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Comparing the Use of Intra Vaginal Progesterone Devices with Standard Ovulation Synchronisation Protocols in a Hungarian Dairy Herd

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Table of Contents

List of abbreviations

DOS: Double Ovulation Synchronisation.

Ovsynch: Ovulation Synchronisation.

P4: Progesterone.

AI: Artificial Insemination.

TAD: Twice A Day.

BCS: Body Condition Score.

NEB: Net Energy Balance

1.Introduction

High reproductive efficiency is a vital factor in the profitability of the dairy industry. Repeat breeders can lead to substantial economic losses for dairy producers due to additional inseminations, longer calving intervals and therefore higher culling rates. The main aim is to reduce calving internal and minimise repeat breeders while improving the fertility and milk output of animals [6]. The performance of a herd is measured by calving interval, days open, conception and pregnancy rates. There is an inverse relationship between days in milk and herd average daily milk production. In this study, our aim is to compare the reproductive efficiency of three herds using traditional double ovulation synchronisation protocol and progesterone protocol.

2. Literature Review

2.1. Estrus synchronization of dairy cattle:

Estrus synchronisation is the manipulation of the estrus cycle of bovine animals to ensure the majority of animals show standing heat in a short period of time. It allows for more efficient AI of animals by the use of better sires and also is less labour intensive for farms using exact time of insemination. Most programs on farms now involve the use of a combination of hormones but the basic principle of estrus synchronisation is manipulation of the luteal phase length in the estrus cycle [10]. This phase can be lengthened with treatment of progestogens or shortened with the use of PGF2a or it's analogues. To reduce the estrus time length and increase conception rate, other hormones such as GnRH have been added into some programs. These allow for manipulation of the development of the follicular wave.
It is believed that in herds with high estrus detection accuracy and efficiency, that estrus synchronisation is associated with reduced conception rates but can increase an overall reproductive performance of herds. This only occurs in cows in the early to mid-stages of their cycle at the time of the second PGFa2 injection and so this reduction is likely caused by the shortening of the luteal phase rather than the PG treatment [10]. There is now a current trend shift towards ovulation synchronisation as estrus synchronisation may be associated with a drop in conception rates at estrus synchronisation but an overall increase in reproductive

performance due to an improvement in detection accuracy and efficiency. Ovulation synchronisation allows for timed breeding as desired, without this detection of estrus. [10].

The breeding of bovine animals-based heat detection by visualisation is an important tool in the management of all programs but relying on this solely can result in a poor reproductive performance. The use of synchronisation protocols alongside visual heat detection can improve this greatly and the human job with reproduction became easier by using hormonal protocol and time insemination. There are several synchronization protocols which were developed from the seventies.

2.1.1 Pre-synchronisation: these programs include the use of prostaglandin +/- GnRH to initiate ovulation in cows that are not cycling and increasing the number of cows synching during the Ovsynch protocol.

2.1.2 Ovsynch: this protocol involves the use of GnRH and a treatment of PGF2A 7 days later and then a second GnRH treatment before AI. There are several options of when to give the second GnRH treatment before AI. The first GnRH treatment induces ovulation and a new follicular wave.

A limitation to this is the suppression of the expression of estrus after GnRH administration as it induces an LH surge. Research has shown that cows given GnRH at 17-36days after AI, less readily show estrus. Other research has shown that cows with a functional CL at the Ovsynch initiation, conceive at rates 50% higher than those without a functional CL [21]. This can prove to be more costly to farmers and so it is beneficial to provide supplementary progesterone during the Ovsynch to cows starting the program without a CL present, while cows bearing a CL at ovsynch may not require the first GnRH injection as the CL is responsive to PGF2a. [21].

2.1.3 Resynch: In the instance when estrus is not detected in cows, they are enrolled into a resynch protocol. This will initiate Ovsynch to help resynchronise ovulation in open cows. This is widely used across the world, especially in the US. This protocol can begin 7 days

before or at a non-pregnant diagnosis [21].

There are several options for using time insemination protocol such as Double Ov Synch and Progesterone synch, Co-synch etc.

2.1.4 Double Ovsynch: In short, this protocol is two Ovsynch protocols in succession over about 4 weeks. The first half of DOS, Ovsynch1 is intended to pre-synchronise the animals and ensure the majority are in the same stage of estrus. It addresses animals that may be anovular before Ovsynch2. In a recent study by Herlihy et al. it showed that DOS reduced the number of potentially anovular animals (low progesterone concentrations) from 25% to just 5%. This stage is then ideal to start the second half, Ovsynch2. In a second study, it was shown by Ayres et al that the use of DOS increased the number of cows starting Ovsynch 2 with a viable CL to 94% from 68%. This is especially evident in first lactation animals.

