

SYNCHRONIZATION OF ESTRUS AND OVULATION IN DAIRY CATTLE

Sincronización de celo y ovulación en vacas lecheras

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ABSTRACT

Development of bovine estrus synchronization protocols started in the 1970's. The aim of these protocols has changed progressively from synchronizing estrus to synchronizing ovulation with main goal of performing insemination without heat detection (Timed artificial insemination). The present paper discusses the physiology behind development of these synchronization protocols and reviews of the main protocols used nowadays in dairy herd reproductive management.

Keywords: Ovsynch, Presynchronization, Resynchronization, Pregnancy risk

RESUMEN

El desarrollo de los protocolos de sincronización del celo en bovinos comenzó en la década de 1970. El objetivo de estos protocolos ha cambiado progresivamente de la sincronización del celo a la sincronización de la ovulación con el objetivo de realizar la inseminación sin detección de celo (inseminación artificial a tiempo fijo). El presente artículo discute la fisiología detrás del desarrollo de estos protocolos de sincronización y las revisiones de los principales protocolos utilizados hoy en día en el manejo reproductivo del hato lechero.

Palabras clave: Ovsynch, Presincronización, Resincronización, Riesgo de preñez

INTRODUCCION

One of the most challenging aspects in the management of dairy cattle reproduction is heat detection particularly in large high producing dairies. Heat detection rate is below 50% in most North American dairies (Senger, 1994; Scanavez et al., 2019). Poor heat detection results in a fewer number of eligible cows and heifers submitted to artificial insemination (AI) in a timely manner. This results in increased age at first calving and poor pregnancy rate. Multiple factors contribute to heat detection inefficiency including inadequate observation, management, cow level of production, social interaction, housing, and lameness, (Fricke et al., 2014; Mendonca et al., 2019). Estrous synchronization strategies have been devised since the 1970's. Early approaches were based on hormonal protocols to synchronize estrus in a group of cows with the objective of improving heat detection and AI submission rates. Synchronization of estrus combined with the use of heat detection aids allowed partial alleviation of this problem (Marquez et al., 2019). In the late 1980's and early 1990's, new techniques were developed to eliminate the need for heat detection all together and led to the development of fixed-time or timed artificial insemination (TAI) (Seguin et al., 1989).

Today, the majority of dairy operations rely on these ovulation synchronization protocols for reproductive management. Two common strategies used by dairy farms are the submission of all cows to TAI or a combination of insemination at detection estrus (EDAI) and TAI (Ferguson and Skidmore, 2013; Stangaferro et al., 2019). Although synchronization protocols are used in heifers and lactating dairy cows, the main financial advantages is in the latter group. The use of ovulation synchronization and TAI allows submission of a significantly higher proportion of eligible cows to AI and reduce the number days to first insemination and days open (days to first conception). Ovulation synchronization have been further improved by the use of presynchronization protocols allowing more precise TAI at specific times after the voluntary waiting

period. Selection of a synchronization protocol should always keep in mind the desired voluntary waiting period, the herd production level and the lactation number of cows enrolled (primiparous vs. multiparous) (Stangaferro et al., 2019).

Advances in the strategies of estrus/ovulation synchronization have been possible because of better understanding of follicular dynamics in cattle. The aim of the present paper is to discuss the physiological principles of estrus and ovulation synchronization and describe the most common protocols used in dairy cattle reproduction as well as factors affecting pregnancy per insemination.

PRINCIPLES OF SYNCHRONIZATION OF FOLLICULAR WAVES AND OVULATION

Ultrasonographic monitoring of follicular dynamics in cattle along with endocrine profiles allowed clinical characterization of the estrous cycle in the cows. The bovine estrous cycle possesses 2 or 3 follicular waves. Four follicular wave cycles are possible but rare. A new follicular wave usually starts right after ovulation. Under the influence of FSH, 4 or 5 antral follicles emerge from the pool of follicles. These emerging follicles grow to a diameter of 4 mm (visible on ultrasonography, Figure 1). After 2 or 3 days, one of these follicles establishes dominance through secretion of estradiol and inhibin forcing the subordinate follicles to undergo atresia and regression. The dominant follicle continues to grow and develops receptors for LH. However, ovulation cannot occur because of the inhibition of an LH surge in presence of a functional corpus luteum (luteal phase, high progesteronemia), and the dominant follicle undergoes atresia. Loss of the endocrine function and regression of a dominant follicle from this first wave alleviates the negative feedback on FSH resulting in the emergence of another follicular wave.

