SYNCHRONIZATION PROGRAMS FOR REPRODUCTIVE MANAGEMENT OF DAIRY HERDS

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ABSTRACT

There are many factors that can influence reproduction of the dairy cow such as management, physiologic factors, nutrition, genetics, and diseases Reproductive efficiency. Reproductive performance is therefore a major concern in dairy herds to success, and it has to be subject of continuous and accurate evaluation in reproductive herd health programs, in order to detect problems and implement adequate solutions. Controlled breeding programs have allowed dairy producers to optimize service rate with little impact on conception and pregnancy losses in lactating dairy cows.

Key words: Estrus synchronization, reproductive efficiency, pregnancy rate

PROGRAMAS DE SINDRONIZACIÓN DE CELOS PARA EL MANEJO REPRODUCTIVO DE GANADERÍAS DE LECHE

RESUMEN

Existen muchos factores que pueden influenciar la reproduccion en ganaderías de leche, tales como el manejo, factores fisiológicos, la nutrición, genéticos, y la presentación de enfermedades, entre otros. Por esta razon la eficiencia reproductiva es de gran importancia en las ganaderías para ser exitosas y debe ser continua y adecuadamente evaluada en los programas reproductivos de salud de hato, con el fin de detectar los problemas y establecer las soluciones adecuadas. Los programas de reproduccion controlados, han permitido a los productores optimizar las tasas de servicios con un muy pequeño impacto en las tasas de concepción y perdida gestacional en ganaderías de leche.

Palabras clave: sincronización de celos, Eficiencia reproductiva, tasa de preñez.

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 estrous detection, establishment and maintenance of pregnancy. Recently, it was estimated that the average value of a pregnancy was US$278 in high-producing herds in the US, whereas the cost of a pregnancy loss was substantially greater (1).

There are multitudes of management, physiologic, nutritional, genetic, and disease issues that can influence reproduction of the dairy cow. Furthermore, consolidation of the industry

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with larger herds poses new challenges with implementation of reproductive programs with large number of cows. In the past, most dairy herds used reproductive programs that relied upon observation of estrus up to a certain number of days in milk (DIM), and subsequent intervention was only implemented in cows with advanced DIM and no insemination. Typically, interventions were based on palpation per rectum of the reproductive tract and a decision was made based upon detection of ovarian structures. These more traditional reproductive programs focused on finding the "problem cow" and "fixing" her; however, in systems based on artificial insemination (AI), the value to the producer of interventions is to find the nonpregnant and get her pregnant on a timely manner. Quite often, key indicators of success of these programs were based on averages, such as for DIM at first AI, days open and calving interval.

Nowadays, reproductive programs have taken a slightly different approach and the goal is now to be more proactive and work with groups of cows. In most cases, the focus is to increase the rate at which eligible cows become pregnant and, for that, use of systematic breeding protocols have become an integral portion of reproductive management in dairy herds (2). Ultimately, the goals are to minimize the variation in the interval from calving to first AI, increase the rate at which eligible cows become pregnant and, consequently, reduce the interval from calving to pregnancy in a consistent manner. Most of the reasons for change are related to the economic values of pregnancy and of when the cow becomes pregnant, the need to manage large groups of cows without creating systems that might not be implemented due to difficulty or lack of compliance, and the need to address deficiencies in cow fertility such as poor estrous expression and detection.

INDICATORS OF REPRODUCTIVE EFFICIENCY

Success of AI programs in dairy herds depends upon accurate and efficient detection of estrus; however, accuracy and efficiency of estrous detection are variable and depend upon animal, environmental, and management factors (3). For the high-producing dairy cow the altered competence of follicles and the smaller circulating concentrations of estradiol during proestrus have been associated with reduced estrous detection rates and fertility (4).

Four main factors affect reproductive efficiency in dairy herds: days postpartum at first AI, estrous detection rate, conception rate, and pregnancy loss. For this manuscript, the estrous detection rate is defined as the number of eligible cows receiving an insemination every 21-d, which is the standard estrous cycle length for cattle. Eligible cows are those that have passed the voluntary waiting period, they are not pregnant, and the producer wants to inseminate her.