2.1.5 Progesterone Synch: There are several ways to manipulate the cycle of dairy cows such as the use of progestogens, prostaglandins, progesterone-prostaglandin combinations, progestogen- estrogen combinations and gonadotropin- prostaglandin combinations +/- progestogens. In this study we look at how the use of PRID devices can impact the cycling of animals. Progesterone intra-vaginal devices have been available since the 1970s and are used to improve the reproductive efficiency of dairy herds. There are some devices commercially available on the market. The farms in this study use PRID Delta (Ceva Santé Animale, France). Progesterone has a negative feedback effect on the hypothalmo-pituitary axis. In particular LH secretion. When the device is inserted, it is absorbed intra-vaginally and prevents the LH surge and supresses estrus and ovulation. When this device is removed after 7 days, the drop in progesterone causes a new estrus cycle.

2.2 Anovular condition of dairy cows:

Anovulation defines a cow which is not ovulating. Hancock in 1948 proposed that cows should be divided in categories based on their ovarian activity; ovulatory cows, anovulatory cows (cystic or non-cystic), and inactive ovaries. Anovular animal can be determined accurately with ultrasound exams or by measurement of progesterone (P4) concentrations in the blood [48]. It

is also possible to easily misdiagnose a cow as anovular in the field when estimation is done by palpation and the detection of estrus. In these cases, there are many reasons the animal may not be showing estrus such as increased level of milk production. In a study by Lopez et al, it was concluded that animals that produce a high milk yield show standing estrus for much shorter durations [16].

There are a number of causes which can lead to a cow becoming anovular.

2.2.1. Physiology Causing Anovulation: The most apparent cause is the presence of a CL on the ovary. The presence of this persistent CL and the absence of ovulation is associated with pregnancy and is not considered a reproductive problem. The issue is some cows have a persistent CL that does not regress after 25days and are not pregnant. This is known as a persistent CL. According to *Ball and McEwan*, the prevalence of a persistent CL in cows that ovulate 25 days after calving increases drastically (25%) when compared to those who ovulate later (0%) [1]. Persistent CLs can easily be treated with the use of PGF2A.

Three main primary follicular growth patterns have been noted in anovular dairy cows. These are a classical anovular or anestrous cow with small follicles (9-15mm), b) follicular cysts (>25mm), c) Anovulation of large follicles (16-24mm)

2.2.2. Classical anestrous with small follicles: Follicles in this pattern only reach a maximum of 9-15mm in diameter. This is usually associated with a NEB and low BCS. This low energy level in the animal causes insufficient LH pulses which lead to poor follicle growth and inadequate estradiol production from the follicle. In these cases, cows do not produce follicles which are large enough or produce enough estradiol to come into estrus and ovulate. This issue most commonly occurs in the early postpartum period when the animal is in its lowest NEB and have a reduced BCS (2.5 or lower). As the cow's energy and nutrient intake increases, the LH pulses will improve and the ability to produce follicles of sufficient size and estradiol production returns. They will then come back into estrus and ovulate.

2.2.3 Follicular cysts: This condition is often diagnosed by veterinarians. These follicles reach up to >25mm in diameter. Here, most cows do not show heat or ovulate, despite having such

high estradiol levels in circulation. This is due to the lack of the positive estradiol feedback to the hypothalamus [13]

2.2.4. Anovulation of large follicles: This condition is the most common in that of lactating dairy cows but still remains the most difficult to diagnose. Here, the follicles grow to 16-24mm in diameter (ovulatory size) but do not ovulate. Cows here have a high circulating estradiol concentration but do not show standing heat or ovulate. Other signs of estrus such as increased activity and mucus production may be present, but these cows remain anovular.

2.3 Farm Factors

The factors on dairy farms that have been found to influence anovulation.

2.3.1 Milk production: There is a clear relationship between milk production and anovulation. It was found in studies in North America, that high levels of milk production or the potential for it due to genetics did not cause anoulvation but of those animals that were anovulatory, they produced more milk compared to their herd mates [5,17]. In a second study done in California in 2006, the results were similar. The number of anovular cows at 49days postpartum was not related to milk production with cows at the lowest and highest production showing similar anovular rates [3]. It can be noted that the incidence of anovulation is more likely to be due to other factors than milk production.

2.3.2. Body Condition

In a number of papers written, there is a strong correlation between BCS and anovulation. Lopez et al. showed that in cows at 71days post-partum, as the BCS increased, anovulation levels decreased from 83.3% (<2.5 BCS) to 8.5% (>3.25 BCS) [16]. Similarly, Chebel et al. noted that the percentage of cows anovular at 49days post-partum with a BCS of <2.75 was 45.2% while those >2.75 was 33.7% [3]. This shows there is a relationship between BCS and anovulation though it does not completely explain the high anovulation levels in dairy cows. It seems other factors may have a role to play as well, not only BCS and NEB as many cows with low BCS have a higher incidence of being anovular, there are also many cows with anovulation that do not have a low BCS.

2.3.3. Age of animals: Most studies done, have shown a higher incidence of anovulation in primiparous cows when compared with multiparous cows. In a paper by Moreira et al. circulating P4 levels were measured and low measurements alongside anovulation were reported in 37.3% of primiparous cows and 15.7% of multiparous cows. Chebel et al, also noted that at 49 days post-partum, primiparous cows showed higher rates of anovulation (54.1%) when compared to multiparous (31.5%) [3]. The relationship between age and anovulation appears to be controversial and varies between herds. A number of factors could play into it such as resource competition between herd mates, nutrition, study methods or production level.