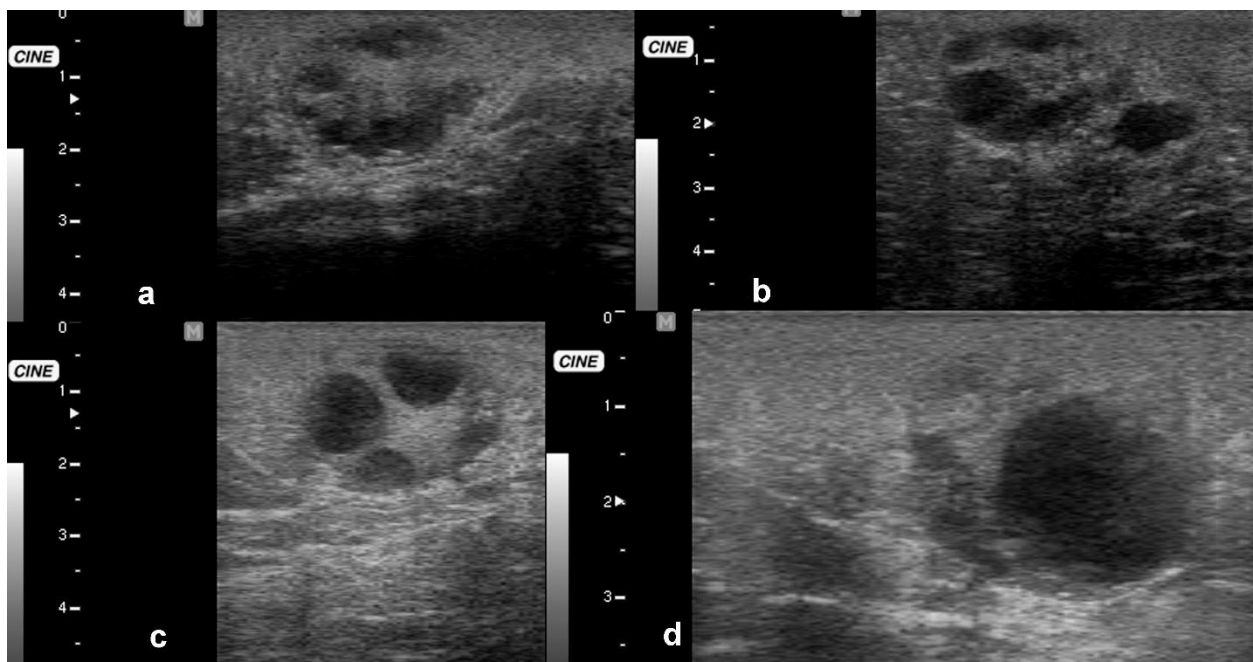


Figure 1. Ultrasonographic imaging of a follicular wave: a) recruitment, b) follicular growth, c) dominance, d) pre-ovulatory follicle.

In a two follicular waves cycle, the second follicular wave emerges between day 9 and 10 of the cycle (Day 0= day of ovulation). The dominant follicle from this wave continues to grow after luteolysis and eventually ovulates in response to the surge of LH (Figure 2). In a 3-wave cycle, the second follicular wave emerges between day 8 and 9 of the cycle ending with regression of the dominant follicle followed by emergence of a

3rd wave on day 15 or 16 of the cycle with a dominant follicle continuing to grow until ovulation (Figure 3). The number of follicular waves in a cycle depends on the length of dominance in the first follicular wave. Two-follicular wave cycles are generally shorter (20 days) than 3-wave cycles (23 days) (Adams, 1999; Adams et al., 2008).

