregnancy and delivery by improving reproductive efficiency is highly valuable and crucial in a successful business.

Reproductive efficiency is considered to be a measurement of the ability for a cow to produce viable offspring[47]. Another measure is the calving interval, meaning the time period between two successive calvings in the same individual cow[47]. An optimal calving interval is usually aimed at approximately 365 day interval between the successive calvings, considering the cow will be pregnant for approx. 9 months and with no more than 80-85 days open[47]. However, the trend in dairy cows today is a generally declining reproductive efficiency due to numerous challenges within the industry[1]. Synchronization protocols allow for a relatively precise onset of estrus at the time of AI, which in return increases the fertility rates and chances for a successful pregnancy[34]. After parturition, it is important for the cows to return to estrus for AI as soon as reasonably possible in order to maintain the optimal calving interval. Maintaining the physiological cyclic ovarian activity is essential also for a sustainable calving-to-conception interval, making sure that the whole process is financially beneficial for the farmers as well[48, 49]. Therefore, estrus synchronization can ensure a higher calving interval by reducing the time of the onset of estrus after parturition,

increasing the fertility rates and improving the chances of pregnancy. This results in an improved reproductive efficiency overall when the synchronization protocols are performed correctly and with desirable results.

When focusing on the effects of the calving interval, sub-optimal calving intervals can have large consequences for the profitability and economic success of the dairy production[19]. Repercussions such as reduced milk yield produced daily by the herd, higher number of culled animals and a reduced number of replacement heifers born, leads to significant economical losses for the producers[19, 50, 51]. More about the financial aspects when it comes to synchronization protocols will be discussed in detail in a later segment.

Another important aspect of estrus synchronization when it comes to the reproductive efficiency of the cows is that it allows for a shorter breeding and calving period. As the cow has a known estrus period of 21 days, it would take as much as 63 days for three separate breeding opportunities to be completed when the cows are in their natural cycles. This is opposed to a much shorter period of 45 days for the same three breeding sessions when the cows have been previously synchronized[52]. A shortened breeding season will significantly reduce labor costs for the workers, as well as shorten the interval between the previous calving and the following one, resulting in an overall improved and optimal calving interval. In addition to this, cows would be inseminated in groups at a predetermined time, in contrast with cows inseminated upon signs of heat individually and more spread out according to their cycles[52]. This therefore benefits the workers with a much more efficient breeding period, even if the estrus detection is still necessary as the animals are grouped according to an approximate stage in estrus[19].

2.3.3 Improves the success of timed AI

Dairy cows in the physiological state of lactation have been associated with a considerably lower rate of reproduction and fertility compared to heifers, due to their excessive milk production as a result of the modern intensified dairy industry[32, 53]. By the use of various estrus synchronization protocols, we have the opportunity to improve the general fertility of lactating dairy cows back to a more normal level[32]. Synchronization protocols used today have truly improved the timed AI strategy to optimize reproductive efficiency, by reducing the number of inseminations needed for a successful conception and fertilization. Therefore, the time between calving and conception of a new pregnancy will be considerably shorter,

meaning the calving interval will be kept at the optimal level when this procedure is successful. It has also been reported that the pregnancy rates of the first insemination postpartum (after the previous pregnancy and calving) using timed AI with synchronization were higher than in case of the following inseminations[54]. The process of controlling the time of follicular development and ovulation as well as the regression of the CL with relatively precise accuracy is what allows the timed AI to be more efficient[32].

It has been stated in previous research that the time for the first AI after pregnancy is considerably shorter in cows synchronized than in cows with a natural estrus cycle. This is also true regarding the second and third insemination, where the AI could be performed even 30 days earlier in the cows which have been pharmaceutically synchronized[25]. This clearly will lead to a positive effect on the reproduction management in making the calving interval shorter, which evidently leads to a shorter time period until the next pregnancy and lactation, compared to a much later successful insemination of cows in their natural estrus cycle. This ensures an efficient and time- and labor-reducing reproductive management which is highly beneficial for the farmers, and in the end a more profitable business overall for producers.

2.4 Financial benefits of synchronization

When considering the reproduction efficacy and management of any dairy farm, the economical aspects and financial benefits cannot be ignored as this is one of the main focus points for any legitimate business. The implementation of the estrus synchronization with timed AI protocols have been reported to greatly improve the reproductive performance of the herd, which inevitably leads to economic growth and a greater source of income. This is especially important in herds where the estrus detection methods are proving to have a low accuracy[55, 56]. Although there are undeniable costs involved with the application of such synchronization programs, considering the costs of the pharmaceutical products used as well as the veterinary bills, the value of these costs when compared to the successful application and outcome can in most cases be overlooked. In a study conducted by Giordano *et al.*[57], the results of comparing two synchronization programs to the AI with estrus detection, revealed that the overall cost of the programs per cow per year were significantly less compared to the overall increase in income per cow per year, including an increase of the pregnancy rates of the first and subsequent inseminations[55, 57]. When considering these improvements in the herd reproduction, it is evident that these protocols are more profitable to the business financially, as it is more economically fruitful to increase the number of

successful pregnancies than the expenses used on the commercial products which ensures such results[55].

A reduced reproductive efficiency in a herd will have significant economical consequences for the affected producers. The main losses associated with this are increased cost of semen, higher levels of reproductive management needed such as labor, veterinary investigations, treatments and frequent heat detection[58]. As synchronization programs are aimed at improving reproductive efficiency, these costs will be markedly reduced as a result. The reduction of costs when using estrus synchronization programs in a dairy herd mainly come from the decreasing number of days open, as well as fewer cullings of cows considered infertile after a period of unsuccessful inseminations[56, 58, 59]. It is important to consider the improved results of the production in addition to the overall cost of the synchronization program if we want to find out the benefits compared to natural estrus cycles. The desired benefits of the synchronization programs are the rise in the number of pregnant cows within the appropriate amount of time, whilst the costs include the drugs and treatments, veterinary assistance and number of AI sessions needed for the confirmed pregnancy[56]. For a synchronization program to be considered useful and profitable for the farmers, it is necessary that the benefits outweigh the costs of the procedure itself. Therefore, the results of such programs should ensure a high response rate to the treatments in various stages of the estrus cycle, a predictable synchrony of the treated animals, normal fertility and return to estrus after, in case of repeated procedures[19]. This would ensure positive results and eventually lead to an increased profit. The final financial gain of synchronization protocols are usually not obvious in an immediate fashion after the cows become pregnant, but become obvious after a more long-term application technique. The increase in income can be seen with the timed subsequent calvings following the succeeding treatments, keeping the animals in earlier lactation for a longer time throughout their lifetime, which is the most productive phase of lactation. This will in return increase the profit of each cow per year on the holding, as well as keeping a steady flow of replacement heifers for future production[58].

2.5 Synchronization used in embryo transfer

The use of estrus synchronization has not only made a huge impact on the day-to-day reproductive management of the farmers and the general dairy industry, but it is also considered of great importance when it comes to various modern embryo transfer and manipulation procedures in cattle, as well as in other mammalian species. Embryo transfer

(ET) is the procedure where an embryo or several embryos are retrieved from the reproductive tract of a female donor animal and then transferred into one or more recipient female animals for the remaining pregnancy and delivery. The process of fertilization of the oocyte(s) can happen inside the live animal (*in vivo*) or outside in a controlled environment (*in vitro*)[60]. ET is only one procedure of a long series of actions taken to impregnate the recipient cow and produce a viable calf, and estrus synchronization is one of these prerequisites to make this ET strategy more successful. Estrus synchronization was primarily used to synchronize the recipient females, inducing estrus in the recipient animals to prepare them to receive the fertilized embryos[61]. Synchronizing the recipient animals is beneficial in ET because it does not require estrus detection of the recipient animals, similar to how it is beneficial during normal reproductive management as well. In addition to this, it has been documented that the number of recipients successfully receiving embryos are higher in synchronized recipients[62]. Many ET protocols using various hormones as part of the superovulation were still dependent on the knowledge of the exact day of the individual animal's estrus cycle. This made the use of estrus synchronization, to initiate superovulation, a lot more favorable as the time of the estrus cycle would be a controlled event[63]. In the past, the use of estrus synchronization of the recipients was crucial to ensure there were animals in the appropriate stage of estrus to receive the embryos when it was necessary. After the development of methods to store the embryos frozen, or cryopreservation, the need of synchronized recipients animals were not as dire anymore, as the embryos did not need to be transferred fresh and could be stored and transported over long distances[64]. The modern ET protocols today, provided by commercial embryo transfer companies and its industry, are predominantly using the frozen embryos due to its convenient storage and possibility of long-distance transportation, reducing the need of synchronized recipients[64, 65].