On top of the above-mentioned other factors such as uterine infections and phytoestrogens in feed can play a role in anovulation. From this, it seems that there is quite a number of possible causes or mechanisms that could lead to anovulation in dairy cows. Management which results in better BCS close to the beginning of breeding should be implemented. In saying that, well fed cows with optimum BCS can still have a high incidence of anovulation. The most used treatment for anovular cows is the Ovsynch protocol. It is used to induce ovulation in a high number anovular cows although in some cows, it does result in a shorter luteal phase [12]. It was also shown that although Ovsynch may induce ovulation in cows that weren't cycling, there was a decrease in their conception rates.

The use of P4 devices, has been effective in the treatment of anovular cows for many years. Progesterone supplementation in an anovular cows helps to reestablish the hypothalamic response to estradiol that is produced by the dominant follicle. This reestablishment allows the LH surge to occur in response to estradiol concentration increasing. According to Fike et al. giving additional progesterone increases estradiol responsiveness in the brain and increases the likelihood of behavioural estrus being shown at first postpartum ovulation and increasing the likelihood of a normal luteal phase duration while also increasing the risk of pregnancy after ovsynch protocol [8].

3. Role of progesterone

Progesterone is an essential hormone for normal reproductive cyclicity. It inhibits estrus and controls follicular growth.

In normal cycle functioning cows, progesterone is produced by the CL and enters circulation in a high enough concentration that allows it to maintain a pregnancy. It has been shown that progesterone has a positive relationship with the fertility of lactating cows.

Circulating progesterone levels are very important in cattle reproduction. Rivera et al. showed that higher levels of circulating P4 have a positive impact on both the degree of follicular growth and embryo quality [14]. It has also been shown that elevating P4 levels during the pre-ovulatory follicular waves can increase pregnancy rates and in turn improve fertility and reduce twin calving rates. It is also important to have low levels of P4 around AI and then an elevation after AI to improve fertility and embryonic development [25]. On days three to seven post insemination, supplementation of progesterone resulted in significantly improved rates of conception, with limited proven benefits if supplementation is given after the first week. [11] Interestingly, when the circulating progesterone levels are low there is a high higher likelihood of twinning by co-dominance of two follicles resulting in a double ovulation. A similar significant risk around progesterone supplementation during days 15-17 post insemination is the prevalence of twinning. During this time, maternal recognition is taking place and the supplementation would aid in saving one of the two embryos that would have been lost had it not been given. This is a risk most farmers would like to avoid.

In some cows, as discussed above, there may be too little in circulation which can lead to issues in reproduction. Due to progesterones ability to control and suppress LH, a key hormone in follicular growth stimulation, a lower concentration of circulating progesterone leads to larger follicles. The conception rates of these larger follicles ovulating is lower when compared to those developed under normal conditions of progesterone concentration. It is also worth noting again that concentrations of progesterone in circulation should not always be high. They are required to drop after PGF2A is given and at the time of AI.

4. Materials and methods

4.1 Animals

All data was collected from BoviSync systems used on the farms. The study was carried out on 2673 Holstein Friesian cows and heifers over 3 herds (1,2,3) in Western Hungary from January- December 2022. The cows are in a twice a day (TAD) milking system and the farms are divided (1,2,3) as follows; Farm 1 is a manual farm that has 904 cows with an average daily milk yield of 37.5 1 litres, Farm 2 is a robotic farm with 1157 cows with an average milk yield of 37.5 l, while farm 3 is manual and has 612 cows with an average of 36.33 l. Farm 1 and 2 have a mix of both cows and heifers, while there are no heifers on farm 3. The animals are kept in large modern free stall barns and were fed a TMR diet that that met or exceeded the nutritional requirements for Holstein Friesian cows and heifers, twice daily. Heifers are an average of 382 days of age at first fertilisation. While cows had an average of 75 days between calving and first service. On farm 1, 925 conceived in the first insemination, 519 in the second, 335 in the third and 352 in the fourth insemination. On farm 2, 850 cows conceived after the first insemination, 480 in the second, 277 in the third and 267 in the fourth insemination. On farm 3, 717 conceived after the first insemination, 475 after the second, 264 after the third and 215 after the fourth insemination. During this year, there was a total of 2131 inseminations on farm 1, 1874on farm 2 and 1671 on farm 3.