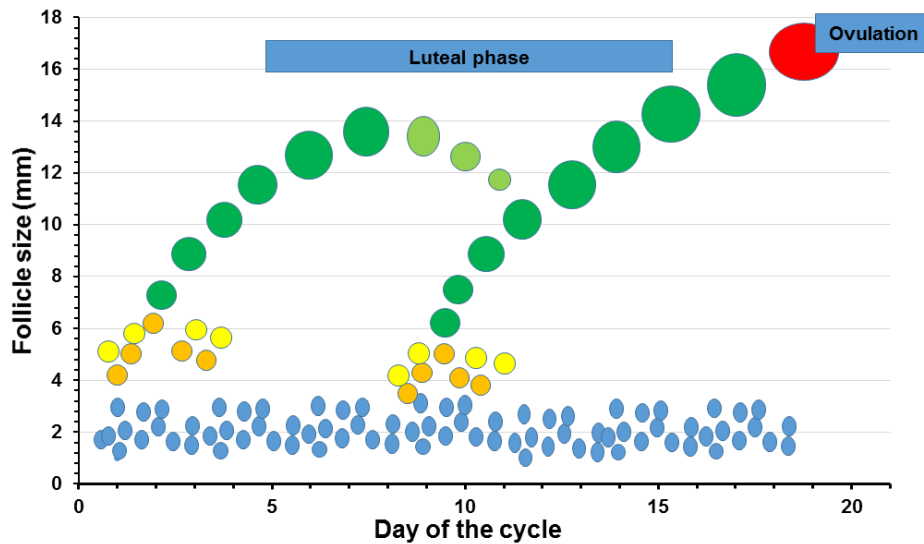


Figure 2. Schematic representation of a 2-follicular wave cycle

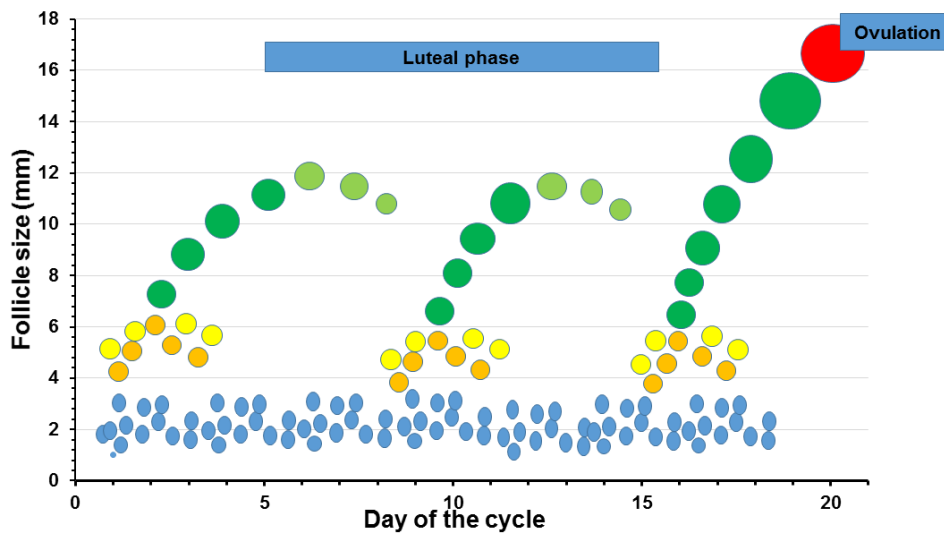


Figure 3. Schematic representation of a 3-follicular wave cycle

Studies characterizing the follicular wave pattern in cattle allowed further development of strategies to control follicular development leading to synchronization programs. The general principle for synchronization of the follicular wave and ovulation is to control emergence of the follicular wave and establishment of a new dominant follicle in a predictable manner. Although control of the follicular wave can be achieved using estrogen and progesterone, the use of the

former is forbidden in several countries (Mapletoft et al., 2018; Yapura et al., 2018; Yu et al., 2018). Today, in North America, Europe, Australia and New Zealand, all synchronization programs are based on the combination of GnRH, PGF2 α and progesterone.

ESTRUS SYNCHRONIZATION AND AI FOLLOWING ESTRUS DETECTION.

The most common methods for synchronization of estrus are the use of a double regimen PGF_{2α} or an intravaginal progesterone releasing devices (Controlled Internal Drug Releasing Devices, CIDR or Progesterone Releasing Internal Devices, PRID). These programs have been established since 1980's.

The standard PGF_{2α} program consists of an administration of two luteolytic doses of PGF_{2α} (Dinoprost tromethamine, 25 mg) or its analogue (cloprostenol, 500μg) at 11 to 14 days interval (Figures 4a). The 11-day interval is better for heifers while the 14 days interval is better for lactating cows (Seguin, 1997).

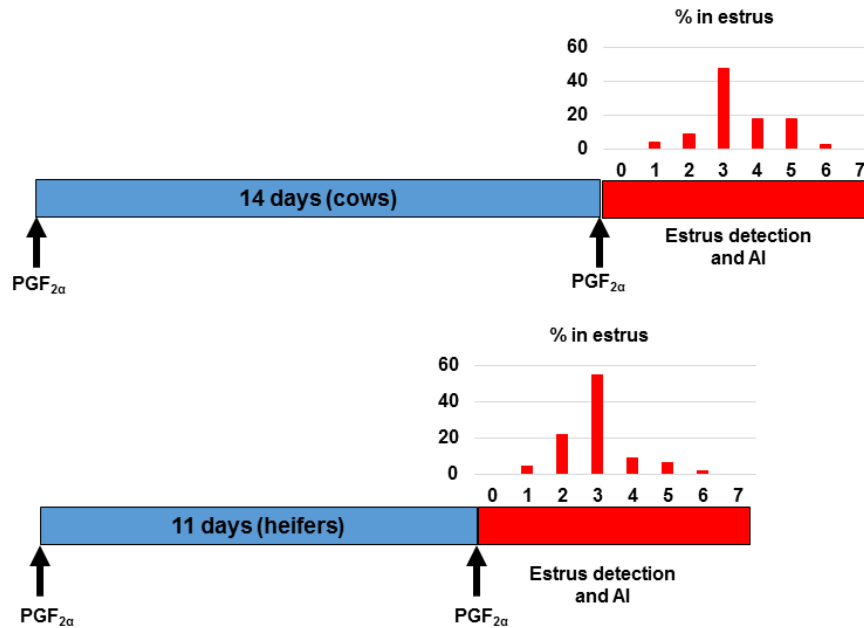


Figure 4a. Synchronization of estrus with double injection of PGF_{2α} 14 days (cows) or 11 days (heifers) apart with predicted distribution of estrus.

Attempt to use these protocols in a fixed-time artificial insemination yielded variable results. In the double PGF_{2α} program, TAI is performed at 72 to 80 hrs. for cows and 60 to 68 hrs. for heifers (Seguin, 1997; Seguin, 1989) (Figure 4b). In one study on dairy cows, there was no difference in conception rates between control (heat detection without synchronization, 50%), AI at 75 to 80 hrs. (46%) or AI at 72 and 96 hrs. (47%).