2.6 Current synchronization protocols

The use of hormonal synchronization protocols have increased in recent years around the world, and is today a largely known part of the reproductive management of dairy producers all around the world in varying degrees[66]. The exact percentage of farms and veterinary practices using such synchronization protocols can be hard to estimate, although several studies have conducted surveys to find an approximate estimation of this. In one study in England (Europe) in 2013[67], 20 veterinary practices regularly working with dairy cattle were asked to answer a questionnaire with some questions about their use of hormonal

preparations in their practice and with their dairy clients. The results of this questionnaire was that only 0.6% of the farms they work with did not use any reproductive hormones to promote breeding at all, excluding the farms working under organic conditions where hormonal products are prohibited to be used overall. In another study from the USA (North-America) in 2015[68], more than 70% of dairy cows are treated with hormonal synchronization as well. This shows the importance of the synchronization protocols in the general reproductive management on a large number of farms in various countries, as well as the overall popularity of such products. Due to this growth in the interest of using estrus synchronization protocols as part of the reproductive management within the dairy industry, many different protocols exist today with numerous application methods, all of them with their own advantages and disadvantages.

2.6.1 Ovsynch

The Ovsynch protocol, first performed and published by *Pursley et al.* in 1995[20], was one of the first synchronization protocols implemented for dairy cattle for estrus synchronization to avoid the need of heat detection as well as maintaining acceptable conception rates. The application of the protocol was first applied with an injection of GnRH into the muscle tissue of all the cows and heifers at various stages of their estrus cycles, not previously known and without a previously detected estrus performed by the workers. Seven days after the GnRH injection, PGF2-alpha would then be injected intramuscularly. Lastly, a second GnRH injection would be given 48 hours after the PGF2-alpha, intending to induce ovulation in the animals. To summarize the stages of this protocol more clearly, on day 0, GnRH would be injected. On day 7, PGF2-alpha is injected as well. Lastly, on day 9, the second GnRH injection would be given. The timed AI of the animals would then ideally happen on day 10, within 16-24 hours after the second GnRH injection[20, 37]. The effect of the initial GnRH injection is to stimulate and induce ovulation of the dominant follicle present in various stages in the different animals, which would then produce a CL after ovulation. When the PGF2-alpha is injected seven days later, the CL would ideally be mature enough to respond and regress to its application. This would result in new follicular growth to start in the now synchronized animals at the same time. Lastly, the second GnRH injection on day 9 would ultimately lead to the ovulation of the new dominant follicle in all the synchronized animals, allowing for AI shortly after and within the same time frame as each other[20, 37].

In the original study performed by *Pursley et al.* (1995), it was demonstrated how an increased number of cows can become pregnant when using such a protocol by means of eliminating the need of time-consuming estrus detection, thus increasing the service rate of the number of AI performed[20]. In other words, fewer sessions of AI would be necessary to result in pregnancy when compared to inseminated based on manual estrus detection. However, the pregnancy rates per AI performed did not improve in the synchronized animals when compared to the AI after a detected estrus[69, 70]. This leaves a very significant limitation to the Ovsynch protocol, as the pregnancy rates of the synchronized animal do not improve compared to the control groups. This can be considered a significant downside to the protocol, as it may not be considered worth implementing in the reproductive management to some farmers due to the costs if the efficiency of the protocol is not generally improving.

One big limitation to the Ovsynch protocol is the general need to start the protocol at nearly optimal times in the estrus cycle to be able to influence the success and efficiency of the synchronization[37]. For the synchronization protocol to be successful in a desirable way, the ovaries need to respond to the first GnRH injection by ovulating the dominant follicle, as well as forming a CL ready to be regressed by the PGF2-alpha injection 7 days later. A failure of that first ovulation and the subsequent luteolysis will result in an ultimately failed synchronization, decreasing the effect of the protocol significantly[37]. Due to these challenges with the Ovsynch program, it has been suggested to include thorough gynecological examinations of all heifers and cows before implementing this protocol, or the use of so-called presynchronization protocols before the Ovsynch in order to better regulate the timing of the application[37, 71, 72].

2.6.2 Presynch-Ovsynch

The idea of a pre-synchronization before the initiation of the Ovsynch is one of the modifications made to the original Ovsynch protocol due to some of its original limitations, as mentioned earlier. It had been reported in previous research that the Ovsynch protocol is the most effective at synchronization of estrus if it is started between day 5 and 12 of the estrus cycle, as the optimal time window for its application[72–74]. Due to this discovery, generally two PGF2-alpha injections would be administered before the initiation of the Ovsynch with the first GnRH application. This is to increase the amount of female animals being present in this time window more precisely, to then begin the Ovsynch protocol at a

more optimal time of the estrous cycle and make the synchronization protocol more effective to more animals treated[72]. This pre-synchronization involves the administration of two PGF2-alpha injections applied 14 days apart and 12 days before the initiation of the Ovsynch protocol with GnRH[75]. The pre-synchronization has been reported to have positive effects on the pregnancy rates, especially in cyclic cows, meaning the cows which show normal signs of heat and cyclicity. It has also been seen how this protocol exhibits little to no effect of anestrus cows with physiologically abnormal estrus cycle, due to them being unresponsive to PGF2-alpha[72, 75]. It can therefore be argued that the use of pre-synchronization before the use of Ovsynch should be considered in order to gain more desirable results.

2.6.3 Double-Ovsynch

The Double-Ovsynch protocol, as suggested by its name, is another protocol developed with the intention of improving the reproductive efficiency of the original Ovsynch. As this protocol also uses hormonal products in order to spike the effect of the following Ovsynch, it can be categorized as a pre-synchronization protocol as well. This protocol was officially developed and introduced by Souza *et. al.* in 2008[76], with the idea to use an initial Ovsynch protocol as a pre-synchronization technique, followed by another Ovsynch. At the end of the final Ovsynch protocol, the timed AI should be performed as usual. The initial hypothesis of this research was that the conception rates would be higher after the use of Double-Ovsynch, due to a higher ovulation response to the first GnRH after such a pre-synchronization protocol[76]. This is again due to increasing the number of animals in that optimal time frame of their estrus cycle for the Ovsynch protocol to be more efficient with a higher success rate[76, 77]. As one would expect, the Double-Ovsynch protocol itself is very similar to the original Ovsynch, with just the timing of the injections being slightly different. The protocol starts 16 days before the Ovsynch, thus the first GnRH injection is administered on day -16. This is followed by the PGF2-alpha on day -9, one week later. Lastly, the second GnRH is administered on day -7, two days after the prostaglandin injection. The original Ovsynch can then be initiated on day 0 of the protocol, seven days after the pre-synchronization and following the same procedure as before. At the end the Ovsynch, timed AI will be performed on day 10 as usual[37].

As mentioned before, the idea behind Double-Ovsynch as a pre-synchronization protocol is to optimize the conditions for the Ovsynch protocol to be as efficacious as possible for the timed AI, leading to a higher fertility rate. This can be achieved by making a greater number

of cows respond by ovulating following the first GnRH injection, as well as responding to the subsequent injection of the Ovsynch protocol after pre-synchronization[78]. Increasing the amount of cows ovulating to the timed treatments will make the Ovsynch with timed AI more successful, enhancing the chances of conception and pregnancy of the herd. In the original study [76], where Double-Ovsynch was compared to Presynch-Ovsynch, the fertility rates were considerably higher for both and the highest for Double-Ovsynch. As indicated previously, the Presynch-Ovsynch was considered to provide low fertility in anestrus animals, which can prove to be a significant limitation to that protocol[75, 76]. Unfortunately, the original study on Double-Ovsynch did not categorize the anestrus animals and could therefore not determine if the protocol had positive effects on their fertility. However, this has been revealed in more recent and larger studies, suggesting that Double-Ovsynch can also improve fertility and conception rates of anestrus animals by reestablishing cyclicity of inactive ovaries, in a larger degree than Presynch-Ovsynch[79, 80]. Anestrus animals and other reproductive disorders are a huge financial problem in the high-yielding dairy industry. This results in a lower milk production, as the days between calving and conception increases, causing the interval between days in production to increase as well[80]. It is therefore encouraging that Double-Ovsynch is showing tendencies to improve the fertility and productivity in high-yielding dairy operations, also for the anestrus animals when used as reproductive treatment.

