

# Controlled Breeding Programs for Reproductive Management

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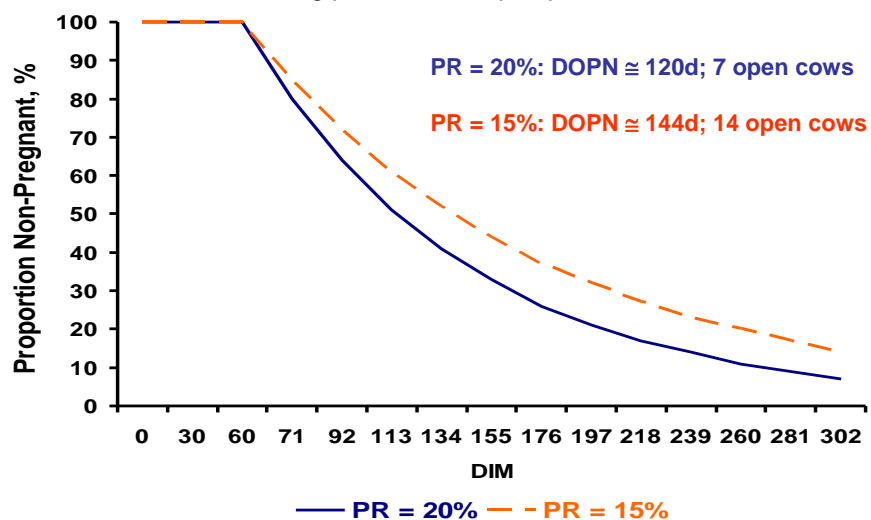
## ■ Introduction

Reproductive efficiency is a major component of economic success in dairy herds. The physiological and environmental stress of high producing dairy cows negatively affects estrus detection and fertility. Success of artificial insemination (**AI**) programs in dairy herds depends upon accurate and efficient detection of estrus. However, accuracy and efficiency of estrus detection is variable and depend upon animal, environmental, and management factors. In the high producing dairy cow the altered competence of follicles and the lower circulating levels of estradiol during proestrus are associated with reduced estrus detection rates and fertility.

Four main factors affect reproductive efficiency in dairy herds: days postpartum at first AI, estrus detection rate, conception rate (**CR**), and pregnancy loss. Of these four factors, the first two, days postpartum at first AI and estrus detection rate can be easily manipulated. However, CR and pregnancy loss are, in many instances, under little human control and more difficult to impact. An additional factor, culling, can bias reproductive indices without being directly related to reproductive activities in the dairy.

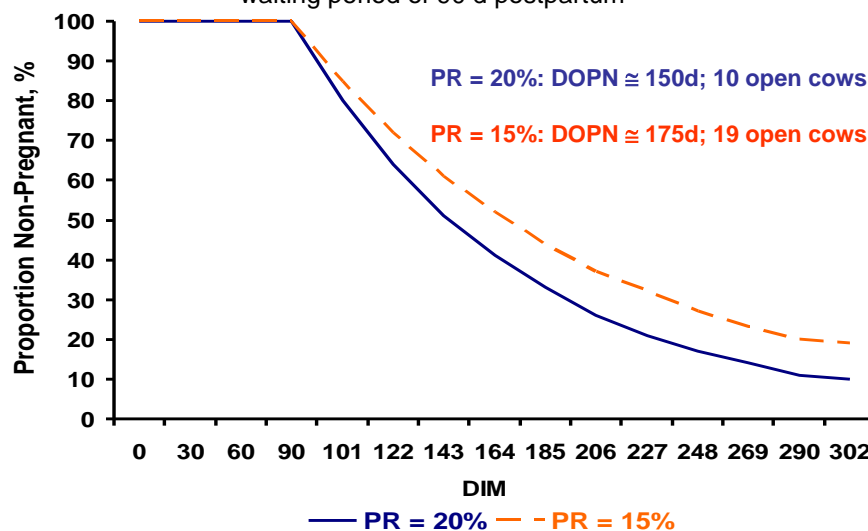
Pregnancy rates (**PR**), the proportion of pregnant cows relative to all eligible cows to become pregnant every estrous cycle past the voluntary waiting period, is the most meaningful reproductive parameter to determine reproductive efficiency in dairy herds. Simply, PR is a function of estrus detection rate and CR.

**Figure 1.** Survival curves for different pregnancy rates (PR) after a voluntary waiting period of 60 d postpartum



In order to maintain low days open and a high proportion of the herd with a calving interval less than 13 months, the voluntary waiting period must be limited to the first 60 d postpartum, and once insemination starts, PR for every 21-d estrous cycle should be above 20% (Figure 1). Manipulation of the interval from calving to first postpartum AI impacts reproductive efficiency in dairy cows and, extending the interval usually increases days open when PR are maintained (Figure 2). Ferguson and Galligan (1993) indicated that PR at first postpartum AI explained 79% of the variation in the calving interval in dairy cows. Therefore, optimizing first postpartum insemination PR is critical to improve reproductive efficiency in dairy herds.