Table 1. Fertility data of cows on the farms

	Farm 1	**Farm 2**	**Farm 3**
No of inseminations	2131	1874	1671
No of Pregnancy	861	755	617
Fertility Rate of Total Inseminations	40.28%	40.4%	36.92%
1st insemination	43.40%	45.35%	42.90%
2nd insemination	24.35%	25.61%	28.42%
3rd insemination	15.72%	14.78%	15.79%
4th insemination	16.53%	14.26%	12.89%
Cull cows	5%	4.8%	5.2%

4.2 Hormonal Treatment Protocol of Oestrus

On the farms, double ovulation synchronisation (DOS) is used for the initial insemination in all animals. This protocol starts every Friday for cows in 47-53 days in milk. A summary of the DOS procedure is show in Fig.1. The animals are then checked for pregnancy at 30 days (D58). If an animal is found open, regardless of if a corpus luteum (CL) is present, PRID sync protocol is used from there on out. The device is inserted vaginally and removed after 7 days (Figure 3, 4). This is summarised in Fig 2. The PRID delta devices used are produced by Ceva Santa Animale and contain 1.55g of progesterone. If after the 4th insemination, cows remain empty they are sent to slaughter. The average percentage of cull animals per all 3 farms is 5%.

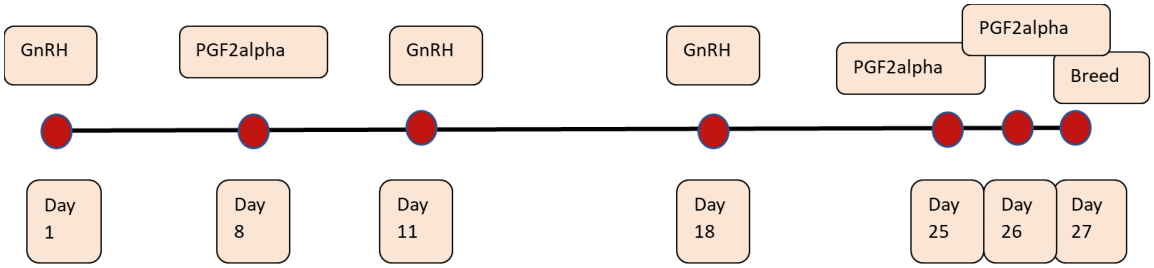

Figure 1; Protocol of DOS is carried out of farm.

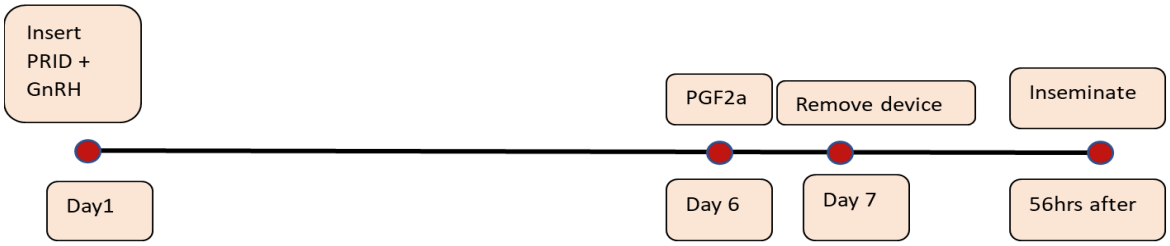

Figure 2; Protocol of Prid-delta

Figure 3. Vaginal insertion of Prid-delta

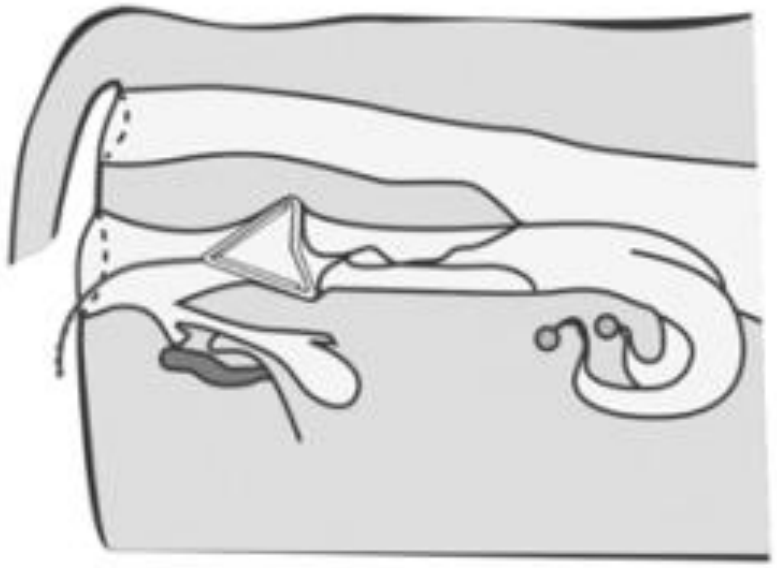

Figure 4. Disposition of Prid-delta into the vagina

4.3 Body Condition Score, Estrus Detection and AI

Body condition score was assessed using a scale of 1-5. Animals were maintained at a BCS of >2.5 and <4 in order to maximise ovulation and conception rates. This was monitored by workers on the farm during milking and yard work. Workers and vets on the farms noted mounting of animals and rubbing of tail paint for estrus detection. AI was performed using the AM/PM rule by the vet on site.