Table 1. Calculation of reproductive indices at different days in milk (DIM)

DIM	Eligible cows	Detected in estrus	Estrous detection (%)	Pregnant	Conception rate (%)	Pregnancy rate (%)
51 – 71	100	50	50,0	20	40,0	20,0
72 – 92	80	50	62,5	20	40,0	25,0
93 – 113	60	30	50,0	10	33,3	16,7
114 – 134	50	25	50,0	9	36,0	18,0
Total	290	155	53,5	59	38,1	20,3

Conception rate is defined as the number of pregnancies divided by the number of AI, which is the typical measure of intrinsic fertility of the cow. Finally, pregnancy loss is defined as the proportion of pregnant cows that have experienced either an embryonic or fetal loss. Table 1 depicts an example of calculation of reproductive indices for every 21-d interval past the 50 DIM in a herd with 100 eligible cows for the first 134 DIM.

Of the factors outlined, the first two, days postpartum at first AI and estrous detection rate can be manipulated and controlled with a certain degree of efficacy. On the other hand, in high-producing dairy cows, conception rate 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 rate, 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, pregnancy rate is a function of estrous detection rate and conception rate.

In order to maintain an adequate interval from calving to pregnancy and a large proportion of the herd with a calving interval inferior to 13 months, the voluntary waiting period must be limited to the first 60 to 70 d postpartum and, once insemination starts, service rate should be 100%, first AI pregnancy rate greater than 35%, and pregnancy rate for every 21-d estrous cycle above 20%. 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 pregnancy rate are maintained. Ferguson and Galligan (5) indicated that pregnancy rate at first postpartum AI explained 79% of the variation in the calving interval in dairy cows. Such high impact is because all cows in the herd eligible to insemination have to receive a first AI. Therefore, optimizing first postpartum insemination pregnancy rate is critical to improve reproductive efficiency in dairy herds. In the so called “well managed” high-producing herds, it not uncommon to obtain 50 to 55% of the lactating cows pregnant before 110 DIM, and achieve median days open of 105 d. These numbers are usually only achieved with manipulations of the interval from calving to first AI, and improvements in service rate and conception rate.

VOLUNTARY WAITING PERIOD AND FIRST POSTPARTUM AI

Duration of the voluntary waiting period is, for most part, a management decision that can be easily manipulated. Traditionally, it varies from 40 to 90 d postpartum in most dairy herds, and decisions on when to begin AI results from physiological windows to optimize conception and economic debate as to when is best to first inseminate cows. There have been suggestions that the best interval from calving to conception in dairy herds is between 100 to 120 d. As production per cow increases, particularly when associated with increased persistence of lactation, delaying first postpartum AI and time when pregnancy is obtained has lesser impact on the value of the pregnancy and the impact of reproduction on the economics of the dairy (1).

Insemination early postpartum usually results in smaller conception rate and delaying first postpartum AI up to 90 to 100 d postpartum tended to increase fertility (6). Earlier review by Britt (7) observed a curvilinear relationship between days postpartum at first AI and conception rates, with maximum conception being achieved at 70 to 90 d postpartum. Part of the improvement in fertility of dairy cows as first AI is delayed past the traditional 60 to 70 d postpartum is the improved uterine health, as involution has completed is almost all cows, but also the reduced prevalence of anovular cows as the lactation progresses. Anovular cows prior to first postpartum insemination experience reduced conception rate and increased pregnancy loss (8).

Delaying first postpartum AI to 70 to 90 d postpartum increases conception rate, but does not necessarily reduces days open and improves overall reproductive performance of the herd. Veterinarians and dairy producers must decide what the main objective of their reproductive program is when choosing a voluntary waiting period for the herd. Typically, for every delay of an estrous cycle, ~ 21 d, in the voluntary waiting period, conception rate has to increase 8 to 10% units to compensate for the delay in first postpartum insemination to result in similar days open and proportion of cows pregnant at different intervals postpartum. In other words, if the voluntary waiting period of a dairy herd is 60 d, and conception rate at first AI is 35%, delaying the voluntary waiting period to 81 d postpartum has to result in increased conception rate to 43% to maintain a similar median and mean days open. Figure 1 depicts an example of the average dairy herd in the state of California, with a voluntary waiting period of 50 d postpartum and a 21-d pregnancy rate of 15% (conception rate of 30% and 21-d estrous detection rate of 50%).