**Figure 2.** Survival curves for different pregnancy rates (PR) after a voluntary waiting period of 90 d postpartum



Manipulation of the estrous cycle to improve service rate and fertility usually impacts positively on PR. Pharmacological control of the estrous cycle involves synchronization of follicular development, control of corpus luteum (CL) regression, and synchronization of ovulation to improve conception and PR. The ability to control the time of ovulation precisely with synchronization of ovulation protocols that combine recruitment of follicle growth associated with CL regression, and ultimately induction of a synchronized ovulation has allowed for successful timed artificial insemination (TAI) with adequate PR (Thatcher et al., 2001). Such programs have become an integral part of reproductive management in herds (Stevenson, 2001) that recognize estrus expression and estrus detection to be inadequate.

Although programmed insemination has the ability to optimize service rate and, in many cases PR, it has little or no impact on CR and pregnancy loss.

## ■ Estrus Synchronization Protocols

Estrus synchronization protocols allow for insemination of cows with little control over time of insemination and the total number of cows serviced. Because estrus synchronization protocols do not control ovulation, detection of estrus is required. Therefore, estrus synchronization protocols are effective only when estrus detection rate is high.

## **Prostaglandins**

Use of prostaglandins to synchronize estrus is the most common protocol implemented in most dairy farms. It consists of a single or multiple injections of  $\text{PGF}_{2\alpha}$  and analogues to regress a responsive CL, which causes the cow to return to estrus in 2 to 7 days.

The CL is generally responsive to  $\text{PGF}_{2\alpha}$  only after d 5 of the estrous cycle and a single injection of  $\text{PGF}_{2\alpha}$  given at random should induce estrus in approximately 60 to 70% of the cycling cows. When 2 injections of  $\text{PGF}_{2\alpha}$  are given 10 to 14 d apart, over 90% of the cycling cows are expected to respond to the second injection. However, frequency of anestrous cows and lack of optimal estrus detection can have a major impact on the number of cows responding to  $\text{PGF}_{2\alpha}$  and observed in estrus. In most dairy farms, utilization of 2  $\text{PGF}_{2\alpha}$  injections in the first 50 d postpartum results in an estrus detection rate following the second injection of 50 to 60% (Santos et al., 2003a).

Because  $\text{PGF}_{2\alpha}$  has no impact upon follicle development and no control over follicle wave emergence is obtained, cows in this program come into estrus at different days following the injection, with little precision over time of insemination and ovulation.

## **Controlling Emergence of Follicle Wave and Induction of Estrus with Prostaglandins**

To achieve precision of onset of estrus it is necessary to control emergence of follicle wave, which allows for a recently selected dominant follicle to be present at the moment of the  $\text{PGF}_{2\alpha}$  treatment, immediately prior to induction of estrus. Such events allow for more precision of estrus with adequate CR.

Turnover of follicles can be achieved by injections of GnRH and estradiol. Treatment of cows with GnRH results in an LH surge and ovulation of a dominant follicle with subsequent recruitment of a new cohort of follicles. However, use of GnRH to recruit a new follicular wave is only effective when the dominant follicle is responsive to LH. Generally, follicles larger than 10 mm in diameter have undergone deviation and developed LH receptors in the granulosa cells (Ginther et al., 1996). This seems to be the threshold diameter for follicles to respond to LH (Sartori et al., 2001). When GnRH is given at random stages of the estrous cycle to induce ovulation and recruitment of a new follicular wave, the highest response to the injection is observed when it is given between days 5 and 9 (Vasconcelos et al., 1999), which coincides with the period of time when the first wave dominant follicle is responsive to LH (Ginther et al., 1996). Utilization of GnRH followed 7 days later by an injection of  $\text{PGF}_{2\alpha}$  results in tight synchrony of estrus with high estrus detection rates (Santos et al., 2001).

An alternative to GnRH to recruit a new follicular wave is the use of estradiol esters such as estradiol cypionate (**ECP**; ECP™, Pharmacia Animal Health, Kalamazoo, MI). Injection of 1.0 mg of ECP on day 3 of a synchronized estrous cycle in dairy heifers resulted in the emergence of a new follicular wave 5.5 days later, and deviation of the new dominant follicle occurred 7.5 days after ECP injection (Galvão et al., 2003). Because of the longer period from ECP injection to follicle deviation and acquisition of ovulatory capacity of the new dominant follicle, a longer interval between ECP and PGF<sub>2α</sub> is required in order to minimize the interval from luteolysis to estrus and improve synchrony of estrus.

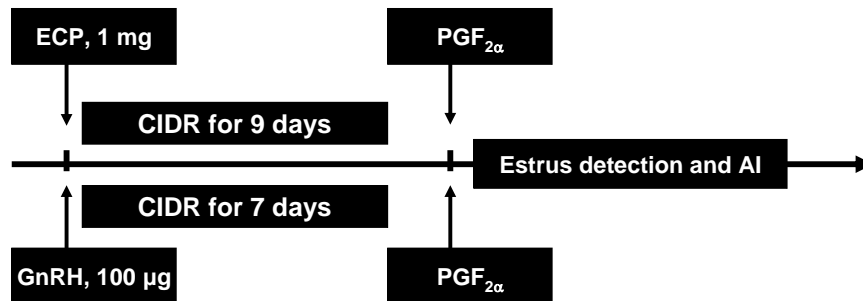
### **Controlling the Day of Estrus with Progesterone and Prostaglandins**

One of the problems with estrus synchronization protocols described above, which combine recruitment of a new follicular wave with CL regression, is the possibility of premature luteolysis with cows coming into estrus prior to the PGF<sub>2α</sub> treatment. This can occur when GnRH is given after day 12 of the estrous cycle. In such case, the original CL and the newly formed CL might regress due to the spontaneous secretion of PGF<sub>2α</sub> by the uterus between days 16 and 18 of the cycle.

