

CHAPTER 2

PRE-SYNCHRONIZATION PROTOCOLS IN DAIRY COWS

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INTRODUCTION

Dairy cows are aimed to produce one calf per year (Alan et al., 2000; Kalkan and Horoz, 2010; Xu et al., 2000). To achieve this goal, oestrus must be detected timely and accurately, cows should be inseminated at the correct time, and they must become pregnant as soon as possible (Kalkan and Horoz, 2010; Stephen et al., 1998). Recently, despite the use of the most modern techniques and technologies, the oestrus detection rate remains around 50% (Dinç and Kutlu, 2015). Failure to detect oestrus in farms practicing artificial insemination reduces insemination success and conception rates. Consequently, inadequate and inaccurate detection of oestrus is one of the major factors reducing reproductive fertility in cows (Lopez-Gatiús and Vega-Prieto, 1990). Detecting oestrus during the postpartum period (PP) is particularly challenging (CEVA Turkey, 2014). More than 60% of the first oestrus cycles observed between 15 and 34 days postpartum are classified as suboestrus (CEVA Turkey, 2014). This condition is attributed to insufficient development of the corpus luteum (CL), leading to a shorter lifespan. Moreover, the short duration or silent (suboestrus) nature of oestrus in high-yielding cows complicates its detection. Another contributing factor is the presence of infertile cows (anoestrus, suboestrus, non-cyclic). Therefore, non-cyclic cows should be identified early during the second half of the postpartum voluntary waiting period (VWP). Furthermore, genital tract infections and metabolic problems delaying the onset of postpartum ovarian activity should be diagnosed early (Dinç, 2013; Hudson et al., 2012).

A useful criterion for assessing oestrus detection is the first oestrus observed after calving. The target should be 80% by the end of VWP (typically 60 days) (Dinç and Kutlu, 2015). The first observed oestrous after calving is a valuable criterion for evaluating oestrus detection. However, the oestrus detection rate alone may not be a fully accurate assessment tool. The best submission rate can be calculated using the oestrus detection efficiency of herds. Submission rate is the most common method used to determine oestrus detection efficiency. It is defined as the number of cows or heifers served within an 18- or 24-day period, expressed as a percentage of the number of cows or heifers that are at or beyond the earliest date at the start of the 18- or 24-day period (CAFRE, 2005; Dinç, 2013; Esslemont et al., 1985). The submission rate responds to changes in the herd much faster and demonstrates trends over time more effectively (Hudson et al., 2012). Cows that complete the VWP are included in the calculation of the submission rate (CAFRE, 2005; Dinç, 2013). The VWP determines the beginning of the submission rate. The target submission rate for farms with a year-round calving policy should be 75% per 3-week period (CAFRE, 2005; Dinç, 2013; Hudson et al., 2012).

Submission Rate = Number of cows served during specific period (18-24 days) / Number of eligible cows (18-24 days) × 100

Pre-Synchronization Protocols to Increase Submission Rate

Oestrus synchronizations may increase the submission rate (Nebel and Jobst, 1998). Ovulation synchronization protocols (Ovsynch), which do not require oestrus monitoring to increase the submission rate, can be applied to problematic cows whose oestrus cannot be detected or to farm with low oestrus detection rates. There are pre-synchronization methods that increase the effectiveness of these protocols. The aim of pre-synchronization is to initiate treatment early after calving, rather than waiting for cows to exhibit oestrus after the VWP and insemination. Using ovulation synchronization protocols, cows are inseminated without the need to observe their oestrus. When this treatment is applied, the oestrus cycle of the cows will be regular and they show oestrus every 18-24 days, even if they do not become pregnant after the first insemination. Therefore, there will be at least 3 or 4 chances for insemination by the 125th day after calving. Different methods are used to increase the submission rate. Information about hormonal applications developed to enhance the submission rate is presented below. In this context, hormonal applications such as Presynch, Double-Ovsynch, G6G, PG-3-G, and PG+G are significant (Figure 1 and Table 1).