In this example, ignoring pregnancy losses and culling of cows, the expected median and mean (± SD) days open for the average herd (PR 15-30) would be 140 and 158 ± 9 days, respectively. In the example herd, if the reproductive program is altered and if service rate at the first 21-d past the voluntary waiting period is increased from the typical 50% estrous detection rate to 100% by manipulating the estrous cycle, but conception rate is maintained (PR 30/15-50), then median and mean days open change to 100 and 139 ± 8 days, respectively. When the voluntary waiting period is now delayed to 70 d, and assuming that conception rate increases from 30 to 40% and all cows are inseminated in the first 21 d past the voluntary waiting period, then median and mean days open change to 120 and 143 ± 8 days, respectively. Finally, if an additional delay in voluntary waiting period is implemented, now to 90 days postpartum, and conception rate increases to 50% with all cows again inseminated in the first 21 d past the voluntary waiting period, then median and mean days open will be 100 and 156 ± 8 days,

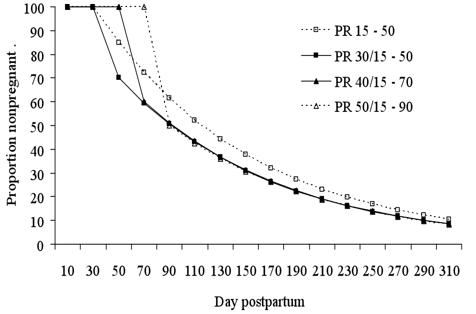

Figure 1. Impact of voluntary waiting period and 1st AI conception rate on days open. Legend indicates the pregnancy rate (PR) at first and subsequent AI (30/15 = 30% at first AI, and 15% thereafter) and the voluntary waiting period of 50, 70 or 90 d postpartum.

respectively. Therefore, these data illustrate that careful consideration must be taken when deciding on what the voluntary waiting period should be for a given herd.

Although delaying the first postpartum AI usually might not necessarily maximize the proportion of pregnant cows at a specific interval postpartum, despite increased conception rate, inseminating cows very early in lactation might not be attractive either. Likely, there is an optimum time postpartum when first AI should be performed, in which improvements in fertility and maximization of pregnant cows with adequate days open reach a balance, which is not really known at this time. In reality, discussions on the optimum time to inseminate a cow have to consider its impacts on reproductive performance of the herd, risk of a cow to be culled or die with more calvings in a lifetime, as calving is the period of highest risk for culling and death, genetic progress of the herd, as more calvings in a lifetime results in more replacement heifers, and milk yield per day of calving interval, all of which will impact the economics of the decision. Generally, for cows with lactation persistence past peak milk yield of less than 95% (decline in milk yield of > 5%/month past peak production), it is a consensus that extending the interval from calving to pregnancy will reduce milk yield per day of calving interval. On the other hand, for cows with high persistence of lactation such as primiparous, and of high milk yield, becoming pregnant early can be a negative event (1).

In order to illustrate the differences in control of interval to first AI, Figure 2 depicts scatter graphs of three different dairy herds with distinct reproductive managements for first postpartum AI. Herd A has a short voluntary waiting period, but it is clear that there is little control of the upper limit to DIM at first insemination. This is typical of herds that rely solely on estrous detection to inseminate cows. These herds usually begin inseminating cows early to compensate for their deficiency in estrous detection and the extended interval to first AI some cows experience. Herd B begins to inseminate cows at approximately 50 days postpartum, but almost every cow receives their first AI no later than 80 DIM. This is typical of herds that initially rely on estrous detection for AI, but eventually cows not inseminated up to a certain number of DIM will receive a timed AI. Finally, in herd C, almost every cow receives the first AI between 65 and 75 DIM. This is typical of herds that decide not to inseminate cows early postpartum, but eventually every cow receives the first AI following an ovulation synchronization protocol.