The development of progesterone delivery devices such as intravaginal devices (EAZI-BREED CIDR, InterAg, Hamilton, New Zealand) and ear implants (Crestar; Intervet Ltd) have the ability to sustain plasma progesterone concentrations that avoid the LH surge in case of absence of a CL. This feature eliminates the problem of cows displaying signs of estrus prior to the desired breeding period.

Estrus response to a combination of progesterone and PGF<sub>2α</sub> treatment is usually high, and more than 80% of the cows that come into estrus do so between 36 and 60 h after removal of progesterone (Diskin et al., 2002). Because of the problem of subluteal concentrations of progesterone causing persistent follicles, which results in lower fertility (Austin et al., 1999), use of progesterone devices should be limited to 7 to 9 d. The most common protocol is the insertion of a CIDR for 7 d, with an injection of PGF<sub>2α</sub> on day 6 or 7. Improvements in this protocol can be attained by the addition of an injection of GnRH or estradiol at CIDR insertion to recruit a new follicular wave (Figure 3).

**Figure 3.** Estrus synchronization with a combination of GnRH, Estradiol, CIDR and  $\text{PGF}_{2\alpha}$



## ■ Ovulation Synchronization Protocols

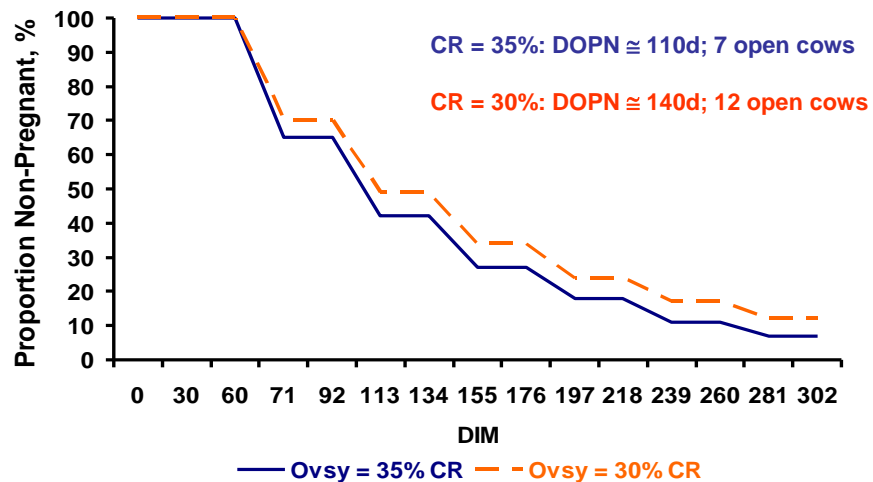
Ovulation synchronization protocols allow for precise synchronization of ovulation for TAI with adequate CR. The most accepted TAI protocol in dairy herds in the US is the Ovsynch protocol, which consists of an injection of GnRH given at random stages of the estrous cycle, followed 7 d later by a luteolytic dose of  $\text{PGF}_{2\alpha}$ , and 48 h later by a second injection of GnRH. Fixed-time AI is performed 12 to 16 h after the second GnRH injection. An alternative protocol to the Ovsynch has been named Heatsynch, which combines an injection of GnRH to recruit a new wave of follicles, followed 7 d later by an injection of  $\text{PGF}_{2\alpha}$  to regress the CL, and a final injection of ECP 24 h after the  $\text{PGF}_{2\alpha}$  to induce a synchronous ovulation approximately 56 hours later. Fertility of TAI following the Heatsynch protocol is similar to that in the Ovsynch protocol, but Heatsynch cows that do not display signs of estrus prior to or at the TAI have low fertility.

Recently, manipulation of follicle development prior to the initiation of the TAI protocol has been shown to increase PR 18 percentage units (25 vs 43%; Thatcher et al., 2001) in lactating cyclic cows at the first postpartum AI. Therefore, association of pre-synchronization with  $\text{PGF}_{2\alpha}$  prior to the initiation of the TAI protocol is advised to enhance fertility in dairy cows subjected to a TAI protocol during the first postpartum service. Furthermore, incorporation of a

CIDR at the moment of the first GnRH injection of the TAI protocols enhances fertility of anestrous cows.

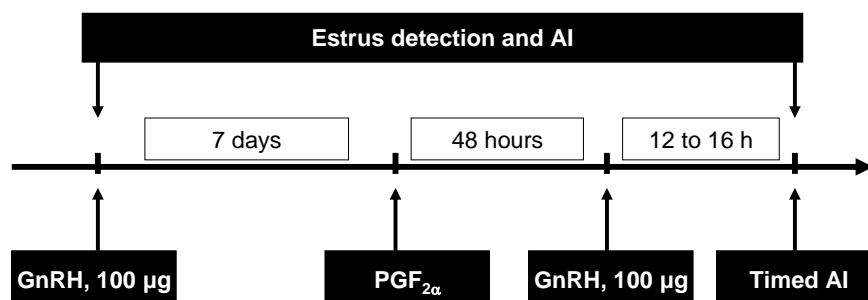
Although TAI protocols can potentially eliminate the need for estrus detection, implementation of fixed time AI with no detection of cows that return to estrus will result in pregnancy rates similar to conventional breeding programs with alternate cycles of high PR and zero PR (Figure 4).