If ovulation synchronization protocols are initiated during the early luteal phase of the oestrus cycle (in the presence of a dominant follicle—dioestrus between days 5 to 9 of the oestrus cycle), a high pregnancy rate can be achieved. The best response is observed when a dominant follicle with a diameter of 10 mm is present. Cows that began Ovsynch on days 5 to 9 also exhibited greater circulating P4 levels at the time of PGF2 α treatment (3.6 ng/ml), likely due to the presence of two CL, compared to cows that initiated Ovsynch on days 1 to 4 (2.5 ng/ml) (Moreira et al., 2000; Souza et al., 2008; Wiltbank and Pursley, 2014). In cows that began Ovsynch on days 1 to 4, the first GnRH treatment rarely resulted in ovulation. Consequently, cows on the day 1 to 4 schedule developed an older and larger follicle that ovulated in response to the second GnRH treatment (19.2 mm), compared to cows that began Ovsynch on days 5 to 9 (16.8 mm). When the Ovsynch protocol is initiated on days 15 to 21 of the oestrus cycle, cows may be in oestrus during the PGF2 α injection. In this case, the follicle is small and does not develop a new CL, resulting in a low P4 concentration (Vasconcelos et al., 1999; Wiltbank and Pursley, 2014).

Pre-synchronization methods (Presynch) containing only PGF2 α may fail to stimulate oestrus and increase fertility in anovular, non-cyclic animals (Bisinotto et al., 2014). This is a common issue in high-yielding cows after the VWP, with rates ranging from 7.1% to 41.7% (Bamber et al., 2009). Since PGF2 α cannot alter the course of follicular development, differences in oestrus and ovulation timing caused by the developmental phase of the follicular wave may lead to complications when applied. To address this, pre-synchronization protocols that combine PGF2 α and GnRH, such as Double-Ovsynch, PG +

G, G6G, and PG-3-G, have been developed. These protocols synchronize the onset of the Ovsynch protocol with the early luteal phase of the oestrus cycle (in the presence of a dominant follicle—dioestrus between days 5 to 9 of the oestrus cycle), resulting in a high pregnancy rate in anovular cows.

Presynch

The Presynch protocol positively affects fertility by increasing the number of oestrus, improving the uterine environment and immune system, and enhancing embryo survival (Cavalieri et al., 2006). It was first applied by Moreira et al. (2001) (Moreira et al., 2001). The aim of Presynch is to achieve a 100% submission rate by the end of the VWP and ensure that animals are inseminated as soon as possible. Before starting the ovulation synchronization protocol, PGF2 α is administered twice at 14-day intervals (Figure 1). In the original Presynch protocol, ovulation synchronization methods are initiated 12 days after the second PGF2 α administration (Moreira et al., 2001). If cows respond to the first PGF2 α , they enter the luteal stage and respond to the second PGF2 α 14 days later. If cows do not respond to the first PGF2 α , they will enter the luteal stage within 14 days and then respond to the second PGF2 α . During Ovsynch, which begins 14 days after the second PGF2 α administration, cows are typically between days 5–14 of the oestrus cycle. When Presynch-14, Presynch-12, Presynch-11, or Presynch-10 is used as a pre-synchronization program, cows are generally between days 9–14, 7–12, 6–11, and 5–10 of the oestrus cycle, respectively, at the time ovulation synchronization begins (Stevenson et al., 2012). Presynch is a pre-synchronization method commonly used during the PP)period. Early initiation of PP administration may reduce problems such as ovarian cysts and pyometra, while increasing luteal activity, oestrus, ovulation, and pregnancy rates (Lopez-Gatius and Vega-Prieto, 1990). Presynch can stimulate oestrus in up to 75% of anovular animals (Thatcher et al., 2004). Presynch-Ovsynch should be regarded as a comprehensive treatment method rather than merely a tool to increase pregnancy rates in high-yielding cows (Wiltbank and Pursley, 2014).