However, the pregnancy rate following PGF_{2α} synchronization and TAI was superior to that of the control (47% vs 30%) because only 61% of the control group cows were detected in heat and submitted to AI (Momont, 1985; Seguin, 1997). In heifers, a single TAI at 61±1 hours after the second injection of cloprostenol resulted in a pregnancy rate of 53% in cyclic animals (Seguin et al., 1989).

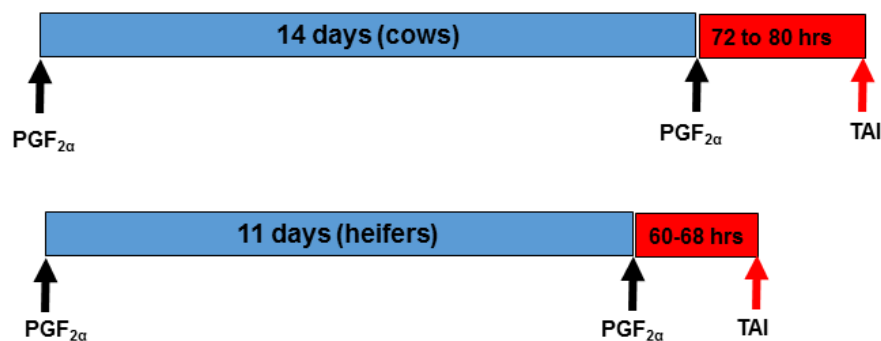


Figure 4b. Fixed time AI in cows and heifers after a double injection of PGF_{2α}

Several types of progestogen have been marketed for synchronization of estrus in cattle. Today the most common

protocol involves the use vaginal dispensing devices CIDR (1.38 g of progesterone or PRID (1.55 g progesterone) (Figure 5).

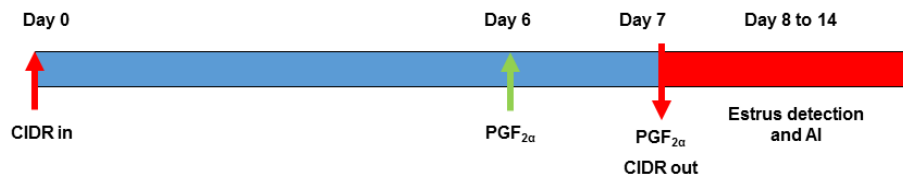


Figure 5. Synchronization protocol with CIDR containing 1.3 mg progesterone. CIDR is inserted for 7 days, PGF_{2α} is administered on day 6 or day 7 and estrus is detected for 7 days after treatment (70% of heifers are expected to show estrus in the 7 days).

SYNCHRONIZATION AND TIMED ARTIFICIAL INSEMINATION

Fixed-time artificial insemination or timed artificial insemination (TAI) presents the advantage of eliminating heat detection resulting in an insemination risk of 100% of eligible females. In North America, the most common synchronization protocol for TAI of dairy cattle is the OvSynch® protocol or one of its modifications. The original protocol developed in 1995 by Pursley et al. (Pursley et al., 1995) has been tweaked in different manners to fit management issues (Bisinotto et al., 2014; Wiltbank and Pursley, 2014) (Figure 6). The protocol consists of a first injection of GnRH to eliminate any dominant follicle (ovulation or luteinization) and to initiate a new follicular

wave within 1 or 2 days. This injection is followed 7 days later by a luteolytic dose of PGF_{2α} or analogues, to eliminate any luteal activity (i.e. regression of the corpus luteum). The dominant follicle is expected to grow to an ovulatory size within 48 hours after the administration of PGF_{2α}. Cows enrolled in this protocol receive a second GnRH injection 56 hours post PGF_{2α} and are inseminated at 72 hours (Pursley et al., 1998). In the original protocol, TAI at 8 to 16 hours after the second GnRH resulted in similar conception rate. This synchronization program has become known as the OvSynch-56 (Pursley et al., 1998; Brusveen et al., 2008). The expected pregnancy risk after TAI ranges from 30 to 40% (Seguin, 1997).



Figure 6. OvSynch-56 protocol: GnRH is administered on day 0, PGF_{2α} on day 7 followed by a second GnRH 56 hours later and timed artificial insemination 16 hours after GnRH.

In order to reduce animal handling, variants of the original OvSynch program were devised where the AI is performed at the same time as the second GnRH injection “CoSynch” at 48 hours (Cosynch-48) or 72 hours (CoSynch-72) (Figure 7) (Alnimer et al., 2009; Karakas et al., 2009; Alnimer and Ababneh, 2014). However, pregnancy risk are lower

compared to the OvSynch protocol and vary between 25 and 35% (Karakas et al., 2009; Alnimer and Ababneh, 2014; Borchardt et al., 2018). Cosynch-56 has been tested as well but produced significantly less pregnancies per AI than the traditional OvSynch 56 when frozen-thawed semen was used (Borchardt et al., 2018).



Figure 7. CoSynch-72 protocol. Estrus detection is performed for 72 hours after administration of PGF_{2α} and all cows not detected in estrus are given GnRH and inseminated at 72 hours.