**Figure 4.** Survival curves for cows inseminated only at fixed time with re-insemination prior to d 42 of the initial AI and varying conception rates (CR)



### Ovsynch Protocol

The Ovsynch protocol (Pursley et al., 1995) was developed as a breeding strategy to eliminate the need for estrus detection. The protocol is composed of an injection of GnRH at random stages of the estrous cycle to induce ovulation of the dominant follicle and synchronize a new follicle wave emergence. Seven days later, PGF<sub>2α</sub> is given to regress the original and the newly formed CL, followed by a second GnRH injection 48 h later to induce a synchronous ovulation 24 to 32 h later that allows for TAI 12 to 16 h after the second GnRH injection (Figure 5).

**Figure 5.** Ovsynch protocol for timed AI

This protocol has been implemented very successfully in many commercial dairy farms as a strategy for AI during the first postpartum service, as well as for re-insemination of non-pregnant cows. Although the Ovsynch protocol allows for TAI without the need for estrus detection, approximately 10 to 15% of the cows will display signs of estrus during the protocol and they should be inseminated promptly if maximum PR are to be achieved.

Pursley et al. (1997a) evaluated CR in lactating dairy cows ( $n = 310$ ) and heifers ( $n = 155$ ) when AI was performed following the Ovsynch protocol or a synchronization program utilizing only  $\text{PGF}_{2\alpha}$  injections. Cow in the  $\text{PGF}_{2\alpha}$  treatment received as many as 3 injections 14 d apart if signs of estrus had not been observed. All control cows not detected in estrus after the third injection of  $\text{PGF}_{2\alpha}$  were TAI 72 to 80 h after that injection. Pregnancy was determined by ultrasonography 25 to 30 d after AI or by rectal palpation 35 to 49 d after breeding. Conception rates for the two programs were similar and it averaged 38%. For the lactating cows, estrus detection rate during the first 2 injections of  $\text{PGF}_{2\alpha}$  averaged 54.0% following each injection, with an overall 81.8% for the 28-d period. Because of the low estrus detection rate in the  $\text{PGF}_{2\alpha}$  group, cows enrolled in the Ovsynch protocol would have had higher PR.

In a subsequent study by the same group (Pursley et al., 1997b), lactating dairy cows from 3 commercial herds ( $n = 333$ ) were randomly assigned to either the Ovsynch protocol or AI based on estrus detection with periodic use of  $\text{PGF}_{2\alpha}$ . Pregnancy was diagnosed 32 to 38 d after AI and non-pregnant cows were re-inseminated using the original treatment. Median days postpartum to first AI (54



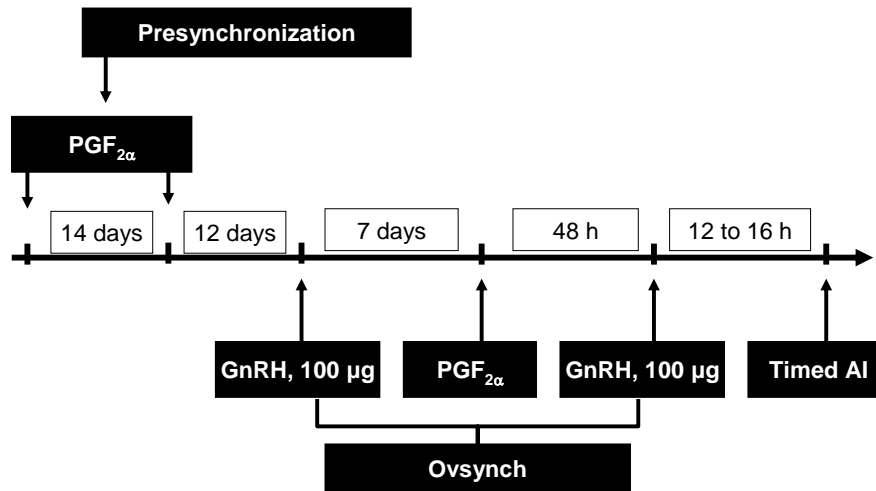
vs 83;  $P < 0.001$ ) and days open (99 vs 118:  $P < 0.001$ ) were lower for cows in the Ovsynch compared to cows inseminated following estrus detection. Therefore, implementation of TAI with the Ovsynch protocol for the first postpartum AI improved reproductive performance in dairy cows and subsequent re-insemination of open cows with TAI resulted in higher PR.

### **Pre-synchronization Prior to TAI**

Response to the Ovsynch protocol is optimized when cows ovulate to the first GnRH injection of the program, and when a responsive CL is present at the moment of the  $\text{PGF}_{2\alpha}$  treatment. Vasconcelos et al. (1999) initiated the Ovsynch protocol at different stages of the estrous cycle and observed that synchronization rate to the second GnRH injection was higher when cows received the first GnRH injection prior to d 12 of the estrous cycle. Also, initiation of the Ovsynch protocol between days 5 and 9 of the cycle resulted in the highest ovulation rate. Ovulation to the first GnRH injection and initiation of a new follicular wave should improve PR because an ovulatory follicle with reduced period of dominance is induced to ovulate (Austin et al., 1999). Furthermore, initiating the Ovsynch protocol prior to day 12 of the estrous cycle should minimize the number of cows that come into estrus and ovulate prior to the completion of the program.