Double-Ovsynch

In the Ovsynch protocol, cows are not found to be in appropriate stages of the cycle in terms of follicular development and lower success is achieved in non-cyclic cows. To address these limitations, the Double-Ovsynch protocol has been developed, which partially overcomes these disadvantages (Souza et al., 2008). The initiation of the second Ovsynch protocol in the Double-Ovsynch protocol during days 5–12 of the oestrus cycle significantly improves synchronization rates. Compared to cows treated with the Ovsynch protocol alone, the Double-Ovsynch protocol offers the advantage of eliminating the need to determine the stage of the oestrus cycle at the start of the protocol (Bilgen and Özenç, 2010). The Double-Ovsynch protocol posi-

tively affects fertility by increasing the number of cows in the early dioestrus stage at the start of the second Ovsynch administration and by raising serum progesterone concentrations during the protocol (Souza et al., 2008). In Double-Ovsynch protocol, ovulation occurs after the first administration of GnRH, followed by the formation of a corpus luteum (CL) and the emergence of a new follicular wave. This is similar to the process observed after the first GnRH injection in the Ovsynch protocol. However, the Double-Ovsynch protocol offers additional benefits, including ovulation before timed artificial insemination (TAI). Seven days after the first administration of PGF2 α , the CL regresses, and ovulation occurs. Three days later, a second administration of GnRH induces a new follicular wave. Seven days after that, a third GnRH injection induces ovulation again. At this point, there are likely two CLs in the ovaries: one formed by the second GnRH and the other by the third GnRH. Seven days later, the administration of PGF2 α causes the regression of these two CLs. A fourth GnRH injection is then administered to stimulate ovulation, followed by TAI. The primary advantage of this protocol is that it is 11 days shorter than the classic Presynch-14 protocol. The high progesterone concentration in the follicular environment in response to the third GnRH administration leads to more cows ovulating and being fertilized after TAI. Both factors—enhanced ovulation rates and progesterone enrichment in the follicular environment—have been shown to improve fertility outcomes with TAI. Additionally, about 40–45% of cows show oestrus during TAI, and the protocol also facilitates the initiation of the first ovulation in anovular cows through the administration of either the first or second GnRH before starting Ovsynch. However, the primary disadvantage of the Double-Ovsynch protocol is its higher cost compared to the Presynch-Ovsynch protocol (Astis and Fargas, 2013; Carvalho et al., 2014; Herlihy et al., 2012; Stevenson, 2012b).

G6G

In this protocol, the goal is to regress a young, middle or old CL that may be present in ovaries by starting with a PGF2 α injection. Forty-eight hours after the injection, GnRH is administered to induce ovulation and the formation of a new CL. The purpose of these two consecutive applications is to initiate a new synchronized oestrus cycle and stimulate a new wave of follicular growth. This pre-synchronization program ensures the presence of a functional dominant follicle ready for ovulation, which will respond to the first GnRH injection of the Ovsynch protocol, initiated six days later. The new CL formed by the G6G protocol increases progesterone concentrations during the seven-day follicular development and maturation period leading up to TAI. These elevated progesterone levels positively affect follicle development and oocyte quality (Astis and Fargas, 2013; Bello et al., 2006; Stevenson, 2012a; Yilmaz et al., 2011). When the G6G protocol is applied, the Ovsynch protocol begins during days 4, 5, or 6 of the oestrus cycle (Pursley, 2015; Ste-

venson, 2011). The G6G protocol is particularly suitable for cows that do not have a CL present during examination (Pursley and Martins, 2011), multiparous cows undergoing their first insemination (Astis and Fargas, 2013) and anovular cows (Ribeiro et al., 2011).

PG-3-G

Cows treated with the PG-3-G protocol were injected with PGF2 α 10 days and GnRH 7 days before the start of the Ovsynch protocol. In this protocol, 90% of cows are in the middle of the luteal phase at the beginning of the ovulation synchronization protocol. Therefore, cows with a dominant follicle in the early stages of a follicular wave (<10 mm) at the time of PGF2 α administration would likely develop a follicle with an antral diameter of ≥ 10 mm and have LH receptors at the time of the subsequent GnRH injection 3 days later, responding with ovulation (Peters and Pursley, 2002). When the G6G protocol is applied, the Ovsynch protocol begins during days 5–6 of the oestrus cycle (Stevenson, 2011). The PG-3-G protocol increases the ovulation rate and 7-day luteal function before Ovsynch, resulting in better synchronization of follicular growth and improving the potential to increase pregnancy rates (Stevenson et al., 2012).