2.6.4 Double PGF2-alpha during Ovsynch

Another modification to the original Ovsynch synchronization protocol is the use of a double PGF2-alpha injection. In this protocol, there is the addition of a second PGF2-alpha injection 24 hours after the PGF2-alpha of the Ovsynch[81]. The reason for this introduction of another injection is to increase the chance of a luteolysis and a complete luteal regression when compared to the one injection of the original protocol, in a greater number of cows than originally[81]. In some individuals, the CL formed after the initial ovulation from the GnRH injection is too young and immature to respond to the following PGF2-alpha, and will therefore not fully regress in the normal Ovsynch protocol[82]. Without a fully regressed corpus luteum, there will be no following follicular wave and development, resulting in a failed estrus synchronization and no longer a possibility of timed AI in accordance with the synchronization protocol[37]. As the Ovsynch protocol is tightly linked to the timed AI, a delay in luteal regression will cause the cow to be in the wrong stage of the cycle for a successful insemination. Therefore, this addition of a second PGF2-alpha will consequently

improve the overall success of the Ovsynch protocol. This is due to the cases of luteal regression in the treated animals increasing, ultimately increasing the pregnancy rates per AI[81]. However, in a study performed by Brusveen *et. al.* in 2009 [83], the addition of a second PGF2-alpha injection showed a very marginal improvement in the fertility of the cows, measured in pregnancies per AI. Nevertheless, this double injection is certainly successful in increasing the luteal regression when compared to the one injection of the Ovsynch protocol[83].

2.6.5 Progesterone-based synchronization protocols

Progesterone and progestin commercial products can also be useful when it comes to estrus synchronization of dairy cows. By administering progestin, either via the feed of the animal or through slow-release insertion devices into the vagina, the animals will demonstrate estrus within a few days after the withdrawal of the treatment[18]. Progesterone insertion devices have been reported to be useful to return the cyclicity of anestrus female animals [16], as well as the return to estrus in postpartum and non-pregnant cows[84]. This can make them very useful for the treatment and management of reproductive problems often seen in the high production dairy cows[15]. This kind of estrus synchronization in such animals can therefore be considered a sort of “resynchronization”, essentially returning the normal function of the ovaries after a time of inactivation[84]. Especially the use of slow-releasing progesterone devices, which are to be inserted into the vagina for a fixed period of time, have gotten more popular in reproductive treatments. These devices, often called progesterone-releasing intravaginal device (PRID) or controlled internal drug release (CIDR), are inserted into the vagina of the desired cows for approx. 7 days before they are removed[85]. The number of days in which the device is kept inside the vagina varies according to different studies. The concept behind slow-releasing progesterone devices is to maintain a stable level of progesterone in the body of the animal, with an initial rapid increase in the levels[85–87]. A high level of progesterone in the body will simultaneously minimize the release of LH from the pituitary gland, resulting in the inhibition of estrus and ovulation. Once the device is removed and the progesterone levels decline, this will consequently stimulate follicular development and a subsequent estrus[86]. However, as mentioned before, it has also been reported how the use of progesterone for synchronization protocols significantly reduces the fertility of the animals, especially when the administration is for 10 days or longer[3, 12, 88], suggesting improved results when used for a shorter period of time[88]. Progesterone together with PGF2-alpha is an example of a

synchronization protocol using CIDR. In one of the original studies on the CIDR devices[88], the use of PGF2-alpha in combination with the progesterone device showed promising results in signs of estrus with the subsequent insemination 1-2 days after the removal. Adding the application of PGF2-alpha is to combine luteinization with synchronized follicular growth, where PGF2-alpha is administered around the time of removal after the CIDR has been present for 7 days[88]. CIDR devices are also combined with other commercial hormones for estrus synchronization, such as GnRH, equine chorionic gonadotropin (eCG) and estradiol[86].

3. Materials and methods

3.1 Description of Experimental Farms

Our study was conducted on two Hungarian Holstein-Friesian dairy farms in Hungary. Both farms housed approximately 1,000 lactating dairy cows each. One of the farms is located in the eastern region of Hungary, while the other is situated in the northern Great Plain region of the country. The study analyzed the results of the estrus synchronization protocol Double-Ovsynch treatments administered on these two farms over the course of three years, between January 1st 2021 and July 7th 2023.

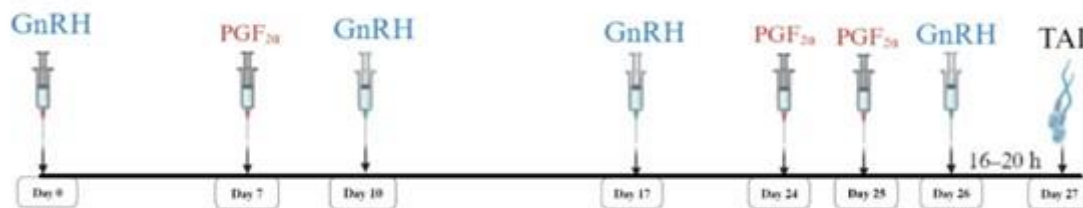
At both farms, the animals were housed in an open housing system with barns equipped with waterbed resting boxes. The barns were climate-controlled and featured misting gates and automated manure scrapers. The barns were also equipped with headlocks at the feeding barriers, where the cows were restrained for treatments and pregnancy checks for this study. Cows were fed a Total Mixed Ration (TMR) appropriate for their production group. On one of the farms, the feed was distributed by a feed delivery robot. On the other farm, a self-propelled feeding cart and a feed push-up robot were used. Cows were milked three times a day on both farms, either in a 2x18 herringbone milking parlor or in a barn equipped with 17 milking robots for the whole herd.

3.2. Synchronization methods

3.2.1 Experimental Animals

Data was collected from the first artificial insemination, from a total of 2,542 cows. All cows were synchronized for ovulation using the Double-Ovsynch presynchronization protocol prior to AI. Cows were enrolled in the program two weeks after a transrectal ultrasound confirmed the end of the involution period, around day 35 postpartum. Cows showing signs of bacterial complications during the ultrasound were excluded from the experiment and only inseminated after treatment. A detailed description of the Double-Ovsynch protocol is provided in Figure 1.

Figure 1 Illustration describing the process of the Double-Ovsynch protocol, which injections were used and on which specific day during the protocol.



3.2.2 Protocol details

On the first day of the protocol, cows received the first GnRH injection (100 micrograms), followed by a PGF_{2α} injection (10 mg) one week later, usually on a Friday. Ten days after the initial GnRH injection, the cows received a second GnRH injection (100 micrograms). Seventeen days after the first GnRH injection, the cows were administered the first GnRH of the Ovsynch protocol, followed by a PGF_{2α} injection one week later. A second PGF_{2α} injection was administered the next day, followed by a third GnRH injection (100 micrograms) on day 26 of the protocol. The cows were inseminated 16-20 hours after the second GnRH injection of the Ovsynch protocol. Cows that exhibited estrus symptoms and returned to estrus were re-inseminated before the pregnancy check. All injections were administered intramuscularly using an 18G needle. During the protocol, gonadorelin (Ovarelin 50 mg/ml, Ceva, France) was used as the GnRH analogue, with a 2 ml dose per injection. Cloprostenol sodium (PGF Vevx, Vevx Pharma, Germany), a PGF_{2α} analogue, was also administered in 2 ml dosages during the protocol. As illustrated in Figure 1, the first GnRH injection of the protocol is considered the starting point of the following injections. It is therefore displayed being administered on day 0 of the protocol, with the succeeding days injections calculated from this first application.

3.3. Pregnancy Diagnosis

Pregnancy checks of the cows included in the protocol were performed 28-41 days after AI using transrectal ultrasound. Two veterinarians conducted the ultrasound exams biweekly throughout the three-year study. The transrectal ultrasounds were performed using a linear transducer (Easi-Scan III, IMV, France). A positive pregnancy diagnosis required the simultaneous confirmation of embryo presence, heartbeat, amniotic fluid, and the presence of a corpus luteum on the ovary, clearly illustrated by the ultrasound machine. In cases of uncertain pregnancy, a follow-up ultrasound was conducted two weeks later, and these cows

were excluded from the study. An additional pregnancy check was performed 57-70 days after AI through transrectal palpation to detect potential pregnancy loss after 41 days.