Moreira et al. (2001) designed a pre-synchronization protocol to optimize response to the Ovsynch program by given 2 injections of  $\text{PGF}_{2\alpha}$  14 days apart, with the second injection given 12 days prior to the first GnRH of the TAI protocol (Figure 6). Pre-synchronization of lactating dairy cows submitted to the Ovsynch protocol with 2 injections of  $\text{PGF}_{2\alpha}$  increased PR at 32 and 74 days after TAI. Therefore, pre-synchronization of cows may be used to increase first service PR to a TAI protocol.

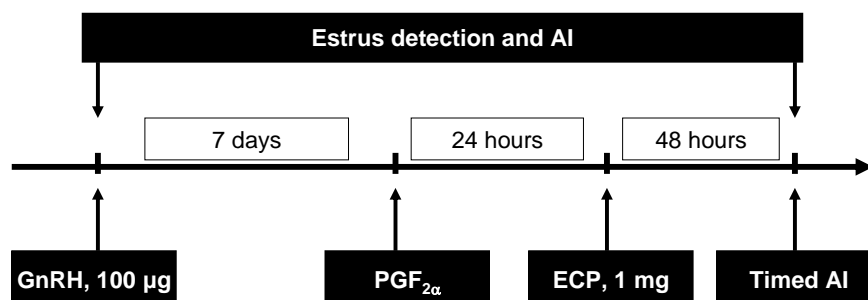
**Figure 6.** Presynch/Ovsynch protocol for timed AI in the first postpartum service



### Heatsynch Protocol

The use of estradiol products to synchronize ovulation in cows immediately after luteolysis has been utilized for many years. However, most of the studies with estradiol utilized estradiol benzoate (Diskin et al., 2002), which is not labeled for use in lactating dairy cattle in the US. An alternative to estradiol benzoate is another esterified form of estradiol, ECP. Incorporation of an ECP injection for TAI in dairy cows was developed by researchers at the University of Florida (Lopes et al., 2000). The use of ECP is an alternative strategy to control the time of ovulation because of the ability of exogenous estradiol to induce a LH surge when given under a low progesterone environment. The protocol consists of an injection of GnRH given at random stages of the estrous cycle to recruit a new wave of follicles to develop. Similar to the Ovsynch protocol, on day 7 after the GnRH injection, cows receive an injection of PGF<sub>2α</sub> to regress the original CL and/or the newly formed CL induced by the GnRH treatment. Twenty-four hours after the PGF<sub>2α</sub>, an injection of ECP is given to induce a surge of LH and synchronize ovulation. Artificial insemination should be performed at any time after the PGF<sub>2α</sub> if the cow displays signs of estrus or they should be timed artificially inseminated 48 h after the ECP treatment (Figure 7).

Figure 7. Heatsynch protocol for timed AI



For the first postpartum AI, it is indicated that cows be treated with 2 injections of  $\text{PGF}_{2\alpha}$  given 14 d apart, with the last injection given 12 d prior to the initiation of the Heatsynch/TAI protocol. Pre-synchronizing cows with two injections of  $\text{PGF}_{2\alpha}$  has been shown to increase PR of cows subjected to the Ovsynch protocol (Moreira et al., 2001). This pre-synchronization protocol increases the proportion of cows in the most favorable stages of the estrous cycle to respond to the first GnRH injection of the TAI protocol, as well as minimizes the number of animals with premature luteolysis, which creates asynchrony of ovulation at the moment of the TAI.

***ECP Dosage.*** Estradiol cypionate is an esterified form of estradiol 17- $\beta$  that is commercially available for use in lactating dairy cows. When low doses of ECP are injected in cows under a low progesterone environment, it induces a LH surge that lasts for approximately 10 h. This is similar to the spontaneous LH surge observed during estrus, but longer than that observed followed an injection of 100  $\mu\text{g}$  of GnRH (gonadorelin), commonly used in the Ovsynch protocol.

The rise in estradiol concentrations when cows are treated with ECP during the proestrus phase of the cycle is dependent upon the dose of ECP used and whether the animal is lactating or dry. Lopes et al. (2000) synchronized the estrous cycle of cows with an injection of GnRH followed 7 d later by an injection of  $\text{PGF}_{2\alpha}$ . Melengestrol acetate, 0.5 mg/cows, was fed for 6 d starting on the day following the GnRH injection. Twenty-four hours after the  $\text{PGF}_{2\alpha}$  treatment, cows were treated with 0, 0.5, 1 or 2 mg of ECP and blood samples

were collected twice daily. Peak plasma estradiol concentrations were higher for cows receiving the higher doses of ECP and they were 12, 25, and 35 pg/ml for 0, both 0.5 and 1, and 2 mg of ECP, respectively. Time of ovulation was shorter and synchronization of ovulation was tighter for cows receiving the 0.5 and 1.0 mg of ECP. Because lactating cows have lower concentrations of estradiol than growing heifers (Sartori et al., 2002), it is indicated that 1 mg of ECP be used for synchronization of ovulation in cows, and 0.5 mg in growing heifers. Suboptimal concentrations of estradiol might result in a suboptimal LH surge. Furthermore, suboptimal concentrations of estradiol might result in premature luteolysis in the subsequent estrous cycle (Mann and Lamming, 2000), which may decrease embryo survival.