PG+G

The PG + G protocol involves the simultaneous administration of GnRH and PGF2 α injections 7 days before the start of Ovsynch. Pregnancy rates are similar when compared to the G6G protocol; however, the potential progesterone concentration during ovulatory follicle growth is lower. In this protocol, the simultaneous injections of GnRH and PGF2 α at the beginning reduce labor costs and shorten the duration of the protocol compared to the G6G and PG-3-G protocols. It serves as an alternative to more complex pre-synchronization protocols (Martins et al., 2017; Yousuf et al., 2016).

Result

As a result, the choice and effectiveness of the pre-synchronization program to be used will depend on the farm's resources, objectives, and the veterinarian's preference (Wiltbank and Pursley, 2014). It is important to note that GnRH administration can potentially reduce oestrus in cows when pre-synchronization programs are implemented (Chebel et al., 2013; Lopes et al., 2013; Mendonca et al., 2012). On the other hand, the duration of the pre-synchronization period before TAI should not pose a significant issue. The main considerations are the costs, the level of fertility on the farm, and the effectiveness of farm management.

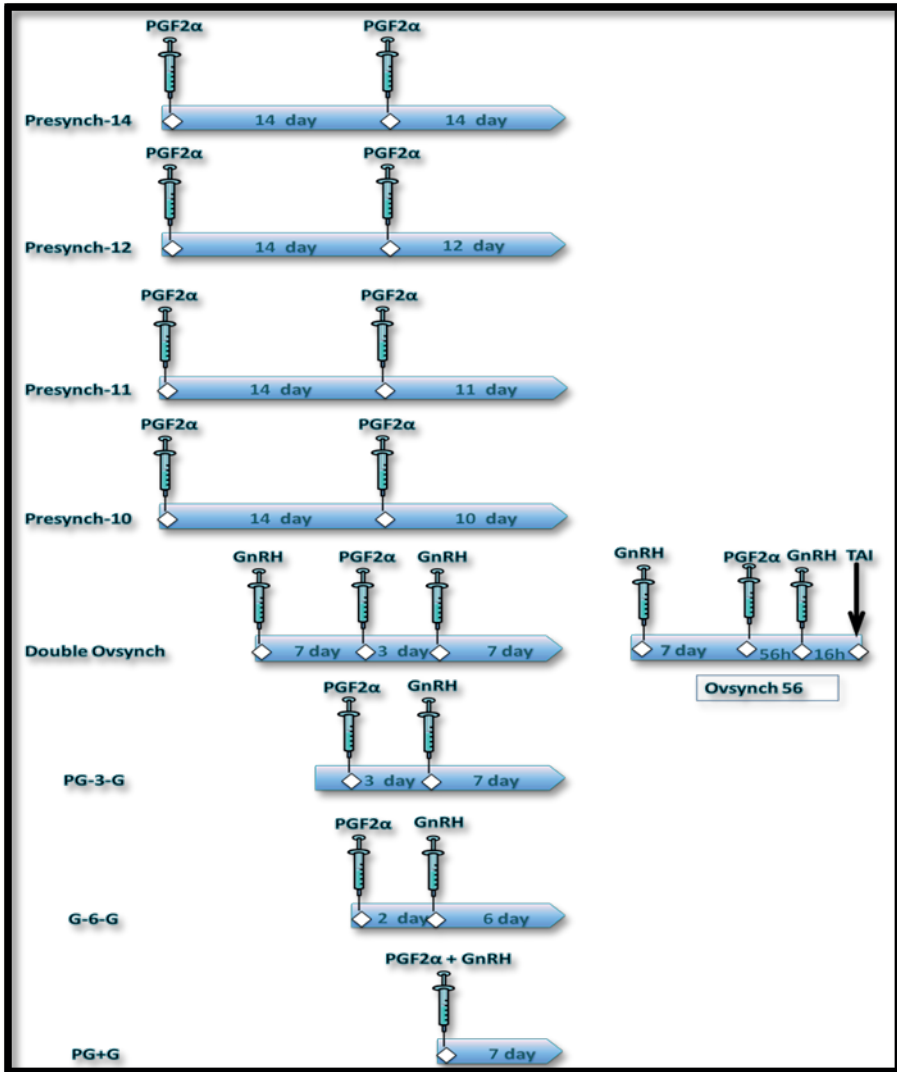


Figure 1. Pre-synchronization protocols

Table 1. Pre-synchronization protocols pregnancy rates

Gebe Kalma Oranları	Ovsynch	Presynch	Double-Ovsynch	G6G	PG-3-G	PG+G
Moreira et al. (2001) (PP 37)(All Cows)	6% (n=97)	36.9% (n=88) (Presynch-12)				
Moreira et al. (2001) (PP 37) (Cycle Cows)	34.4% (n=67)	46.9% (n=66) (Presynch-12)				
El-Zarkouny et al. (2004) (PP 59-79)	37.5% (n=304)	46.8% (n=310) (Presynch-12)				
Navanukraw et al. (2004) (PP 60>)	37.3% (n=134) (PP 99)	49.6% (n=135) (PP 89) (Presynch-14)				
Aköz et al. (2008) (n=104) (PP 26-41)		43% (n=54)(Presynch-14)				
Carvalho et al. (2014) (n=661)			52.6% (n=306)			
Herlihy et al. (2012) (n=1687) (PP 57-68)		Primipar=42.3% Multipar=34.3% (Presynch-12)	Primipar= 52.5% Multipar=40.3%			