3.4 Data Collection

The data required for the study were recorded using the Riska (Systo Kft, Hungary) and Afimilk (Afilact, Israel) herd management systems used by the farms and were organized using Microsoft Excel (Microsoft, Redmond, USA). The excel table included the date of the first GnRH injection of the Double-Ovsynch protocol, AI dates, name of the bull used for insemination, the result of the first AI (pregnant or open), the date of the insemination that resulted in pregnancy, the number of inseminations, the date of the pregnancy confirmation, and the voluntary waiting period. Additionally, the date of calving, lactation number, calf sex, and any incidents of stillbirth, dystocia, or twin births were recorded. Other recorded data included milk yield and duration of the previous lactation, the dry period length, the interval between calvings, average daily milk yield in the current lactation, and milk composition (protein, fat, and sugar content). Postpartum diseases were also recorded, including retained placenta, metritis (0-21 days postpartum), endometritis (21-42 days postpartum), lameness (0-42 days postpartum), milk fever, metabolic diseases, and mastitis (1-10 and 0-30 days postpartum). Cows that died or were culled by day 60 postpartum were also recorded.

3.5. Data Analysis

The data were analyzed using the SPSS statistical software (IBM® SPSS®, Version 27, Chicago, USA).

4. Results

4.1 Number and description of animals in the farms

Figure 2 describes the number of first services carried out with the Double-Ovsynch method during the study years on the two different farms included in our study. We collected data from the years 2021, 2022 and 2023, whereas the largest number of data were collected from 2022. Farm F did not initiate the protocol until 2022, which explains why there is no visible data from 2021 from this farm.

Figure 2 Distribution of dairy cows included in this study (n=2542) and synchronized with the Double-Ovsynch protocol, categorized by two farms across 3 years.

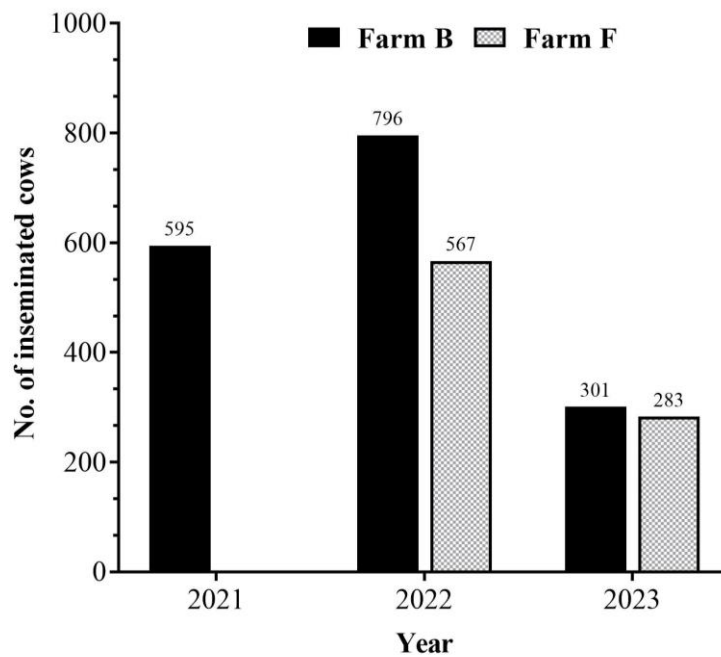


Table 1 describes the lactation performance of the study animals from each farm separately. The lactational production was higher in Farm B with 1300 kgs milk yield. However, both of the farms represent large scale dairy farms and production in Hungary. In accordance with this, the first 100 days milk production was also significantly higher in Farm B. We can also see in this table that the levels of the milk components are much higher in Farm B than in Farm F. As seen below, Table 1 contains all the production data of the two farms in terms of the study animal's milk production. The table shows significant differences between the two farms. However, both farms are in the high milk yield categories, with a total milk production of 11,515 kilograms in Farm B vs. 10,263 kilograms seen in Farm F, recorded by the study population. In accordance with this, the produced total milk solids in the current lactation,

when ovulation synchronization was performed, are also differing significantly between the two farms. Although, the farm with the higher milk production also produced more milk solids during the lactation. There was also a difference seen in the milk yield of the first 100 days, as well as in the milk solid production.

Table 1 Lactation performance of dairy cows synchronized using Double-Ovsynch protocol at the two farms included in the study.

Parameters	Farm		P-value
	Farm B	Farm F	
No. of cows	1692	850	
Milking days (d)	281.0 ± 1.8 ^b	302.2 ± 2.5 ^a	<0.001
Average DMY (Kg)	41.1 ± 0.2 ^a	33.8 ± 0.3 ^b	<0.001
Largest DMY (Kg)	50.9 ± 0.3 ^a	44.0 ± 0.4 ^b	<0.001
Persistence (d)	80.5 ± 0.3 ^a	77.2 ± 0.4 ^b	<0.001
Total MY (Kg)	11515.5 ± 87.2 ^a	10263.2 ± 122.9 ^b	<0.001
Total fat (Kg)	437.3 ± 3.3 ^a	393.6 ± 4.7 ^b	0.003
Total fat (%)	3.82 ± 0.01 ^b	3.89 ± 0.02 ^a	<0.001
Total protein (Kg)	412.0 ± 3.1 ^a	356.1 ± 4.3 ^b	<0.001
Total protein (%)	3.58 ± 0.01 ^a	3.47 ± 0.01 ^b	<0.001
Total lactose yield (Kg)	565.8 ± 4.2 ^a	469.8 ± 5.9 ^b	<0.001
Total lactose (%)	4.93 ± 0.01 ^a	4.59 ± 0.01 ^b	<0.001
SCC (10 ³ cells/mL)	133.8 ± 6.1	136.0 ± 8.6	0.83
D100-MY	4428.5 ± 28.6 ^a	3771.5 ± 40.1 ^b	<0.001
D100-Fat (Kg)	170.4 ± 1.2 ^a	147.3 ± 1.6 ^b	<0.001
D100-Fat (%)	3.81 ± 0.02 ^b	3.96 ± 0.03 ^a	<0.001
D100-Protein (Kg)	152.3 ± 1.0 ^a	125.9 ± 1.3 ^b	<0.001
D100-Protein (%)	3.41 ± 0.01 ^a	3.35 ± 0.02 ^b	0.003

*DMY: daily milk yield; MY: milk yield; SCC: somatic cell count; D100: day 100 of lactation; D305: day 305 of lactation.

4.2 Results of Double-Ovsynch protocols in the different farms

In Table 2, we can see the first service conception rates performed with the Double-Ovsynch protocol in both farms during the period of our study. There was no significant or immediately exciting difference between the two farms, as the data remains relatively similar between them. However, a clear difference was shown in the services per conception parameter. This parameter described the number of inseminations, or services, needed for the cows to finally become pregnant on an average. From this fact alone, the calving to conception (days open) and calving interval parameters also differed significantly when using the same protocol on both farms. To evaluate this point further, we also assessed the culling rate from the study animals in the subsequent lactation, which can also be seen in

Table 2. The culling rate was found to be low and did not differ significantly between the two farms.

Table 2 Fertility parameters of dairy cows synchronized using the Double-Ovsynch protocol at two farms included in the study.

Parameters	Farm		P-value
	Farm B	Farm F	
No. of cows	1692	850	
1 st AI conception (n, %)	763 /1692 (45.1%)	401 /850 (47.2%)	0.32
Total conception (n, %)	1467 /1692 (86.7%)	725 /850 (85.3%)	0.33
Calving rate (n, %)	1353 /1467 (92.2%) ^a	648 /725 (89.5%) ^b	0.03
Pregnancy loss (n, %)	114 /1467 (7.8%) ^b	77 /725 (10.6%) ^a	0.03
Services per conception	1.9 ± 0.04 ^b	2.1 ± 0.05 ^a	<0.001
Calving to 1 st AI (d)	70.1 ± 0.19 ^a	68.2 ± 0.27 ^b	<0.001
Calving to conception (d)	98.2 ± 1.3 ^b	110.9 ± 1.9 ^a	<0.001
Gestational length (d)	277.4 ± 0.15 ^a	276.9 ± 0.22 ^b	0.04
Calving interval (d)	375.6 ± 1.4 ^b	387.2 ± 2.0 ^a	<0.001
Culling rate* (n, %)	439/1692 (25.9%)	214 /850 (25.2%)	0.68

4.3 Some factors providing effect on pregnancy rates in the Double-Ovsynch protocol

In Table 3, we can see the differences in reproductive parameters between primiparous and multiparous cows during the use of the synchronization protocol at the two farms. In this table, primiparous animals is defined as cows which had their first calf, whilst multiparous are cows have had two or more calves in the past. The biggest differences we can see between primiparous and multiparous animals in this study, according to Table 3, are the decrease in the total conception parameter of multiparous animals. This is likely due to the decrease in fertility of multiparous animals and their increased age compared to young primiparous cows. We can also see a clear increase in the culling rate of multiparous cows when compared to primiparous, with an increase of almost 1.7 times higher incidence rate. This might be explained by the increased chance of complications postpartum, especially when the cow has given birth to more calves in the past. Also, the chance of more age and metabolic related problems are higher in multiparous animals. Twinings are also more common in multiparous cows, which can also be a greater risk for the life and health of the cow postpartum and during calving.