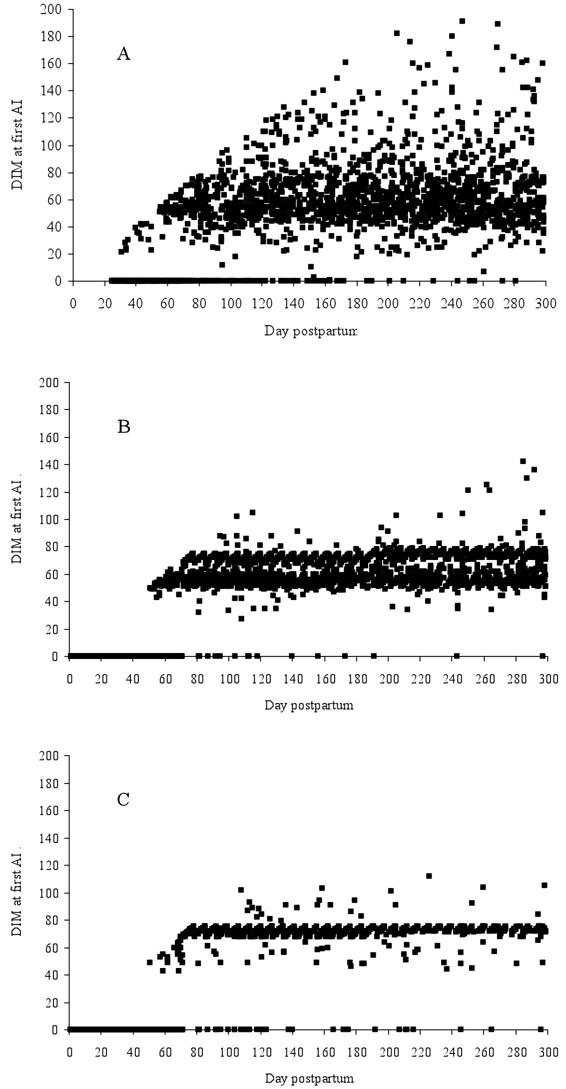

Figure 2. Scatter graphs of days in milk (DIM) at first AI relative to current day postpartum for cows in three dairy herds. Each square represents one or more cows.

The approach taken by herd A (Figure 2) is the least effective as a large proportion of cows receive their first AI either too early or too late. Early inseminations results in poor conception rates and might compromise milk yield in the current and future lactations. Cows that become pregnant very early postpartum have short lactation and might not have time to recover body condition for the subsequent lactations. The approaches taken by herds B and C likely result in similar reproductive performance (9), and both have their advantages and disadvantages. In herd B, expenses with labor and hormones for synchronization of estrus or ovulation is reduced, although conception rate is likely to be lesser for cows inseminated early in the voluntary waiting period than those inseminated at 70 to 80 DIM (10, 6).

IMPLEMENTING REPRODUCTIVE PROGRAMS FOR FIRST AI

It is clear that high-producing lactating dairy cows have compromised duration and intensity of estrous expression (4, 11). Therefore, implementation of reproductive programs based on synchronization of estrus, ovulation, or both is needed to optimize reproductive efficiency in dairy herds.

MANAGING ANOVULAR COWS

In high-producing dairy herds, 6 to 59% of the postpartum Holstein cows did not resume cyclicity by 60 d postpartum or before the first postpartum AI (8). These cows experience reduced conception rate and increased pregnancy loss following the first insemination (10, 12, 8).

A method to induce cyclicity in anovular cows is to administer exogenous progesterone by using controlled internal drug releasing (CIDR) impregnated with progesterone (13). When anovular cows were treated with a new or a 7-d used autoclaved CIDR originally containing 1,38 g of progesterone (Table 2), induction of cyclicity was increased and short-cycling was reduced in cows receiving supplemental progesterone, but these effects were not sufficient to improve proportion of cows pregnant and pregnancy loss (14).

Table 2. Effect of a new or 7-d used autoclaved controlled internal drug-releasing (CIDR) containing progesterone on reproductive responses of anovular cows[1]

Item	Treatment			*P* [2]	
	Control	New CIDR	Used CIDR	CIDR	Type
		% (number of cows)			
Cyclic	34,1 (120)	50,3 (199)	46,4 (196)	0,02	0,55
Short-cycling[3]	21,6 (74)	11,8 (110)	14,2 (120)	0,09	0,67
Pregnant					
d 38	36,8 (117)	43,6 (195)	37,8 (193)	0,86	0,36
d 66	32,5 (117)	39,4 (76/193)	35,4 (192)	0,61	0,57
Pregnancy loss	11,6 (43)	8,4 (83)	5,6 (72)	0,40	0,48

[1] Adapted from Cerri et ál. (14).

[2] CIDR = effect of CIDR (control vs. new + used CIDR); Type = effect of type of CIDR.

[3] Reinsemination of cows between 6 and 17 d after the initial AI.