4.4 Cost of the hormonal protocol

The cost of the treatments is a very important aspect to reproduction. Balancing the efficiency and the costs of treatments can pose a challenge to farmers. DOS treatment protocol includes three injections of prostaglandins and one injection of GnRH while the Prid-synch protocol includes one prostaglandin injection, one GnRH injection and the cost of the Prid-delta device. A summary of the costs of these individual injection and device can be seen in Table 2. The total cost of one animal's DOS treatment is €9.24 while the cost of one Prid-synch treatment is €16.78. This is a difference of €7.44 but a high percentage of animals are requiring both DOS and Prid-synch the cost is upwards of €26.02 per animal.

Table 2 Cost of the DOS and Prid-synch protocol/cow

Cost	DOS/Treatment/cow	Prid-synch/Treatment/cow
Pg	**3.30€**	**1.10€**
Gnrh	**6.04€**	**1.68€**
Prid-delta	**0**	**14.00€**
Total	**9.34 €**	**16.78€**

5. Results

The following results are from the cows on the farms collecting from BoviSync. Animals are first started with double Ovsync methods and then checked for pregnancy at 30 days and if found open, PRID protocol was initiated.The total number of cows inseminated was 5,676 in all 3 farms. Of those, 2131 were on farm 1 and had 861 pregnancies, 1874 on farm 2 with 755 pregnancies and 1671 on farm 3 with 617 pregnancies. The fertility rate on each farm is as follows; 40.4%, 40.2% and 36.9%, respectively. The average calving to conception interval on the 3 farms was 112 days. This data is demonstrated in Table 1. Table 3 represents the conception rate of cows on each farm. On farm 1 of the total 2131 inseminations, 46% conceived after one insemination, 28% after two inseminations and 26% after three or more inseminations. On farm 2 of a total of 1874 inseminations, 40% conceived after one insemination, 27% after two inseminations and 33% after three or more inseminations. On farm 3 of a total of 1671, 39% conceived after one insemination, 27% after two and 34% after three or more inseminations. Animals that remained empty after 5 inseminations were culled. On farm 1 this totalled 47 cows, 41 on farm 2 and 35 animals on farm 3.

Table 3. Distribution of percentage of pregnancy after each insemination.

	Farm 1	**Farm 2**	**Farm 3**
1x insemination	46%	40%	39%
2x inseminations	28%	27%	27%
3x + inseminations	26%	33%	34%

Heifers

Data were collected from the heifers on Farms 1 and 2 and there are no heifers housed at farm 3.

Farm 1

On farm 1, heifers were inseminated at 381 days of age. Of a total of 875 inseminations, 409 were confirmed pregnant. A fertility rate of 46.74% and a fertility index of 2.13 was calculated form these results. 49.3% of heifers were fertilised on their first insemination and a further 24% using 3 or more inseminations. The results of 3 or more inseminations, was a fertility rate of 48%. Of all the heifers fertilised on the farm, 79% conceived.

Farm 2

On farm 2, heifers were inseminated at 382 days of age. 1773 inseminations were made, 828 conceived and were confirmed pregnant. A fertility rate of 46.7% with a fertility index of 2.14. 48.4% of heifers on this particular farm conceived on their first insemination after double Ovsync protocol while a further 27% were fertilised after 3 plus inseminations and the PRID Delta protocol. The results of the 3 plus fertilisations was 47%. Of all heifers fertilised on the farm 75% became pregnant after one or both protocols. The percentage of those pregnant to those fertilised was 73%.

Table 4. Summary of results of reproduction in farm of heifers 1, 2

Farm	**1**	**2**
Number inseminated	875	1773
Heifers pregnant	409	828
Fertility rate %	46.74%	46.7%
Fertility index	2.13	2.14
Conceived after 1 insemination	49.3%	48.8%
% pregnant vs those fertilised	77%	73%

6. Discussion and Conclusion

In this study we aimed to compare the fertility results of dairy cattle using double ovulation synchronisation (DOS) and the PRID protocol. Based on the above results we can see that this was successful. From the results on Farm 1 43.40% of cows were successfully fertilised using DOS protocol and a further 56.6% were fertilised using PRID protocol. While Farm 2 showed 43.4% DOS fertility and 56.6% PRID. Farm 3 had the lowest DOS fertility rate and the highest PRID rate with 42.9% and 57.1%, respectively. On all 3 farms there is quite a low percentage of animals sent to slaughter as they are persistently open after 5 inseminations, at 5.5%. This also indicates the effectiveness of the Prid-sync treatment. This data is shown in Table1. These results clearly show an overall improved fertility rate in the cows on all 3 farms when treated with progesterone implants after failed DOS attempts.

In a study completed by *Stevenson et al,* in 2006 in USA with the use of progesterone device implants inserted at the time of the first GnRH injection during Ovsynch and removed 1-2 hours before the PGF2a injection, they noted an overall conception rate change of 10 and 5 percent in cows treated with P4 at 28days and 56days after TAI. In this study, P4 blood concentrations were measured regularly. At the time of the PGF2a injection, P4 levels influenced the conception rate of these cows. Those with a low serum P4 before the injection had greater conception rates when treated with progesterone device (CIDR, Zoetis, USA), regardless of cycling or not. They did note that only cows without an active CL before the

PGF2a injection showed improved conception rates. The hypothesis of this study was that cycling and non-cycling cows with low P4 levels before the PGF2a injection should have improved conception rates and so this was not fully proven [23]. In a similar study *Robinson et al*, concluded the use of a supplemental progesterone given at early gestation resulted in pregnancy rates improvements from 30% to 60% from that of the control cows. These results coincide with another study by *Wiltbank et al* to agree that progesterone supplementation given to cows with reduced fertility improves their pregnancy rates [20,25].