Another modification of the protocol consisting of shortening the interval between the first GnRH injection and administration of PGF_{2α} from 7 days to 5 days has been proposed by other authors (Figure 8). In this short protocol, 2 injections of PGF_{2α}

or analogues are required (on day 5 and day 6) to insure luteolysis (Ribeiro et al., 2012; Say et al., 2016; Macmillan et al., 2017). In addition, the interval from PGF_{2α} to TAI should be extended to 72 hours (5 day CoSynch-72).

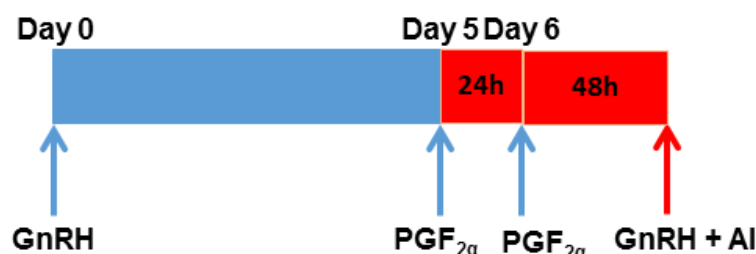


Figure 8. 5-day CoSynch-72 protocol.

A major modification of the OvSynch protocol consists of the insertion of a CIDR or PRID for the period between the first GnRH injection and the PGF_{2α} injection (Rivera et al., 2005; Stevenson, 2011; Bisinotto et al., 2015a). This progesterone supplementation during the OvSynch protocol seems to benefit cows that are anovular or not in diestrus at the beginning of the program. An analysis of several studies showed a significant improvement (5% percentage point higher) in pregnancy rate for cows supplemented with progesterone (Stevenson et al., 2006; Stevenson, 2016b). In other studies, progesterone supplementation (one or 2 CIDR devices) significantly increased pregnancy rates of cows without CL at the time of initiation of OvSynch protocol (Bisinotto et al., 2013; Bisinotto et al., 2015a; Bisinotto et al., 2015b; Bisinotto et al., 2015c).

The main factors affecting pregnancy risk following the OvSynch protocols are the cow cyclicity and the timing of the first injection of GnRH in relationship to the stage of the cycle (Vasconcelos et al., 1999). Cows enrolled in the OvSynch protocol between days 5 and 12 after ovulation have greater pregnancy rate than cows enrolled in other stages of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000). Also, pregnancy outcome in the OvSynch protocol is greatly affected by the proportion of anovular cows and that of cows with a functional corpus luteum (high progesteronemia) at the time of PGF_{2α} (Stevenson et al., 2012; Stevenson, 2016a; Voelz et al., 2016). These observations led to the development of presynchronization programs to ensure that cows are cyclic and at the ideal stage of the estrous cycle prior to initiation of the OvSynch and TAI.

SYNCHRONIZATION AND TAI FOR DAIRY HEIFERS

The best pregnancy rate in heifers is obtained after heat detection either on natural estrus or after synchronization with PGF_{2α}. In these conditions, per service conception rate can surpass 60 to 65% (Seguin, 1997). However, TAI has been adopted by several dairies for heifers because of the same limitations in heat detection described above. In most situations, the resulting drop in pregnancy risk (generally 50 to 55%) is compensated for by the reduced loss of time when heat detection is not adequate.

One of the earlier common estrus synchronization programs is the a 7-day CIDR/CoSynch protocol combined with heat detection for 72 hours after PGF_{2α}. Heifers that do not show estrus during the 72 hours, receive and injection of GnRH and inseminated at the same time. In one study, there was no difference in pregnancy rate in heifers following 7-day CIDR with a PGF_{2α} on day 6 and TAI at 48 and 72 hrs. (PR=54.6±4.6%) or at 56 hrs. (60.2±4.5%) after CIDR removal (Walsh et al., 2017).

More recently, a 5-day CIDR/CoSynch-72 program was shown to be more reliable (Figure 9) (Lima et al., 2009, Masello, 2019; Rabaglino et al., 2010a; Rabaglino et al., 2010b; Rabaglino et al., 2010c). Pregnancy risk varies between 45 and 60%. There has been a debate on whether a single or 2 injections of PGF_{2α} are necessary at the time of CIDR withdrawal in this protocol. Overall it seems that a double injection of PGF_{2α} is not necessary if GnRH is not given at the time of CIDR insertion.