The use of supplemental progesterone to reestablish ovulatory cycles in high-producing anovular cows does not seem to be warranted. When used prior to first post-partum insemination to induce cyclicity in anovular cows, the resulting conception rates are usually not altered (14, 10). When incorporated as part of a timed AI program, the efficacy of progesterone inserts in improving fertility of anovular cows is also questionable (15). When compared with timed AI protocols, treatment of anovular cows (cystic) using intravaginal inserts containing progesterone were less economical, a difference of approximately US$11,4 (1). Therefore, other methods than just progesterone inserts are recommended to induce cyclicity and increase the risk or a cow to become pregnant to an insemination.

ESTROSU SYNCRONIZATION PROTOCOLS

Estrous synchronization protocols allow for insemination of cows with little control over time of insemination and the total number of cows serviced. Because estrous synchronization protocols do not control moment of ovulation, detection of estrus is required and these protocols only become effective when estrous detection rate is good to excellent. Two major impediments for the success of programs that are based solely on estrous synchronization are the poor expression of estrus in high-producing cows and the high prevalence of anovular cows in the first 60 d postpartum (4, 8). Estrous expression and detection can be further compromised by environmental and animal factors such as poor footing and inadequate surface for mounting activity, lameness, and lack of individual animal attention as the industry consolidates and farms become larger with fewer employees per cow.

Synchronization of estrus can be accomplished simply by systematic use of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Use of $PGF_{2\alpha}$ to synchronize estrus is the most common protocol implemented in most dairy farms. It consists of single or multiple injections of PGF_{2a} and analogues to regress a responsive corpus luteum (CL), which causes the cow to return to estrus in 2 to 7 days.

The CL is generally responsive to PGF_{2a} only after day 5 of the estrous cycle and a single injection of PGF_{2a} given at random should induce estrus in approximately 60 to 70% of the cycling cows. When 2 injections of PGF_{2a} are given 10 to 14 d apart, over 90% of the cycling cows are expected to respond to the second injection. However, frequency of anovular cows and lack of optimal estrous detection can have a major impact on the number of cows responding to PGF_{2a} and observed in estrus. In most dairy farms, utilization of 2 PGF_{2a} injections in the first 50 d postpartum results in an estrous detection rates following the second injection between 50 and 60% (16). Because PGF_{2a} 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.

Response to PGF_{2a} can be improved by controlling follicle growth and assuring that a responsive CL is present at the moment of treatment. Utilization of gonadotropin-releasing hormone (GnRH) followed 7 days later by PGF_{2a} is a simple program that increases response to PGF_{2a} and improves synchrony of estrus. Another method to improve estrous response to PGF_{2a} is to combine with an intravaginal progesterone insert. Inserts such as the CIDR result in subluteal concentrations of progesterone in high-producing dairy cows (14), which are sufficient to block estrus and ovulation and increase tightness of estrous detection after removal of the insert. When combined with an injection of PGF_{2a}, the use of progesterone inserts should be limited to no longer than 7 days to avoid persistent follicle and reduction in fertility. The most common protocol is the insertion of the intravaginal device for 7 d, with an injection of PGF_{2a} on day 6 or 7. Improvements in this protocol can be attained by the addition of an injection of GnRH at the insertion of the progesterone device to recruit a new follicular wave. When postpartum cows were treated with CIDR for 7 days and received PGF_{2a} at insert removal, distribution of estrus was altered and cows were inseminated sooner when compared with PGF_{2a} alone (10).

TIMED AI PROTOCOLS

Manipulation of the estrous cycle to improve service rate and fertility usually impacts positively on pregnancy rate. Timed AI protocols rely on control of the estrous cycle by synchronizing follicular development, CL regression and, ultimately, ovulation to allow for insemination at fixed time with adequate conception rate (17). Such programs have become an integral part of reproductive management in herds (2), and adoption has been widespread because of the recognized problems with expression and detection of estrus in dairy cows.

The most accepted timed AI protocol in dairy herds in the US is the Ovsynch and CoSynch protocols, which consist of an injection of GnRH given at random stages of the estrous cycle, followed 7 d later by a luteolytic dose of PGF_{2a}. For the Ovsynch, a final GnRH injection is given at 48 to 56 h after PGF_{2a} and fixed-time AI is performed 12 to 16 h later. When the CoSynch is utilized, cows are fixed-time inseminated 48 or 72 h after the PGF_{2a}, and GnRH is given concomitantly with timed AI. These protocols have 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 nonpregnant cows. Although timed AI protocols allow for insemination without the need for estrous detection, approximately 10 to 15% of the cows will display signs of estrus during the protocol and they should be inseminated promptly if maximum pregnancy rate is to be achieved.