**Timing of Ovulation.** During spontaneous estrus, the LH surge occurs at approximately 1 to 3 h after the onset of estrus with ovulation of a mature follicle some 24 to 30 h later. Similarly, when cows were treated with ECP in the Heatsynch protocol ovulation was detected at 55.4 h after the treatment, which is approximately 27.5 h after the onset of estrus (Pancarci et al., 2002). These data suggest that treatment with ECP to induce ovulation in the Heatsynch protocol results in a sequence of events that culminate with ovulation within a period similar to that observed for cows displaying spontaneous estrus.

Based on data from 32 cows, Pancarci et al. (2002) observed that, when cows were timed artificially inseminated 48 h after the ECP injection, highest pregnancy rates were achieved when ovulation was detected between 48 and 72 h from the ECP treatment. In fact, 75% of the cows ovulated within that interval, which reinforces the importance of scheduling the TAI at 48 h after the ECP treatment.

**Signs of Estrus and Fertility.** In a study in California (Cerri et al., 2003), 82% of the cows in the Heatsynch protocol displayed signs of estrus within 48 h of the ECP injection. When cows were monitored by radiotelemetry, the mean interval from ECP injection to estrus was 29.0 h with a range of 11 to 46.6 h (Pancarci et al., 2002). The duration of standing estrus averaged 12.5 h and cows observed in estrus were mounted 20.3 times. Cows that display signs of estrus during the Heatsynch protocol have higher fertility than those that are not found in estrus. This effect of estrus expression on fertility is probably related to cyclicity of cows prior to or during the synchronization protocol. In fact, cows that displayed signs of estrus had a larger follicle 48 h after the PGF<sub>2α</sub> than those that did not display signs of estrus (17.7 vs 15.9 mm;  $P < 0.04$ ).

It is possible that implementation of the Heatsynch protocol for the first postpartum insemination in herds with high incidence of anestrus in the first 60 d postpartum might result in poor PR. However, use of ECP to induce ovulation in lactating cows enrolled in the Heatsynch protocol may be used as a tool for early identification of animals that have lower fertility due to postpartum

anestrous, allowing for subsequent intervention. One might decide not to inseminate those cows that do not display signs of estrus, and perhaps re-synchronize those animals with the Ovsynch protocol since it induces cyclicity in over 95% of the anestrous cows, which might result in better PR.

### **Heatsynch vs Ovsynch**

A possible advantage of the Heatsynch over the Ovsynch protocol is timing of injections relative to the TAI. The Ovsynch requires that, for optimum fertility, cows be inseminated 12 to 16 h after the second GnRH injection, which forces farm personnel to handle cows twice within a 24 hr period. In the Heatsynch, injections and insemination can be performed only in one period of the day. Furthermore, a high proportion of cows display signs of estrus in the Heatsynch protocol, which might encourage the adoption of controlled breeding programs by dairy producers and AI technicians.

Pregnancy rates were evaluated in lactating dairy cows when ECP was used to induce ovulation as part of a TAI in comparison to Ovsynch for lactating dairy cows in Florida (371 cows) and Texas (321 cows). In both studies, cows were pre-synchronized with two injections given 14 d apart of 25 mg of PGF<sub>2α</sub>. Fourteen days after the second PGF<sub>2α</sub> injection, cows were enrolled in the Ovsynch or Heatsynch, with TAI taken place 16 to 24 h after the last GnRH injection in the Ovsynch or 48 h after the ECP injection in the Heatsynch protocol. Pregnancy rates were similar for Heatsynch and Ovsynch in either site (Figure 8). Therefore, the use of ECP within the TAI protocol to induce ovulation may offer dairy producers an alternative reproductive management system to manipulate the time of first postpartum AI, as well as increase subsequent service rates in non-pregnant cows.

**Figure 8.** Effect of TAI protocol on pregnancy rates in lactating dairy cows at the first postpartum AI. Black solid bar: Heatsynch; white bars with vertical shading: Ovsynch. Florida site: n=371 cows; Texas site: n=321 cows. (Pancarci et al., 2002).



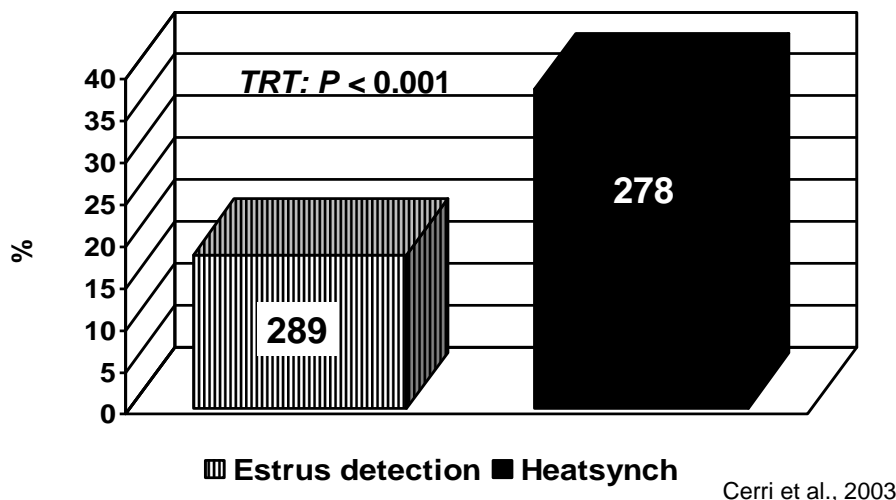
### Timed AI (Ovsynch and Heatsynch) vs Estrus Detection