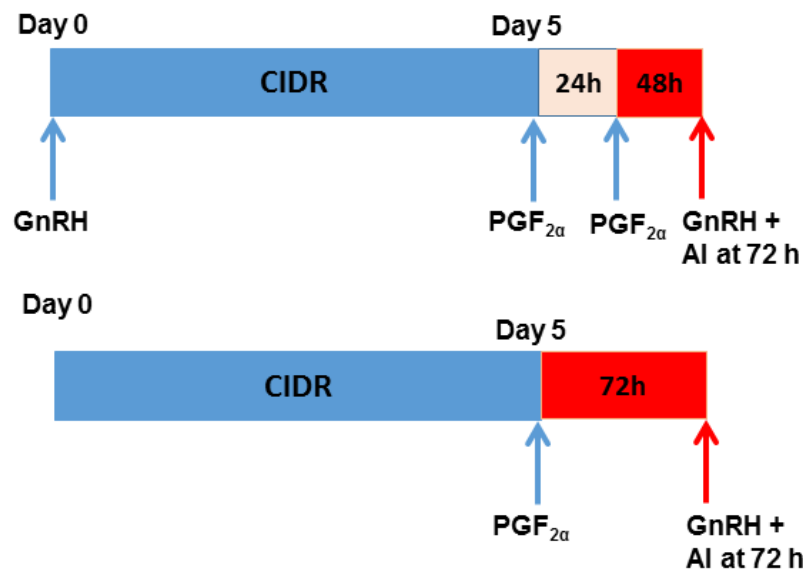


Figure 9. 5-day CIDR/CoSync protocols for heifers

PRESYNCHRONIZATION FOLLOWED BY OVSYNCH.

The main protocols for presynchronization of cows prior to implementation of the OvSynch protocol are based either on a PGF_{2α} or the addition of another OvSynch protocol (i.e. Double OvSynch). Other presynchronization protocols have been developed based on the use of GnRH.

Presynchronization with PGF_{2α} consists of the administration of 2 injections of a luteolytic dose of PGF_{2α} at 14 days interval (Figure 10). This treatment synchronizes estrus in the majority of cows between 2 to 4 days after the second injection of PGF_{2α}. The OvSynch protocol is then initiated 10, 11 or 14 days after

the second PGF_{2α} injection, which would be the ideal stage of the cycle (i.e. 5 to 12 days post-ovulation). The choice of the interval between the second injection of PGF_{2α} and initiation of the OvSynch protocol is generally dictated by the herd management (preferred day of the week of injections and TAI). Despite the complicated nature of the program, the majority of field trials have shown an increase in pregnancy rate by 6 to 12 percentage points using the presynchronization-OvSynch compared to OvSynch alone (Chebel et al., 2005; Melendez et al., 2006; Chebel and Santos, 2010; Chebel et al., 2013; Wiltbank and Pursley, 2014; Stangafarro et al., 2019). Presynch-11 was found to be superior to the presynch-14 (Galvao et al., 2007).

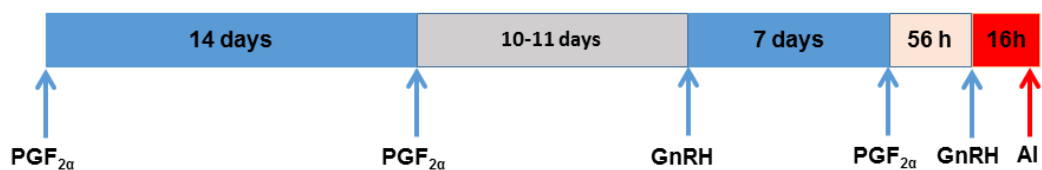


Figure 10. PreSynch-OvSynch protocol.

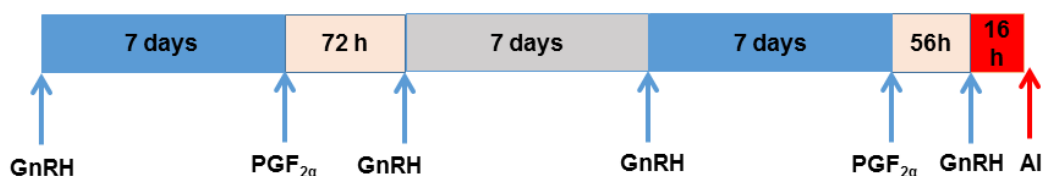


Figure 11. Double-OvSynch protocol

The double OvSynch protocol (Figure 11) and other presynchronization protocols based on GnRH have the additional advantage of improving cyclicity and pregnancy rate in anovular cows recruited into the program. Studies have shown that the proportion of dairy cows that are anovular at the end of the voluntary waiting period may be as high as 42% (Sterry et al., 2007; Bamber et al., 2009; Bisinotto et al., 2014; Fricke et al., 2014; Borchardt et al., 2017).

Several studies have shown the superiority of the double OvSynch program and other GnRH based presynchronization programs compared to the PGF2 α presynchronization in terms of pregnancy rate (Souza et al., 2008; Bilgen and Ozenc, 2010; Herlihy et al., 2012; Ayres et al., 2013; Dirandeh et al., 2015; Karakaya-Bilen et al., 2019; Luchterhand et al., 2019; Stangaferro et al., 2019). In one study, pregnancy per AI for primiparous and multiparous lactating dairy cows was

respectively 52.5% and 40.3% following Double-Ovsynch and 42.3% and 34.3% after Presynch-Ovsynch (Herlihy et al., 2012).