Pursley et ál. (18) evaluated conception rate in lactating dairy cows (n = 310) and heifers (n = 155) when AI was performed following the Ovsynch protocol or a synchronization program utilizing only PGF_{2a} injections. Cow in the PGF_{2a} 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 PGF_{2a} were timed AI 72 to 80 h after that injection. Conception rates for the two programs were similar and it averaged 38%. For the lactating cows, estrous detection rate during the first 2 injections of PGF_{2a} averaged 54,0% following each injection, with an overall 81,8% for the 28-d period. Because of the low estrous detection rate in the PGF_{2a} group, cows enrolled in the Ovsynch timed AI protocol experienced greater pregnancy rate. In a subsequent study by the same group (19), lactating dairy cows from 3 commercial herds (n = 333) were randomly assigned to either the Ovsynch protocol or AI based on estrous detection with periodic use of PGF_{2a}. Nonpregnant 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 reduced in cows receiving the Ovsynch compared with cows inseminated following detection of estrus.

An alternate program for induction of ovulation is the use of estrogens in place of GnRH. Estradiol products have been used to synchronize ovulation in cows immediately after luteolysis for many years;

however, in many countries use of estradiol products are no longer available for reproductive management of dairy cattle. Most of the studies with estradiol for synchronization of estrus and ovulation utilized estradiol benzoate (20). An alternative to estradiol benzoate is another esterified form of estradiol, estradiol cypionate (ECP). Incorporation of an ECP injection for timed AI in dairy cows was developed by researchers at the University of Florida (21). 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 during proestrus. 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 $PGF_{2\alpha}$ to regress the original CL and/or the newly formed CL induced by the GnRH treatment. Twenty-four hours after the $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$ if the cow displays signs of estrus or they should be timed artificially inseminated 48 h after the ECP treatment.

Because high-producing lactating dairy cows often experience suboptimal fertility and have reduced concentrations of estradiol, it is possible that increased estradiol concentrations with ECP during proestrus may enhance fertility. Cerri et ál. (22) compared fertility of cows undergoing a Presynch-Heatsynch reproductive management program with cows inseminated at detected estrus following a Presynch-Selectsynch program. The Presynch-Heatsynch cows had both greater conception rate (43,0 > 35,6%) and pregnancy rate (48,5 > 23,6%) at day 30 after insemination than cows detected in estrus and inseminated with the Presynch-Selectsynch program. Pregnancy losses were similar (12%) between day 30 and 58 after insemination. Apparently, increased estradiol concentration in the periovulatory period of lactating dairy cows at first service enhanced conception rate. In fact, recent work from the University of Wisconsin indicated that supplemental estradiol 8 h prior to induction of ovulation with GnRH in the Ovsynch protocol improved timed AI CR in cows with small follicles.

An important aspect of the use of ECP to induce ovulation is that cows that display estrus at timed AI have substantially greater conception rate (22, 23). Cows that display estrus during the Heatsynch protocol had a larger follicle 48 h after the $PGF_{2\alpha}$ than those that did not display signs of estrus (17,7 vs. 15,9 mm) (22), and experienced increased incidence of synchronized ovulation (23).

It is important to note that, regardless of the program utilized to synchronize ovulation for fixed-time insemination, the positive effects of timed AI compared with more traditional reproductive programs based on detection of estrus on reproductive efficiency of a herd are only observed when conception rates are not reduced with timed AI, and detection of estrus is deficient (24). When timed AI was implemented in 2 herds with distinct reproductive performance, the benefits from a systematic breeding program were more clearly demonstrated in the herd with poor estrous detection rate (24).

IMPROVING RESPONSE TO TIMED AI

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 PGF_{2a} treatment (10). Vasconcelos et ál. (25) 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 day 12 of the estrous cycle. Also, initiation of the Ovsynch protocol between days 5 and 9 of the cycle resulted in the greatest ovulation rate. Ovulation to the first GnRH injection and initiation of a new follicular wave should improve pregnancy rate because an ovulatory follicle with reduced period of dominance is induced to ovulate (26). 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.