Souza et al. (2008) (PP 42>)		Primipar=45% Multipar=39% (Presynch-12) (n=180) (PP 42±3)	Primipar= 65.2% Multipar=37.5% (n=157) (PP 51±3)			
Bilgen and Özenç (2010) (n=51) (PP 25, 32, 39)			Primipar= 75% (PP 32) Primipar=25%(PP 25,39) Multipar= 75% (PP 39) Multipar=37.5%(PP 25)			
Astiz and Fargas (2013) (n=7805) (PP 87±10)			Primipar= 44.3% Multipar=31.4% (n=6783)	Primipar=34.7% Multipar=34.8% (n=1022)		
Bello et al. (Bello et al.) (PP 62-72)	27% (n=34)	-	-	50% (n=32)		

Yilmaz et al. (2011) (Cows) (PP 50-100)	37.8% (n=37) (PP 83±2)	-	-	53.8% (n=119) (PP 88±3)
Pursley and Martins (2011)				61.4% (n=800)
Riberio et al. (2012)				28.7% (n=178) 5-day cosynch72 (PG) 45.4% (n=185) 5-day cosynch72 PG ^{1/2+1/2})
Riberio et al. (2011)	49.1% (n=632) (Presynch-11)			49.9% (n=625) 5-day cosynch72 (PG ^{1/2+1/2})
Sonat et al. (2014) (PP 45-90)				45% (n=40)
Dirandeh et al. (2015)				39.5% (n=250)
Heidari et al. (2017) (PP 28 ± 3)				32.9% (n=149)(PG) 37.5% (n=144) (PG ^{1/2+1/2})
Peters and Pursley (2002) (PP 49-55) (n=427)	%38.3			41.5%
Stevenson et al. (2012)	33.3% (n=105) (45.7% cold season) (n=71) (PP 50) (Presynch-11)			40% (n=105) (59.1% cold season) (n=66) (PP 36)
Stevenson and Pulley (2012) (PP 42)	35.0% (n=1247) (44.3% cold season) (26.7% hot season) (Presynch-10)			41.2% (n=1286) (46.8% cold season) (n=465) (35.9% hot season) (n=421)
Pulley et al. (2015)	52.6% (n=19) (GnRH 56) 22.2% (n=18) (GnRH 72) (Presynch-10) (PP 34)			57.1% (n=14) (GnRH 56) 56.3% (n=16) (GnRH 72) (PP 45)

Stevenson et al. (2018)		39.1% 7-day ovsynch (PG) 30.0% 7-day ovsynch (PG1/2+1/2) 35.0% 5-day ovsynch (PG) 38.7% 5-day ovsynch (PG1/2+1/2) (n=194)		52.1% 7-day ovsynch (PG) 44.0% 7-day ovsynch (PG1/2+1/2) 25.2% 5-day ovsynch (PG) 39.4% 5-day ovsynch (PG1/2+1/2) (n=201)
Kutlu (2015) (PP 28-40)			42.9% (n=35)	51.4% (n=35)
Yousuf et al. (2016) (PP 58-64)			57% (n=114)	50% (n=121)
Martins et al. (2017) (PP 41-47)	%48 (n=214) (Presynch-10)			%46 (n=218)

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