One of the major inconveniences of the Double-Ovsynch protocol is its length (28 days) and number of cow handling. To circumvent these problems a synchronization program known as G6G (Figure 12) (Bello et al., 2006; Yu et al., 2018) was devised. It has the advantage of being 9 days shorter and it was associated with higher pregnancy/AI in multiparous cows (Astiz and Fargas, 2013). A modification of this protocol (PG3-G) (Figure 13) has also been described and shown to improve follicular synchrony and pregnancy per AI (Stevenson and Pulley, 2012; Stevenson et al., 2012).

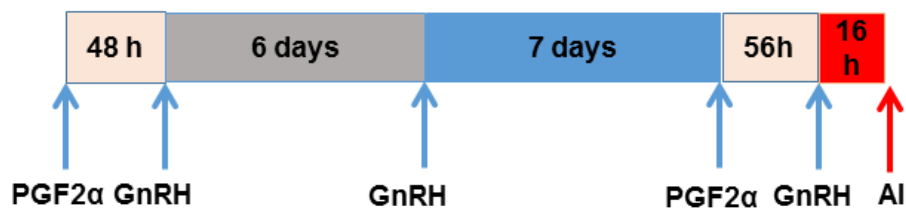


Figure 12. G6G presynchronization-OvSynch protocol.

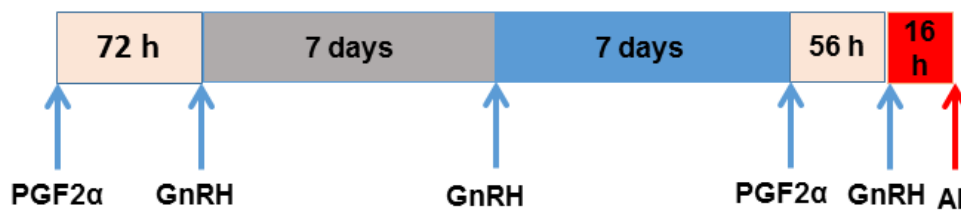


Figure 13. PG3-G presynchronization-OvSynch protocol.

The GnRH programs improve follicular activity and the proportion of cows responding to the OvSynch GnRH injection and to the Ovsynch PGF2 α (increased proportion of cows luteal activity and high progesteronemia) (Stevenson et al., 2012; Ayres et al., 2013). The type of GnRH used in these programs seems to have an impact on ovulation rate and fertility. In one study, cows treated with a hydrochloride-based GnRH product

in a double Ovsynch protocol had lower pregnancy rates than with other products (Luchterhand et al., 2019).

Another approach of presynchronization using a progesterone pretreatment combined with GnRH (Figure 14) was shown to be as efficacious as the Double-Ovsynch protocol in terms of pregnancy per AI at 30 and 60 days (Silva et al., 2018).

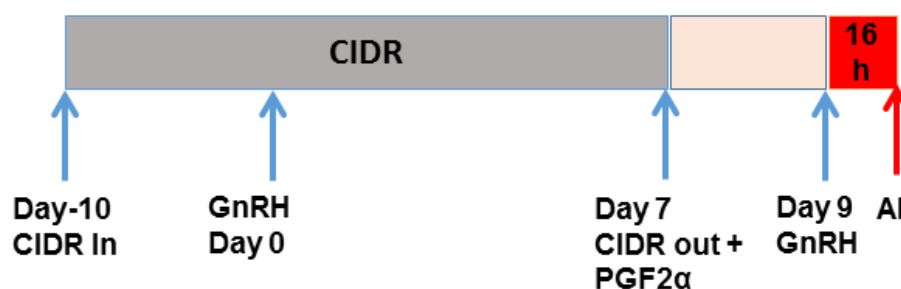


Figure 14. Progesterone presynchronization / OvSynch.

RESYNCHRONIZATION AFTER FIRST AI

As stated above, the primary reason for development of the synchronization and TAI protocols in dairy cattle is the poor AI submission rate that results from poor heat detection. This is even more important in non-pregnant (open) cows after the first insemination. One of the driving factors in reproductive efficiency is the early identification of open cows and prompt resubmission to insemination. Therefore, resynchronization of cows as soon as possible after the diagnosis of a non-pregnant status is critical. Several approaches have been considered and depend on method of pregnancy diagnosis and management considerations (i.e. cattle handling, heat detection strategies). Pregnancy diagnosis is generally performed with excellent accuracy by transrectal ultrasonography between 30 and 36 days after AI or by transrectal palpation between 37 and 43 days after AI.

A common practice, when heat detection is possible and adequate, is to administer PGF2 α at the time of negative pregnancy diagnosis followed by a resynchronization with OvSynch if cows are not seen in heat. In general the PGF2 α injection is given 7 or 11 days prior to the OvSynch (Silva et al., 2007; Bruno et al., 2013; Mendonca et al., 2019). An advantage of this system is that cows that are detected in heat

and re-inseminated become pregnant earlier (i.e. reduction of days open) (Chebel et al., 2013).