A large field trial was designed to determine the effects of bST and reproductive management on reproductive performance in lactating dairy cows (Santos et al., 2003b). Lactating Holstein cows, 840, were randomly assigned to one of four treatments in a 2x2 factorial design at 37 d in milk. Treatments consisted of either bST (500 mg/14 d) starting at day 63 postpartum or no bST (control), with cows submitted to TAI following the Ovsynch protocol or inseminated following estrus detection. After pre-synchronization with two injections of PGF<sub>2α</sub> at 37 and 51 d postpartum, cows received an injection of GnRH at 63 d in milk, followed 7.5 d later by PGF<sub>2α</sub>. Cows in the estrus detection treatments were inseminated after observed in estrus during a 7 d period. Cows in the TAI treatments received a second GnRH injection 48 h after the last PGF<sub>2α</sub> and were inseminated 16 to 18 h later. Pregnancy was diagnosed by ultrasound at 31 d after AI and reconfirmed 14 d later by rectal palpation. Insemination rates were higher for the Ovsynch compared to cows in the estrus detection groups ( $P < 0.001$ ). Conception rates were higher in cows inseminated at detected estrus compared to TAI following the Ovsynch protocol, but pregnancy losses in the first 45 d after AI were similar for cows inseminated at detected estrus or at fixed time.

Conception and pregnancy rates were evaluated in 750 lactating dairy cows subjected to two reproductive management programs in three commercial dairy herds in central California (Cerri et al., 2003). Treatments consisted of TAI following the Heatsynch protocol or AI at detected estrus. In both groups, cows were pre-synchronized with two injections of 25 mg of PGF<sub>2α</sub> given 14 d apart. Fourteen days after the second PGF<sub>2α</sub> injection, cows received 100 μg of GnRH

followed 7 d later by an injection of 25 mg of PGF<sub>2α</sub>. Cows in the estrus detection group were artificially inseminated when observed in estrus during the next 7 days, while cows in the Heatsynch/TAI group received an injection of 1 mg of ECP 24 h after the PGF<sub>2α</sub> and were inseminated 48 h later. Any cow observed in estrus prior to the TAI in the Heatsynch group was inseminated at detected estrus. Pregnancy was diagnosed by ultrasonography at day 31 after AI, and pregnant cows had their pregnancy reconfirmed by rectal palpation at 45 and 65 d after AI. Pregnancy rates on d 45 after AI were higher for cows in the Heatsynch compared to those subjected to AI following estrus detection (Figure 9).

**Figure 9.** Pregnancy rate at 45 d after the first postpartum AI in lactating dairy cows



### ■ Impact of Breeding Program on Pregnancy Losses in Lactating Dairy Cows

The advent of ultrasonography and early pregnancy diagnosis has allowed researchers to determine losses of pregnancy in lactating dairy cows with initial pregnancy diagnosis as early as 25 d after AI. Late losses of pregnancy, between 28 and 45 d after AI are estimated at 10 to 20% in several studies (Cerri et al., 2003; Chebel et al., 2002; Moreira et al., 2001; Santos et al., 2003b; Santos et al., 2001). Some have suggested that implementation of TAI programs might have increased late losses of pregnancy (Lucy, 2001). We have attempted to determine whether different breeding protocols impact late losses of pregnancy in lactating dairy cows.

Chebel et al. (2002) studied factors involved in losses of pregnancy between 31 and 45 d after AI in 1,503 pregnancies from 1,376 lactating dairy cows in 3 commercial dairy farms in central California. Pregnant cows that had been inseminated either following estrus detection ( $n = 1,110$ ) or at fixed time ( $n = 393$ ) with the Ovsynch protocol had similar losses of pregnancy (13.7 vs 11.7;  $P = 0.19$ ). In two subsequent controlled studies (Cerri et al., 2003; Santos et al., 2003b) involving 1,590 cows and over 700 pregnancies in which cows were AI following estrus detection or TAI with the Heatsynch (Cerri et al., 2003) or Ovsynch (Santos et al., 2003b) protocols, losses of pregnancy between 31 and 45 d after AI did not differ. Therefore, implementation of controlled breeding programs with TAI protocols does not seem to affect pregnancy losses when compared to insemination following estrus detection.

## ■ Protocols for Re-synchronization of Non-Pregnant Cows

Re-synchronization of non-pregnant cows is required if optimum PR are to be achieved. At any given AI, only 30 to 45% of the inseminated cows are pregnant at 40 d after insemination, and the remainder needs to be re-inseminated as quickly as possible.