Like this, *El- Zarkouny et al*, in USA, showed that supplementation of P4 during Ovsynch protocol improved both the pregnancy rates and embryo survival rates of cows after TAI. They also noted that the combination of both Presynch and Ovsynch protocols caused an increase in P4 levels at the time of the PGF2a before TAI, increased the number ion cows in diestrus before the start of the Ovsynch protocol and so increased the pregnancy levels in their 1st experiment. This did not occur in the 2nd experiment as cows in this group were already in their first lactation and more anestrus was noted [4].

In a similar study performed in Mexico, cows were presynched before Ovsynch and then progesterone device implant was inserted. Here, there was also a similar positive effect in conception rates [18].

The results of the heifers located on farms 1 and 2 also supported this data. An average of 46.7% of heifers were fertilised with DOS protocol, leaving a further 53.3% open for PRID protocol. This is a very large percentage of animals to be unsuccessful.

A further aspect of this study is the cost of each round of the protocols. Summarised data can be seen in Table 5**.** For one round of DOS and one of PRID it costs €9.24 and €16.78 respectively.

On farm 1, a total of 925 treatments of DOS was used 2022 and an additional 1206 Prid-sync treatments. This is a total of €19,690 DOS and an additional €20,236 for Prid-sync. This is a total of €39,926 for the year 2022.

On farm 2, a total of 850 rounds of DOS were used and a further 1024 Prid-sync treatments were used after 1 failed insemination per animal. This is a total of €17,315 for DOS treatment and an additional €17,182 for Prid-sync treatment. This is a total of €34,497 for this year. This total does not include labour costs.

Farm 3 holds a total of 1671 cows which received 717 DOS treatments and a further 954 Prid-sync treatments. This is a cost of €15,440 and €16,008, respectively. A total cost of €31,448, without labour costs.

There are small differences between yearly cost of DOS and Prid-sync protocol in all farms.

Table 5. Summary of protocol costs on farms

	Farm 1	**Farm 2**	**Farm 3**
Total cows	925	850	717
Total DOS used/year	2131	1874	1671
DOS cost/year	€19,690	€17,315	€15,440
Total Prid-sync used/year	1206	1024	954
Prid-sync cost/year	€20,236	€17,182	€16,008
Total DOS & Prid-sync cost/year	€39,926	€34,497	€31,448

If this study was to be conducted again or further research made, the use of progesterone levels taken by blood would be a very informative addition to the process. Similarly, based on the study by van Werven et al, in the Netherlands, it can be concluded that the use of milk P4 levels can be a valuable tool for predicting fertility and its incorporation into milking machines may be extremely helpful to enhance reproductive efficiency [24].

Other improvements that could have been made to this study is the sample size and division. A control group with divided experimental groups would make for ease of comparison. Prior to commencement of the study, full reproductive examinations and semen quality tests should be carried out. The use of follicular wave measures using US could also be helpful.

In one study by *Forro et al.,* in Germany, it was published that the use of P4 devices or supplementation showed no advantage in their studies [7]. This is a minority response.

In the vast majority of studies, as it is in this one, P4 supplementation or the use of progesterone devices have been a major advantage to conception rates and fertility in dairy cows. *El-Zarkouny et al.,* like many authors, concluded that the use of P4 supplementation during

ovsynch improved both embryo survival rates and pregnancy rates in dairy cows [5].

In conclusion, the understanding of how progesterone works pharmacologically, and the impacts of its supplementation can lead to a better understanding of what causes reduced fertility and so can in turn, help us to improve fertility among lactating dairy cows. In this current study, the use of progesterone device improved fertility and conception rates among the majority of cows treated and such is the reason for low levels of anovular animals on the farms mentioned.

7. Summary

In this study we compared the fertility of 2673 Hungarian dairy cows over 3 herds using double ovsynch protocol and progesterone supplementation reproduction protocol. Animals in these herds were treated with the double ovsynch protocol for their first insemination and if they failed to conceive, they were automatically placed on the PRID protocol, regardless of cyclicity. The device was implanted vaginally for 7 days. Empty animals after the 4th insemination were culled.

On Farm 1, after the first insemination only 43.40% of cows conceived. 925 animals given DOS treatments in 2022. After the Prid-sync treatment, a further 24.35% conceived following a second insemination and 15.72% after a third insemination. 16.53% conceived after a 4th insemination. A total of 47 empty animals, 5 % of the herd were culled after this insemination. On farm 2, after the initial insemination 45.35% of cows conceived. 850 cows treated with DOS protocol. Following Prid-sync treatment, a further 25.61% conceived after a second insemination, 14.78% following the third insemination and 14.26% after the fourth and final insemination. 41 cows, 4.8 % of the herd were culled on this farm after the fourth insemination. On farm 3, 42.90% of cows conceived after the first insemination. 717 cows were treated with DOS. At the second insemination following Prid-sync treatment, 28.42% conceived, 15.79% after the third and 12.89% after the fourth insemination. A total of 35 empty cows, 5.2 % of the herd were culled. Of the 1237 heifers inseminated, on all farms, only 49% conceived after 1 insemination and a further 25.5% after the PRID protocol.