A resynchronization protocol that seems to offer an advantage in term of pregnancy rate consists of an injection of GnRH or hCG 5 or 7 days prior of pregnancy diagnosis. Open cows are then enrolled in an OvSynch protocol on the same day of negative pregnancy diagnosis. This protocol resulted in an increased pregnancy risk of resynchronized cows (Dewey et al., 2010; Giordano et al., 2012a; Giordano et al., 2012b; Bruno et al., 2014). However in one study presynchronization with GnRH 7 days prior to CoSynch did not improve pregnancy/AI (Alkar et al., 2011)

In a recent study, resynchronization with PGF2 α and GnRH "P7G7" (Figure 15) was shown to achieve better pregnancy outcome in herds with good heat detection (Mendonca et al., 2019).

An alternative resynchronization protocol is to include a progesterone (CIDR) treatment prior to initiation of the OvSynch. However, this treatment showed an increase in pregnancy risk with the 5-day OvSynch but not with 7-day OvSynch. The addition of a CIDR increased the pregnancy/AI particularly in cows without a corpus luteum at pregnancy diagnosis (Bilby et al., 2013; Chebel et al., 2013; Bisinotto et al., 2015a; Giordano et al., 2016).

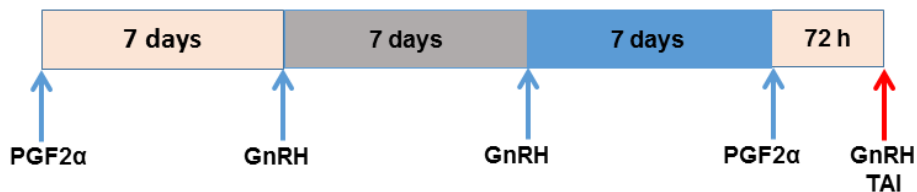


Figure 15. P7G7 resynchronization protocol. PGF2 α is administered at the time of negative pregnancy diagnosis.

INCORPORATION OF PRESYNCHRONIZATION-SYNCHRONIZATION AND TIMED AI IN A HERD

The objective of reproductive management in a dairy herd is to ensure that all eligible cows receive a first insemination in a timely manner after the voluntary waiting period (VWP). The

mean interval from calving to first insemination should then be VWP + 11 days (i.e. 71 days for dairies with a 60 day VWP). Based on this principle a presynchronization-synchronization program can be started at a specific day of lactation as illustrated in Figures 16 and 17.

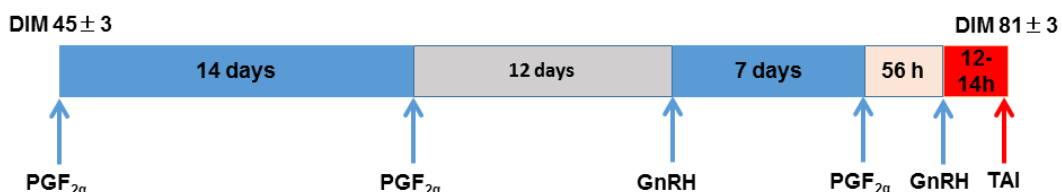


Figure 16. Example of presynch-OvSynch program started at 45 ± 3 days postpartum (DIM=days in milk) with the objective of realizing a first insemination at 81 ± 3 DIM. (adjusted pregnancy rate was 47.5% for primiparous and 34.3% for multiparous cows) (Herlihy et al., 2012).



Figure 17. Example of Double-OvSynch program started at 54 ± 3 days postpartum (DIM=days in milk) with the objective of realizing a first insemination at 81 ± 3 DIM. (Adjusted pregnancy rate was 52.5% for primiparous and 40.3% for multiparous cows) (Herlihy et al., 2012).

In one study, Double-OvSynch with TAI at 60DIM and Presynch-OvSynch with combined heat detection and TAI was shown to increase profitability for primiparous cows while Double-OvSynch with TAI at 80 DIM and Presynch-OvSynch was more advantageous for multiparous cows (Stangaferro et al., 2019). It is important to keep in mind that the timing of initiation of these protocols should take into consideration several factors including parity, herd exit dynamics and production level (Stangaferro et al., 2018a; Stangaferro et al., 2018c; b). In one study a combined estrus detection and TAI was more suitable for primiparous cows (Machado et al., 2017).

CONCLUSION

A tremendous research effort was spent on the development of synchronization programs since the 1970's. Today, it is estimated that most large dairies are using at least one type of estrus synchronization protocol and TAI. The choice of a protocol must be reasoned based on the management system, cost of labor, cost of drugs, facilities, and animal group size. One of the major challenges in implementation of these protocol is compliance as far as timing of treatment and proper hormone handling. Additionally, dairy managers may be attempted to change a protocol or add a new protocol without prior preparation of staff which can be very confusing. Finally, these synchronization protocols can help improve reproductive efficiency only if other health and production requirements are met. With TAI, technically all eligible cows can be submitted to artificial insemination. However, conception rate may be affected by several factors including reproductive health, body condition, metabolic disorders, cow comfort as well as season. Another factor that seems to influence pregnancy risk following TAI that was not discussed in the present paper is the bull (semen) used for insemination. Finally, implementation of synchronization protocols requires complete reproduction, health and production records. Analysis of these records is the only reliable way to compare efficiency of synchronization protocols.

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