### Use of CIDR to Increase Returns to Estrus in Non-Pregnant Cows

The use of CIDR devices was first introduced in New Zealand as a tool to deliver progesterone for estrus synchronization and induction of cyclicity in cattle. The CIDR was initially engineered to contain 1.9 g of progesterone, but a similar product is now available containing 1.38 g of progesterone. Insertion of the CIDR in cows with no CL raises plasma progesterone concentrations immediately, with a decline to basal levels within 2 to 5 h of CIDR removal.

Re-synchronization of lactating dairy cows with CIDR can be achieved by insertion of the device on d 14 after AI and removal 7 days later. Such protocol is being tested in a large pivotal study for approval in the US and data should soon be available. An adaptation of this protocol was designed by El-Zarkouny et al. (2002) in which the CIDR device was inserted on d 13 after AI with or without estradiol treatment (1 mg of estradiol benzoate or 0.5 or 1.0 mg of ECP). The CIDR was removed 8 d later and cows in the estradiol treatments received an additional injection of estradiol benzoate or ECP. These protocols resulted in 61% of the non-pregnant cows returning to estrus between 21 and 26 d after the pre-synchronized AI, with similar CR during the re-synchronization period.



### **Use of GnRH 7 d Prior to Pregnancy Diagnosis to Re-Synchronize Follicle Growth in Non-Pregnant Cows**

A study was conducted to determine the effects of re-synchronization with GnRH on day 21 after AI on PR and losses of pregnancy in lactating dairy cows (Chebel et al., 2003b). Holstein cows, 585, on two dairy farms were assigned to one of two treatments in a randomized complete block design. On day 21 after the pre-enrollment AI, animals assigned to the re-synchronization group received an injection of 100 µg of GnRH, whereas animals in the control group received no treatment. All animals were examined by ultrasound on days 21 and 28 after AI, and blood was sampled for progesterone measurement on day 21. Pregnancy was diagnosed on day 28 and reconfirmed 14 d later. Non-pregnant cows on day 28 were timed inseminated after the completion of the Ovsynch protocol 10 and 17 d after the enrolment in the study for re-synchronized and control groups, respectively. Progesterone concentration  $\geq$  2.35 ng/ml was used as an indicator of pregnancy on day 21. For re-synchronized and control cows, PR at days 21 (70.9 vs. 73.0%,  $P < 0.56$ ), 28 (33.1 vs 33.6%;  $P < 0.80$ ) and 42 (27.0 vs 26.8%;  $P < 0.98$ ) after the pre-enrollment AI did not differ. Administration of GnRH on day 21 after AI had no effect on the losses of pregnancy between re-synchronized and control groups from 21 to 28 (53.4%;  $P < 0.94$ ) and 28 to 42 d (17.9%;  $P < 0.74$ ) after AI. Pregnancy rate after the re-synchronization period was similar for both treatment groups and it averaged 29.4%. Therefore, re-synchronization with GnRH given on d 21 after AI for initiation of a timed AI protocol prior to pregnancy diagnosis did not affect PR and pregnancy loss in lactating dairy cows and it can potentially expedite re-insemination of non-pregnant cows.

### **Use of ECP and PGF<sub>2α</sub> to Re-synchronize Cows between 28 and 34 d after AI**

A new protocol to re-synchronize cows at a known interval after the previous AI is being tested at the University of Florida and at the University of California Davis. The protocol has been named "Quicksynch" and it consists on re-synchronizing cows diagnosed as non-pregnant between days 28 and 30 after AI with an injection of PGF<sub>2α</sub> followed 24 h later by an injection of 1.0 mg of ECP to induce ovulation, with TAI 48 h later. These cows are expected to be on days 6 to 8 of the estrous cycle based on average interestrus interval for lactating cows of 22 days. At the UC Davis (Chebel et al., 2003a), cows diagnosed as non-pregnant based on ultrasonography are enrolled in the Quicksynch protocol when a CL is observed in the ovaries and a follicle greater than 10 mm is present. Those cows with follicles of less than 10 mm and those between 31 and 34 d after the previous AI are enrolled in the Heatsynch protocol. Pregnancy was diagnosed by ultrasonography 28 to 31 d after TAI. Preliminary results indicate similar PR for primiparous cows, but a decrease in PR for multiparous cows enrolled in the Quicksynch compared to Heatsynch.

The decrease in PR for multiparous cows inseminated at fixed time after the Quicksynch protocol demonstrates the need for a GnRH treatment 7 d prior to the PGF<sub>2α</sub> injection. In that case, a treatment with GnRH on day 21 after AI would probably result in improvements in pregnancy rates with no detrimental effect on fertility of pregnant cows (Chebel et al., 2003b).

## ■ Conclusions

Controlled breeding programs have allowed dairy producers to optimize service rate with little impact on conception and pregnancy losses in lactating dairy cows. In herds where estrus detection is high (> 60%) implementation of TAI protocols is expected to have little impact on reproductive efficiency, except during the first postpartum AI. Because PR at first postpartum AI explains most of the variation in the calving interval, implementation of controlled breeding programs is expected to have the biggest impact during the first postpartum AI, when the entire herd is eligible to be pregnant. However, protocols that maximize returns to estrus and re-insemination of non-pregnant cows should optimize PR and overall reproductive efficiency.

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