In conclusion, the use of progesterone devices in the form of Prid-delta helps to increase chances to conceive by reestablishing the hypothalamic response to estradiol that the dominant follicle produces which in turn allows for the LH surge to occur. This estradiol responsiveness increases the likelihood of estrus and normal luteal phase to allow for pregnancy to occur. This is especially important in anovular cows and so helps reduce cull cow numbers per year (around 5%).

8. Összefoglalás

A tanulmányban egy Nyugat-magyarországi tejelő szarvasmarha állomány három telepén tartott 2673 állat szaporodásbiológiai mutatóit hasonlítottuk össze dupla-ovsynch és progeszteron intravaginális készítmény használata után. Az állomány minden egyede dupla-ovsynch protokol után lett először inszeminálva, Azok az állatok, amelyek az első termékenyítés után nem lettek vemhesek, Prid-delta, progoszteron tartalmú készítményt kaptak, intravaginálisan behelyezve. A hüvelyi implantátum hét nap után lett eltávolítva. Azok az egyedek, amelyek nem lettek vemhesek, a harmadik és negyedik termékenyítés előtt is Prid-synch protokollal lettek szinkronizálva. A negyedik, eredménytelen termékenyítés után az állatok selejtezésre kerültek.

Vizsgálataink alapján megállapítható, hogy az első telepen az első termékenyítés után a termékenységi (fertilitási) ráta 43,40%, a második telepen 45,35%, a harrmadik telepen 42,90% volt a dupla-ovssynch protokol után.

Az első telep esetében a második, harmadik illetve negyedik termékenyítés utáni fertilitási ráta 24,35%, 15,72% és 16,53% volt a progeszteron implantátum használata után. A negyedik eredménytelen termékenyítés után az állatok selejtezésre kerültek, mindösszesen 47 (5%) egyed.

A második telep esetében a második, harmadik illetve negyedik termékenyítés utáni fertilitási ráta 25,61%, 14,78% és 14,26% volt a progeszteron implantátum használata után. E telep esetében 41 (4,8%) állat került selejtezésre.

A harmadik telep esetében a második, harmadik illetve negyedik termékenyítés utáni fertilitási ráta 28,42%, 15,79% és 12,89% volt a progeszteron implantátum használata után.

Két üszőtelepen tartott állatoknál szintén dupla-ovsynch protokollt alkalmaztak az első termékenyítés előtt, majd utána az üresen maradt állatoknál progeszteron tartalmú intravaginális készítményt használnak a következő termékenyítés előtt, ugyanolyan protokoll szerint, mint a teheneknél. Az első termékenyítés eredményességi rátája 49% volt, a második termékenyítésé, a Prid protokoll után 25,5%.

Eredményeink azt mutatják, hogy a progeszteron tartalmú intravaginális készítmények használata, mind a tehenek, mind a tenyészüszők esetében hatásos módszer a fertilitás növelésére, különösen a többszöri termékenyítések esetén, hiszen ebben az esetekben gyakran

acikliások az állatok, így működő sárgatest hiányában, alacsony a progeszteron szintjük, ami nagyban befolyásolja a termékenységi mutatókat. Vizsgálatunk során elemeztük a felhasznált hormonok költségét, ezen belül a felhasznált progeszteron implantátum költségét, amely egyéb, a protokollban résztvevő hormon költséggel együtt 16,78 euró kezelésenként, szemben a dupla ovsync protokoll 9,34 eurós költségével. Összeségében, azonban elmondható, hogy a fertilitási eredmények javulása a többszöri termékenyítések után igazolja a progeszteron tartalmú implantátumok használatát, hiszen az eredménytelen termékenyítések száma és az ezt követő selejtezés alacsony szinten (5%) maradt.