Table 3 Fertility parameters of dairy cows synchronized with the Double-Ovsynch protocol influenced by parity.

Parameters	Parity		P-value
	Primiparous	Multiparous	
No. of cows	1174	1368	
1 st AI conception (n, %)	561 /1174 (47.8%)	603 /1368 (44.1%)	0.06
Total conception (n, %)	1060 /1174 (90.3%) ^a	1132 /1368 (82.7%) ^b	<0.001
Calving rate (n, %)	986 /1060 (93.0%) ^a	1015 /1132 (89.7%) ^b	0.005
Pregnancy loss (n, %)	74 /1060 (7.0%) ^b	117 /1132 (10.3%) ^a	0.005
Services per conception	1.92 ± 0.04	1.95 ± 0.04	0.68
Calving to 1 st AI (d)	69.4 ± 0.22	69.5 ± 0.22	0.76
Calving to conception (d)	101.0 ± 1.6	103.4 ± 1.6	0.29
Gestational length (d)	278.0 ± 0.2 ^a	277.5 ± 0.2 ^b	0.05
Calving interval (d)	377.7 ± 1.6	380.9 ± 1.6	0.16
Culling rate* (n, %)	220 /1174 (18.7%) ^b	433 /1368 (31.7%) ^a	<0.001

Table 4 demonstrates the fertility parameters of the two farms together over the course of the three-year period of the protocol, separated into different columns according to the year. An interesting finding in this table is the steady increase in the calving to 1st conception parameter over the three-year period, increasing from 64.5 days in 2021 to 71.6 days in 2023. This means a longer interval between the previous calving until the first AI is performed on the same cow, suggesting that the involution period is increasing over the three-year period.

Table 4 Fertility parameters of dairy cows synchronized using Double-Ovsynch protocol over three different years of the study.

Parameter	year			P-value
	2021	2022	2023	
No. of cows	595	1363	584	
1 st AI conception (n, %)	255 /595 (42.9%)	619 /1363 (45.4%)	290 /584 (49.7%)	0.06
Total conception (n, %)	511 /595 (85.9%)	1176 /1363 (86.3%)	505 /584 (86.5%)	0.09
Calving rate (n, %)	463 /511 (90.6%)	1080 /1176 (91.8%)	458 /505 (90.7%)	0.61
Pregnancy loss (n, %)	48 /511 (9.4%)	96 /1176 (8.2%)	47 /505 (9.3%)	0.61
Services per conception	2.0 ± 0.06 ^a	2.0 ± 0.04 ^a	1.8 ± 0.06 ^b	0.006
Calving to 1 st AI (d)	64.5 ± 0.30 ^c	70.8 ± 0.20 ^b	71.6 ± 0.30 ^a	<0.001
Calving to conception (d)	98.3 ± 2.3 ^a	106.0 ± 1.5 ^b	97.8 ± 2.3 ^a	0.002
Gestational length (d)	277.7 ± 0.25 ^a	276.9 ± 0.17 ^b	277.5 ± 0.26 ^a	0.01
Calving interval (d)	376.0 ± 2.3 ^a	382.6 ± 1.5 ^b	375.4 ± 2.4 ^a	0.009
Culling rate* (n, %)	159 /595 (26.7%)	359 /1363 (26.3%)	135 /584 (23.1%)	0.25

5. Discussion

5.1 Ovulation synchronization results in two Hungarian high yielding dairy farms

The recently developed ovulation synchronization programs are challenging in modern dairy farming. There are several circumstances which should be optimal for the successful application of these programs. On one hand, several injections must be administered in a strict order and within a strict timeframe. Another important factor is the fact that usually more programs or protocols are running at the same time, under practical circumstances. Therefore, strict regulation of these factors are required at farm level when speaking about dairy farming. Another aspect is that these protocols, such as the Double-Ovsynch used in our study, contain 6-7 injections per cow, meaning higher cost compared to the original Ovsynch protocol, where only 3 injections were part of the synchronization. Thirdly, these protocols are designed to synchronize the first insemination, to achieve good results in pregnancy rate and to successfully modify farm level reproductive protocols.

Another aspect is, that due to these aforementioned factors, the application of these protocols are mainly achievable only on well-managed farms. This also means that these farms are typically better in milk production due to appropriate nutrition, better cow comfort, better housing conditions etc. These farms are able to make use of the advantages of the “high fertility cycle” resulting from such synchronization protocols. These higher fertility cycles are not a single reproductive cycle, but a complex one with a low rate of peripartial clinical diseases and good transition of the animals.

Under the circumstances of this study, nearly half of the animals are being declared pregnant within 100-120 days after calving, when the synchronization can be ruled successful. In the long run, this will affect calving intervals lower than 400 days, making round-year calving achievable in high producing dairy cattle. This is highly desirable for the farmers, as it provides a more efficient and larger amount of production when the cows are more in the productive state. Also in a more distant future, with a lower incidence of clinical peripartial problems, a robust pressure can be achieved in the genetic forestepping.

It is also worth mentioning that in those farms where the application of the protocol does not bring good results, due to the aforementioned reasons for those farms, a shift in the reproductive strategy for the first insemination is required. This level of success is between

35 to 40 percent pregnancy rate. This result was achieved in our retrospective data collection even in the summer months during the study. This validates the method for further application.

5.2 Factors affecting the successful fertilization of the first synchronized ovulation

Numerous factors may influence pregnancy outcomes of synchronisation protocols. Among practical circumstances, farm level differences vary in a great range. Our study showed nonsignificant difference between the two farms, whereas the year of the study was also not influenced by the data. With year distribution, our results were ranging between 43 and 49 percent conception after first insemination, indicating a non-significant trendline difference in the results. It is worth mentioning that in the year of 2022, an extreme drought was present all alongside Hungary, causing serious problems in corn silage production. As our results are showing, this has influenced the data as well. However, even in the conditions of a serious drought and climate, the pregnancy rate did not decrease under 40 percent. In other words, extreme weather conditions are accompanied by only a 5 percent decreased pregnancy rate under farm circumstances, according to our study.

Another interesting issue was the parity distribution. Multiparous animals had 3.5 percent lower pregnancy rate in the study, but these data also did not differ significantly from the pregnancy results of primiparous animals. Therefore, this can be considered yet another great advantage of the utilisation of these protocols, that we can achieve almost similar good results in cases of older cows like in heifers after calving. As shown in Table 3, culling rate for multiparous animals is 1.7 times higher than primiparous animals. This finding is considered to be strongly significant. This means that older animals, which are representing higher value in a farm overall, are able to achieve nearly the same pregnancy rates as younger animals. In addition, these animals are producing 10-15 percent more milk during their lactation period, making the lactation more profitable. Earlier studies in the literature reported a much lower pregnancy rate of these animals (1,12,26). Therefore, the reproductive management of these animals is essential.

During the study, every grouping of our data showed lower calving intervals than 400 days. This calving interval is generally accepted as an indicator number of herd level reproductive performance. If we achieve more than 40 percent pregnant animals at herd level, then under optimal circumstances, 80-95 percent of our cows will become pregnant within 3

inseminations. If this fact is connected with a low herd level culling rate, like in our case, then within 2 years, most of the animals can be replaced through this higher genetic progress. It is also worth mentioning that low milk production and poorly managed herds may result in lower pregnancy rates for first service. For them, continuous monitoring of the results will be necessary. Also in case when these protocols are not beneficial, protocol change is then required. At the moment this means a shift to lesser synchronised ovulation like G6G protocol a ProvSynch.