9. References

1. Ball, P.J.H. and McEwan, E.E.A. (1998) 'The incidence of prolonged luteal function following early resumption of ovarian activity in post partum dairy cows', Proceedings of the British Society of Animal Science, 1998, pp. 187–187. doi:10.1017/s1752756200598391.
2. Cardoso Consentini, C.E., Wiltbank, M.C. and Sartori, R. (2021) 'Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs', *Animals*, 11(2), p. 301. doi:10.3390/ani11020301.
3. Chebel, R.C. et al. (2006) 'Reproduction in Dairy Cows Following Progesterone Insert Presynchronization and Resynchronization Protocols', Journal of Dairy Science, 89(11), pp. 4205–4219. doi:10.3168/jds.s0022-0302(06)72466-3.
4. Denicol, A.C. *et al.* (2012) 'Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows', *Journal of Dairy Science*, 95(4), pp. 1794–1806. doi:10.3168/jds.2011-4650.
5. El-Zarkouny, S.Z. *et al.* (2004) 'Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone', *Journal of Dairy Science*, 87(4), pp. 1024–1037. doi:10.3168/jds.s0022-0302(04)73248-8.
6. Erb, HN. 1984. High milk production as a cause of cystic ovaries in dairy cows: Evidence to the contrary. Comp. Cont. Edu. 6:215-219.
7. ERGENE, O. (2012) 'Progesterone concentrations and pregnancy rates of repeat breeder cows following postinsemination prid and GnRH treatments*', *Turkish Journal of Veterinary & Animal Sciences* [Preprint]. doi:10.3906/vet-1103-3.
8. Fike, K E, et al. "Prolonged Increased Concentrations of 17beta-Estradiol Associated with Development of Persistent Ovarian Follicles Do Not Influence Conception Rates in Beef Cattle." *Journal of Animal Science*, vol. 75, no. 5, 1997, p. 1363, https://doi.org/10.2527/1997.7551363x. Accessed 28 Oct. 2021.
9. Fishman-Holland, H. *et al.* (2019) 'Comparison of follicular development, timing of ovulation and serum progesterone, estradiol and luteinizing hormone concentrations in dairy heifers treated with 4- or 5-day COSYNCH + CIDR protocols', *Veterinary Medicine and Science*, 5(3), pp. 379–389. doi:10.1002/vms3.171.
10. Forro, A. *et al.* (2015) 'Factors affecting the success of resynchronization protocols with or without progesterone supplementation in dairy cows', *Journal of Veterinary Science*, 16(1), p. 121. doi:10.4142/jvs.2015.16.1.121.
11. Fuquay, J.W. and Fox, P.J. (2011). *Encyclopedia of dairy sciences*. Editorial: Amsterdam: Elsevier Science.
12. Garcia-Ispierto, I. and F. López-Gatius. 2017. Progesterone supplementation in the early luteal phase after artificial insemination improves conception rates in high-producing cows. Theriogenology 90:20-24.
13. Gumen, A. J.N. Guenther, and M.C. Wiltbank. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J. Dairy Sci. 86:3184-3194.

14. Gumen, A., and M.C. Wiltbank. 2002. An alteration in the hypothalamic action of estradiol due to lack of progesterone exposure can cause follicular cysts in cattle. Biol. Reprod. 66:1689-1695.
15. Hancock JL. The clinical analysis of reproductive failure in cattle. Vet Rec 1948;60:513-517.
16. Hubner, A.M, et al. "Effect of GnRH 7 Days before Presynchronization with Simultaneous PGF2α and GnRH on Reproductive Outcomes in Holstein Dairy Cows." FrontiersinVeterinaryScience,vol.7,22Oct.2020, https://doi.org/10.3389/fvets.2020.574516. Accessed 16 Aug. 2023.
17. Lopez, H., D.Z. Caraviello, L.D. Satter, P.M. Fricke, and M.C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J. Dairy Sci. 88:2783-2793.
18. Lopez, H., L.D. Satter, and M.C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81:209-223
19. Moreira, F., R. Flores, J. Boucher, and J. Chenault. 2004b. Effects of CIDR inserts on first service pregnancy rates of lactating dairy cows submitted to a presynch program and on re-resynchronization of second service in Mexico. J. Dairy Sci. 87 (Suppl.1):256. (Abstr.)
20. Moreira, F., O. Orlandi, C.A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 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.
21. Robinson, N.A., Leslie, K.E. and Walton, J.S. (1989) 'Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in holstein cows', *Journal of Dairy Science*, 72(1), pp. 202–207. doi:10.3168/jds.s0022-0302(89)79098-6.
22. Sauls, J. A., and J. S. Stevenson. "Resynchronization of Lactating Dairy Cows at Open Pregnancy Diagnosis Based on the Presence or Absence of a Corpus Luteum: A Practical Approach." *Kansas Agricultural Experiment Station Research Reports*, vol. 3, no. 8, 1 Jan. 2017, https://doi.org/10.4148/2378-5977.7520.
23. Stevenson, J.S. *et al.* (2008) 'Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol', *Journal of Dairy Science*, 91(7), pp. 2901–2915. doi:10.3168/jds.2007-0856.
24. Stevenson, J.S. *et al.* (2006) 'Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch', *Journal of Dairy Science*, 89(7), pp. 2567–2578. doi:10.3168/jds.s0022-0302(06)72333-5.
25. van Werven, T. *et al.* (2013) 'Comparison of two intravaginal progesterone releasing devices (Prid-Delta vs CIDR) in dairy cows: Blood progesterone profile and field fertility', *Animal Reproduction Science*, 138(3–4), pp. 143–149. doi: 10.1016/j.anireprosci.2013.02.010.
26. Wiltbank, M.C. *et al.* (2014) 'Physiological and practical effects of progesterone on reproduction in dairy cattle', *Animal*, 8, pp. 70–81. doi:10.1017/s175173111400058

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