6. Summary

In order to study the first service conception rate in dairy cattle with recent ovulation synchronization protocols, we synchronized 2,524 dairy cows in two Hungarian large scale dairy farms using the Double-Ovsynch protocol. The study was carried out over the course of 3 years, in which most of the animals were involved in the year of 2022. Both farms gave excellent results in terms of fertility after the synchronization protocols. As a result of our study, it has turned out that the good results of fertility programs also have consequences on the herd level fertility parameters. Both days open, calving interval and P/AI were influenced after the usage of the protocol. In all the animals included in our study, we achieved less than 2.5 P/AI, less than 120 days open, and less than 400 days calving interval.

We evaluated retrospectively multiple factors, which influence our results, parity and partially the year, while the specific farm effect was not present in our data. In the future we plan further evaluation of our data and novel studies to improve herd level progression in reproduction.

7. References

1. Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvão KN (2004) The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. *Anim Reprod Sci* 82–83:513–535. <https://doi.org/10.1016/j.anireprosci.2004.04.015>
2. DeJarnette JM, Marshall CE, Lenz RW, Monke DR, Ayars WH, Sattler CG (2004) Sustaining the Fertility of Artificially Inseminated Dairy Cattle: The Role of the Artificial Insemination Industry. *J Dairy Sci* 87:E93–E104. [https://doi.org/10.3168/jds.S0022-0302\(04\)70065-X](https://doi.org/10.3168/jds.S0022-0302(04)70065-X)
3. Stevenson JS, Britt JH (2017) *A 100-Year Review: Practical female reproductive management*. *J Dairy Sci* 100:10292–10313. <https://doi.org/10.3168/jds.2017-12959>
4. Lauderdale JW (2009) ASAS Centennial Paper: Contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. *J Anim Sci* 87:801–812. <https://doi.org/10.2527/jas.2008-1407>
5. Bartlett JW, Edwards J, Terrill CE, Berliner V, Jeffrey FP, Leonard EP, Henderson JA, Reece RP (1945) *Artificial Insemination of Farm Animals*, Second edition. Vail-Ballou press, Binghamton, New York, Rutgers College New Jersey
6. Foote RH (2002) The history of artificial insemination: Selected notes and notables1. *J Anim Sci* 80:1–10. https://doi.org/10.2527/animalsci2002.80E-Suppl_21a
7. Veler CD, Thayer S, Doisy EA (1930) THE PREPARATION OF THE CRYSTALLINE FOLLICULAR OVARIAN HORMONE: THEELIN. *J Biol Chem* 87:357–371. [https://doi.org/10.1016/S0021-9258\(18\)76871-3](https://doi.org/10.1016/S0021-9258(18)76871-3)
8. Allen WM (1930) PHYSIOLOGY OF THE CORPUS LUTEUM. *Am J Physiol-Leg Content*. <https://doi.org/10.1152/ajplegacy.1930.92.3.612>
9. Fevold HL, Hisaw FL, Leonard SL (1931) THE GONAD STIMULATING AND THE LUTEINIZING HORMONES OF THE ANTERIOR LOBE OF THE HYPOPHYSIS. *Am J Physiol-Leg Content*. <https://doi.org/10.1152/ajplegacy.1931.97.2.291>
10. Hansel W, Trimberger GW (1952) The Effect of Progesterone on Ovulation Time in Dairy Heifers. *J Dairy Sci* 35:65–70. [https://doi.org/10.3168/jds.S0022-0302\(52\)93675-8](https://doi.org/10.3168/jds.S0022-0302(52)93675-8)
11. Mauer RE, Weibel SK, Brown MD (1975) OVULATION CONTROL IN CATTLE WITH PROGESTERONE INTRAVAGINAL DEVICE (PRID) AND GONADOTROPIN RELEASING HORMONE (GnRH). *Ann Biol Anim Biochim Biophys* 15:291–296. <https://doi.org/10.1051/rnd:19750216>
12. Gellersen B, Fernandes MS, Brosens JJ (2009) Non-genomic progesterone actions in female reproduction. *Hum Reprod Update* 15:119–138. <https://doi.org/10.1093/humupd/dmn044>
13. Graham JD, Clarke CL (1997) Physiological Action of Progesterone in Target Tissues*. *Endocr Rev* 18:502–519. <https://doi.org/10.1210/edrv.18.4.0308>
14. Schindler AE (2015) Pharmacology of Progestogens. In: Carp HJA (ed) *Progestogens in Obstetrics and Gynecology*. Springer International Publishing, Cham, pp 33–40
15. Chebel RC, Santos JEP, Cerri RLA, Rutigliano HM, Bruno RGS (2006) Reproduction in Dairy Cows Following Progesterone Insert Presynchronization and Resynchronization Protocols. *J Dairy Sci* 89:4205–4219. [https://doi.org/10.3168/jds.S0022-0302\(06\)72466-3](https://doi.org/10.3168/jds.S0022-0302(06)72466-3)
16. Rhodes FM, McDougall S, Burke CR, Verkerk GA, Macmillan KL (2003) Invited Review: Treatment of Cows with an Extended Postpartum Anestrous Interval. *J Dairy Sci* 86:1876–1894. [https://doi.org/10.3168/jds.S0022-0302\(03\)73775-8](https://doi.org/10.3168/jds.S0022-0302(03)73775-8)
17. Trimberger GW, Hansel W (1955) Conception Rate and Ovarian Function Following Estrus Control by Progesterone Injections in Dairy Cattle. *J Anim Sci* 14:224–232. <https://doi.org/10.2527/jas1955.141224x>
18. Yizengaw L (2017) Review on Estrus Synchronization and Its Application in Cattle. *Int J Adv Res Biol Sci IJARBS* 4:67–76. <https://doi.org/10.22192/ijarbs.2017.04.04.010>
19. Larson LL, Ball PJH (1992) Regulation of estrous cycles in dairy cattle: A review.

- Theriogenology 38:255–267. [https://doi.org/10.1016/0093-691X\(92\)90234-I](https://doi.org/10.1016/0093-691X(92)90234-I)
20. Pursley JR, Wiltbank MC (1995) SYNCHRONIZATION OF OVULATION IN DAIRY COWS USING PGF₂, AND GnRH. *Theriogenology* 9
 21. Britt JH, Cox NM, Stevenson JS (1981) Advances in Reproduction in Dairy Cattle. *J Dairy Sci* 64:1378–1402. [https://doi.org/10.3168/jds.S0022-0302\(81\)82710-5](https://doi.org/10.3168/jds.S0022-0302(81)82710-5)
 22. Horton EW, Poyser NL (1976) Uterine luteolytic hormone: a physiological role for prostaglandin F₂α. *Physiol Rev* 56:595–651
 23. Asselin E, Goff AK, Bergeron H, Fortier MA (1996) Influence of Sex Steroids on the Production of Prostaglandins F₂a and E₂ and Response to Oxytocin in Cultured Epithelial and Stromal Cells of the Bovine Endometrium. *Biol Reprod* 54 371–379
 24. Okuda K, Miyamoto Y, Skarzynski DJ (2002) Regulation of endometrial prostaglandin F₂α synthesis during luteolysis and early pregnancy in cattle. *Domest Anim Endocrinol* 23:255–264. [https://doi.org/10.1016/S0739-7240\(02\)00161-3](https://doi.org/10.1016/S0739-7240(02)00161-3)
 25. Pursley JR, Kosorok MR, Wiltbank MC (1997) Reproductive Management of Lactating Dairy Cows Using Synchronization of Ovulation. *J Dairy Sci* 80:301–306. [https://doi.org/10.3168/jds.S0022-0302\(97\)75938-1](https://doi.org/10.3168/jds.S0022-0302(97)75938-1)
 26. Lucy MC, Stevenson JS, Call EP (1986) Controlling First Service and Calving Interval by Prostaglandin F₂α, Gonadotropin-Releasing Hormone, and Timed Insemination. *J Dairy Sci* 69:2186–2194. [https://doi.org/10.3168/jds.S0022-0302\(86\)80652-X](https://doi.org/10.3168/jds.S0022-0302(86)80652-X)
 27. Momont HW, Seguin BE (1983) Treatment of unobserved estrus in lactating dairy cows with prostaglandin F₂α products. *Compend Contin Educ Pract Vet Reprod Manag Food Anim Univ Minn St Paul* 28
 28. Odde KG (1990) A review of synchronization of estrus in postpartum cattle. *J Anim Sci* 68:817–830. <https://doi.org/10.2527/1990.683817x>
 29. Moore K, Thatcher WW (2006) Major Advances Associated with Reproduction in Dairy Cattle. *J Dairy Sci* 89:1254–1266. [https://doi.org/10.3168/jds.S0022-0302\(06\)72194-4](https://doi.org/10.3168/jds.S0022-0302(06)72194-4)
 30. Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R (1971) Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) ovine origin. *Biochem Biophys Res Commun* 44:205–210
 31. Matsuo H, Arimura A, Nair RMG, Schally AV (1971) Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. *Biochem Biophys Res Commun* 45:822–827
 32. Thatcher WW, Moreira F, Pancarci SM, Bartolome JA, Santos JEP (2002) Strategies to optimize reproductive efficiency by regulation of ovarian function. *Domest Anim Endocrinol* 23:243–254. [https://doi.org/10.1016/S0739-7240\(02\)00160-1](https://doi.org/10.1016/S0739-7240(02)00160-1)
 33. Rabiee AR, Lean IJ, Stevenson MA (2005) Efficacy of Ovsynch Program on Reproductive Performance in Dairy Cattle: A Meta-Analysis. *J Dairy Sci* 88:2754–2770. [https://doi.org/10.3168/jds.S0022-0302\(05\)72955-6](https://doi.org/10.3168/jds.S0022-0302(05)72955-6)
 34. Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JEP (2006) Strategies for improving fertility in the modern dairy cow. *Theriogenology* 65:30–44. <https://doi.org/10.1016/j.theriogenology.2005.10.004>
 35. DeJarnette JM, Salverson RR, Marshall CE (2001) Incidence of premature estrus in lactating dairy cows and conception rates to standing estrus or fixed-time inseminations after synchronization using GnRH and PGF₂α. *Anim Reprod Sci* 67:27–35. [https://doi.org/10.1016/S0378-4320\(01\)00107-5](https://doi.org/10.1016/S0378-4320(01)00107-5)
 36. Pankowski JW, Galton DM, Erb HN, Guard CL, Gröhn YT (1995) Use of Prostaglandin F₂α as a Postpartum Reproductive Management Tool for Lactating Dairy Cows. *J Dairy Sci* 78:1477–1488. [https://doi.org/10.3168/jds.S0022-0302\(95\)76770-4](https://doi.org/10.3168/jds.S0022-0302(95)76770-4)
 37. 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* 61:329–336. <https://doi.org/10.1515/jvetres-2017-0043>
 38. Firk R, Stamer E, Junge W, Krieter J (2002) Automation of oestrus detection in dairy cows: a review. *Livest Prod Sci* 75:219–232. [https://doi.org/10.1016/S0301-6226\(01\)00323-2](https://doi.org/10.1016/S0301-6226(01)00323-2)
 39. DuPonte MW (2007) The Basics of Heat (Estrus) Detection in Cattle. *Co-op Ext Serv*

Univ Hawaii Manoa LM-15 Series:

40. Roelofs J, López-Gatius F, Hunter RHF, van Eerdenburg FJCM, Hanzen Ch (2010) When is a cow in estrus? Clinical and practical aspects. *Theriogenology* 74:327–344. <https://doi.org/10.1016/j.theriogenology.2010.02.016>
41. Reith S, Hoy S (2018) Review: Behavioral signs of estrus and the potential of fully automated systems for detection of estrus in dairy cattle. *Animal* 12:398–407. <https://doi.org/10.1017/S1751731117001975>
42. Fesseha H, Degu T (2020) Estrus detection, Estrus synchronization in cattle and its economic importance. 3:1001
43. Shahriar MdS, Smith D, Rahman A, Freeman M, Hills J, Rawnsley R, Henry D, Bishop-Hurley G (2016) Detecting heat events in dairy cows using accelerometers and unsupervised learning. *Comput Electron Agric* 128:20–26. <https://doi.org/10.1016/j.compag.2016.08.009>
44. Stevenson JS, Britt JH (1977) Detection of Estrus by Three Methods. *J Dairy Sci* 60:1994–1998. [https://doi.org/10.3168/jds.S0022-0302\(77\)84135-0](https://doi.org/10.3168/jds.S0022-0302(77)84135-0)
45. Abdisa T (2018) Review on the Reproductive Health Problem of Dairy Cattle. *J Dairy Vet Sci* 5:. <https://doi.org/10.19080/JDVS.2018.05.555655>
46. Hare E, Norman HD, Wright JR (2006) Trends in Calving Ages and Calving Intervals for Dairy Cattle Breeds in the United States. *J Dairy Sci* 89:365–370. [https://doi.org/10.3168/jds.S0022-0302\(06\)72102-6](https://doi.org/10.3168/jds.S0022-0302(06)72102-6)
47. Ball PJH, Peters AR (2004) *Reproduction in Cattle*, Third Edition. Blackwell Publishing
48. Beckett SD, Lean IJ (1997) Gonadotrophin-releasing hormone in postpartum dairy cattle: a meta-analysis of effects on reproductive efficiency. *Anim Reprod Sci* 48:93–112. [https://doi.org/10.1016/S0378-4320\(97\)00016-X](https://doi.org/10.1016/S0378-4320(97)00016-X)
49. Benmrad M, Stevenson JS (1986) Gonadotropin-Releasing Hormone and Prostaglandin F_{2α} for Postpartum Dairy Cows: Estrous, Ovulation, and Fertility Traits¹. *J Dairy Sci* 69:800–811. [https://doi.org/10.3168/jds.S0022-0302\(86\)80469-6](https://doi.org/10.3168/jds.S0022-0302(86)80469-6)
50. Pelissier CL (1982) Identification of reproductive problems and economic consequences. *Proc Natl Invit Dairy Cattle Reprod Workshop* 9–18
51. Britt JH (1985) Enhanced Reproduction and Its Economic Implications¹. *J Dairy Sci* 68:1585–1592. [https://doi.org/10.3168/jds.S0022-0302\(85\)80997-8](https://doi.org/10.3168/jds.S0022-0302(85)80997-8)
52. Odde KG (1990) A review of synchronization of estrus in postpartum cattle. *J Anim Sci* 68:817–830. <https://doi.org/10.2527/1990.683817x>
53. Badinga L, Collier RJ, Thatcher WW, Wilcox CJ (1985) Effects of Climatic and Management Factors on Conception Rate of Dairy Cattle in Subtropical Environment¹. *J Dairy Sci* 68:78–85. [https://doi.org/10.3168/jds.S0022-0302\(85\)80800-6](https://doi.org/10.3168/jds.S0022-0302(85)80800-6)
54. Momcilovic D, Archbald LF, Walters A, Tran T, Kelbert D, Risco C, Thatcher WW (1998) Reproductive performance of lactating dairy cows treated with gonadotrophin-releasing hormone (GnRH) and/or prostaglandin F_{2α} (PGF_{2α}) for synchronization of estrus and ovulation. *Theriogenology* 50:1131–1139. [https://doi.org/10.1016/S0093-691X\(98\)00214-3](https://doi.org/10.1016/S0093-691X(98)00214-3)
55. Ribeiro ES, Galvão KN, Thatcher WW, Santos JEP (2012) Economic aspects of applying reproductive technologies to dairy herds. *Anim Reprod* 9:370–387
56. Tenhagen B-A, Drillich M, Surholt R, Heuwieser W (2004) Comparison of Timed AI After Synchronized Ovulation to AI at Estrus: Reproductive and Economic Considerations. *J Dairy Sci* 87:85–94. [https://doi.org/10.3168/jds.S0022-0302\(04\)73145-8](https://doi.org/10.3168/jds.S0022-0302(04)73145-8)
57. Giordano JO, Fricke PM, Wiltbank MC, Cabrera VE (2011) An economic decision-making support system for selection of reproductive management programs on dairy farms. *J Dairy Sci* 94:6216–6232. <https://doi.org/10.3168/jds.2011-4376>
58. LeBlanc S (2001) The OvSynch breeding program for dairy cows - A review and economic perspective. *Bov Pract* 35:13–22
59. Risco CA, Moreira F, DeLorenzo M, Thatcher WW (1998) Timed artificial insemination in dairy cattle - part II. *Compend Contin Educ Pract Vet* 20:1284–1289

60. Hasler J (2004) Factors influencing the success of embryo transfer in cattle. *Med VETERINAIRE QUEBEC* 33:66–66
61. Coleman DA, Dailey RA, Leffel RE, Baker RD (1987) Estrous Synchronization and Establishment of Pregnancy in Bovine Embryo Transfer Recipients¹. *J Dairy Sci* 70:858–866. [https://doi.org/10.3168/jds.S0022-0302\(87\)80084-X](https://doi.org/10.3168/jds.S0022-0302(87)80084-X)
62. Bó GA, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tribulo R, Tribulo H, Mapletoft RJ (2002) The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology* 57:53–72. [https://doi.org/10.1016/S0093-691X\(01\)00657-4](https://doi.org/10.1016/S0093-691X(01)00657-4)
63. Bó GA, Mapletoft RJ (2014) Historical perspectives and recent research on superovulation in cattle. *Theriogenology* 81:38–48. <https://doi.org/10.1016/j.theriogenology.2013.09.020>
64. Hasler JF (2003) The current status and future of commercial embryo transfer in cattle. *Anim Reprod Sci* 79:245–264. [https://doi.org/10.1016/S0378-4320\(03\)00167-2](https://doi.org/10.1016/S0378-4320(03)00167-2)
65. Moore SG, Hasler JF (2017) *A 100-Year Review: Reproductive technologies in dairy science*. *J Dairy Sci* 100:10314–10331. <https://doi.org/10.3168/jds.2017-13138>
66. Haile-Mariam M, van den Berg I, Ho PN, Pryce JE (2023) Synchronization of breeding and its impact on genetic parameters and evaluation of female fertility traits. *J Dairy Sci* 106:392–406. <https://doi.org/10.3168/jds.2022-22232>
67. Higgins HM, Ferguson E, Smith RF, Green MJ (2013) Using Hormones to Manage Dairy Cow Fertility: The Clinical and Ethical Beliefs of Veterinary Practitioners. *PLOS ONE* 8:e62993. <https://doi.org/10.1371/journal.pone.0062993>
68. Brotzman RL, Döpfer D, Foy MR, Hess JP, Nordlund KV, Bennett TB, Cook NB (2015) Survey of facility and management characteristics of large, Upper Midwest dairy herds clustered by Dairy Herd Improvement records. *J Dairy Sci* 98:8245–8261. <https://doi.org/10.3168/jds.2014-9264>
69. Fricke PM, Wiltbank MC (2022) *Symposium review: The implications of spontaneous versus synchronized ovulations on the reproductive performance of lactating dairy cows**. *J Dairy Sci* 105:4679–4689. <https://doi.org/10.3168/jds.2021-21431>
70. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL (1997) Pregnancy Rates Per Artificial Insemination for Cows and Heifers Inseminated at a Synchronized Ovulation or Synchronized Estrus¹. *J Dairy Sci* 80:295–300. [https://doi.org/10.3168/jds.S0022-0302\(97\)75937-X](https://doi.org/10.3168/jds.S0022-0302(97)75937-X)
71. Gümen A, Guenther JN, Wiltbank MC (2003) Follicular Size and Response to Ovsynch Versus Detection of Estrus in Anovular and Ovular Lactating Dairy Cows. *J Dairy Sci* 86:3184–3194. [https://doi.org/10.3168/jds.S0022-0302\(03\)73921-6](https://doi.org/10.3168/jds.S0022-0302(03)73921-6)
72. Ayres H, Ferreira RM, Cunha AP, Araújo RR, Wiltbank MC (2013) Double-Ovsynch in high-producing dairy cows: Effects on progesterone concentrations and ovulation to GnRH treatments. *Theriogenology* 79:159–164. <https://doi.org/10.1016/j.theriogenology.2012.10.001>
73. Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC (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* 52:1067–1078. [https://doi.org/10.1016/S0093-691X\(99\)00195-8](https://doi.org/10.1016/S0093-691X(99)00195-8)
74. Moreira F, de la Sota RL, Diaz T, Thatcher WW (2000) Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers¹. *J Anim Sci* 78:1568–1576. <https://doi.org/10.2527/2000.7861568x>
75. Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW (2001) Effects of Presynchronization and Bovine Somatotropin on Pregnancy Rates to a Timed Artificial Insemination Protocol in Lactating Dairy Cows. *J Dairy Sci* 84:1646–1659. [https://doi.org/10.3168/jds.S0022-0302\(01\)74600-0](https://doi.org/10.3168/jds.S0022-0302(01)74600-0)
76. Souza AH, Ayres H, Ferreira RM, Wiltbank MC (2008) A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating

- dairy cows. *Theriogenology* 70:208–215.
<https://doi.org/10.1016/j.theriogenology.2008.03.014>
77. Vazquez Belandria R, Denholm K, Pepler PT, Cook JG, Pinho P, Randi F, Viora L (2023) Comparison of three reproductive management strategies for lactating dairy cows using combination of estrus detection or ovulation synchronization and Fixed-Timed Artificial Insemination. *Anim Reprod Sci* 257:107331.
<https://doi.org/10.1016/j.anireprosci.2023.107331>
 78. Dirandeh E, Roodbari AR, Colazo MG (2015) Double-Ovsynch, compared with presynch with or without GnRH, improves fertility in heat-stressed lactating dairy cows. *Theriogenology* 83:438–443.
<https://doi.org/10.1016/j.theriogenology.2014.10.011>
 79. Herlihy MM, Giordano JO, Souza AH, Ayres H, Ferreira RM, Keskin A, Nascimento AB, Guenther JN, Gaska JM, Kacuba SJ, Crowe MA, Butler ST, Wiltbank MC (2012) Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J Dairy Sci* 95:7003–7014.
<https://doi.org/10.3168/jds.2011-5260>
 80. Li Z, Luan S, Yan L, Xie C, Lian Z, Yang M, Mei M, Lin P, Wang A, Jin Y (2024) Effect of Double-Ovsynch and Presynch-Ovsynch on postpartum ovarian cysts and inactive ovary in high-yielding dairy cows. *Front Vet Sci* 11:1348734.
<https://doi.org/10.3389/fvets.2024.1348734>
 81. Carvalho PD, Fuenzalida MJ, Ricci A, Souza AH, Barletta RV, Wiltbank MC, Fricke PM (2015) Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with gonadotropin-releasing hormone 6 d before initiation of Ovsynch and addition of a second prostaglandin F_{2α} treatment. *J Dairy Sci* 98:8741–8752. <https://doi.org/10.3168/jds.2015-9719>
 82. Nascimento AB, Souza AH, Keskin A, Sartori R, Wiltbank MC (2014) Lack of complete regression of the Day 5 corpus luteum after one or two doses of PGF_{2α} in nonlactating Holstein cows. *Theriogenology* 81:389–395.
<https://doi.org/10.1016/j.theriogenology.2013.10.009>
 83. Brusveen DJ, Souza AH, Wiltbank MC (2009) Effects of additional prostaglandin F_{2α} and estradiol-17β during Ovsynch in lactating dairy cows. *J Dairy Sci* 92:1412–1422.
<https://doi.org/10.3168/jds.2008-1289>
 84. Chenault JR, Boucher JF, Dame KJ, Meyer JA, Wood-Follis SL (2003) Intravaginal Progesterone Insert to Synchronize Return to Estrus of Previously Inseminated Dairy Cows1. *J Dairy Sci* 86:2039–2049. [https://doi.org/10.3168/jds.S0022-0302\(03\)73793-X](https://doi.org/10.3168/jds.S0022-0302(03)73793-X)
 85. van Werven T, Waldeck F, Souza AH, Floch S, Englebienne M (2013) Comparison of two intravaginal progesterone releasing devices (PRID-Delta vs CIDR) in dairy cows: Blood progesterone profile and field fertility. *Anim Reprod Sci* 138:143–149.
<https://doi.org/10.1016/j.anireprosci.2013.02.010>
 86. de Graaff W, Grimard B (2018) Progesterone-releasing devices for cattle estrus induction and synchronization: Device optimization to anticipate shorter treatment durations and new device developments. *Theriogenology* 112:34–43.
<https://doi.org/10.1016/j.theriogenology.2017.09.025>
 87. Rathbone MJ, McDowell A (2012) Controlled release intravaginal veterinary drug delivery. Springer N Y NY Long acting animal health drug products: fundamentals and applications:247–270. https://doi.org/10.1007/978-1-4614-4439-8_11
 88. Macmillan KL, Peterson AJ (1993) A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. *Anim Reprod Sci* 33:1–25.
[https://doi.org/10.1016/0378-4320\(93\)90104-Y](https://doi.org/10.1016/0378-4320(93)90104-Y)

8. Acknowledgements

I would like to thank PhD student Mrs. Eman Mostafa, for her help in the work and for the performance of the statistical analyses of the research. I also would like to thank Ms Nikoletta Torák, another thesis student for the collaboration.