The importance of inducing follicle turnover is demonstrated vividly by evaluating fertilization rates and embryo quality after timed AI following the induction of follicle turnover or not (27). Our hypothesis was that initiating Ovsynch on day 3 of the estrous cycle would lead to continued development of an ovulatory follicle that would result in poorer embryo quality compared to recruitment of a new ovulatory follicle following follicle turnover initiated by an injection of GnRH on day 6 of the cycle. Cows receiving the Ovsynch protocol initiated on day 3 of the estrous cycle had lesser ovulation rate to the initial GnRH than those initiating the program on day 6 of the estrous cycle. The reduced ovulation rate (7,1 vs. 83%) was associated with smaller dominant follicles (9,5 vs. 14,8 mm) at the moment of the initial GnRH injection. Because ovulation was lesser in cows receiving the GnRH on day 3 than day 6, a new follicular wave was observed in fewer cows when the first injection of GnRH was administered during metestrus than diestrus, thereby extending the period of follicle dominance during the Ovsynch protocol. When embryos were flushed on day 6 after AI, fertilization rate was similar among treatments, but cows initiating the Ovsynch on day 3 had embryos that were less developed and with fewer cells than those of cows initiating the Ovsynch on day 6. These findings substantiate the importance of regulating follicle dynamics to optimize the period of follicle dominance to obtain high quality embryos.

Moreira et ál. (28) designed a presynchronization protocol to optimize response to the Ovsynch program by given 2 injections of PGF_{2a} 14 days apart, with the sec-

Table 3. Effect of artificial insemination protocol on embryos recovered from uterine flushings at day 6 after AI1

	Treatment[2]			
	DE	OV3	OV6	OVE
Percent excellent and good structures[b]	61,0	40,0	72,0	65,9
Percent excellent and good embryos[a]	71,4	47,0	83,7	74,3
Number of blastomeres[a]	42,3±3,4	29,7±3,4	42,5±3,3	44,1±3,2
Proportion of live blastomeres[b]	94,3±2,2	90,1±2,4	97,7±2,1	97,6±2,0

[1] Adapted from Cerri et ál. (27).

[2] Detected estrus (DE) = GnRH on day 6 of the estrous cycle, followed by PGF_2 7 days later and AI at estrus; Ovsynch (GnRH, followed by PGF_2 7 days later, then 2 days later GnRH followed by a timed AI 12 h later) as OV3, OV6 and OVE, which correspond to the injection of the first GnRH on day 3, 6 and 6 of the estrous cycle, respectively, but OVE also received an injection of 0,5 mg of estradiol cypionate 36 h before the timed AI.

[a] $P<0,01$, [b] $P<0,05$

ond injection given 12 days prior to the first GnRH of the timed AI protocol. This presynchronization program increased pregnancy rates at 32 and 74 days after timed AI in cyclic cows. Because of the convenience of giving injections on the same day of the week, many producers have opted for administering the $PGF_{2\alpha}$ injections of the presynchronization protocol on the same day of the injection of the Ovsynch protocol, which results in an interval between presynchronization and initiation of the Ovsynch of 14 days. Although presynchronizing cows 14 days before initiating the Ovsynch also improved conception rate compared with no presynchronization (29), the interval is not optimal and results in poor ovulation rate to the initial GnRH of the Ovsynch (10, 30, 31).

We have recently demonstrated that reducing the interval between presynchronization and initiation of the timed AI from 14 to 11 days increased ovulation rate to the initial GnRH of the timed AI protocol and increased conception rate (30, 31).

INSEMINATION OR NOT DURING PRESYNCHRONIZATION

A common program adopted in many farms in California is to administer 2 $PGF_{2\alpha}$ injections at 14 d interval, with the second injection given at approximately 50 to 55 days postpartum. Cows are then inseminated following the second injection, and those not inseminated in the following 11 days are enrolled in the timed AI protocol. Because 45 to 55% of the cows display estrus and are inseminated following the second $PGF_{2\alpha}$ of the presynchronization, these cows

Table 4. Effect of presynchronization treatment on ovulatory responses to the first GnRH of the timed AI, pregnancy per AI and pregnancy loss in dairy cows[1]

	Treatment[2]			*P*[3]	
	Control	PShort	PShortG	Interval	GnRH
Ovulation to 1st GnRH[4]	------------------- % (no.) -----------------				
Overall	44,7 (340)	61,4 (337)	62,2 (323)	<0,001	0,28
Cows with CL	37,2 (274)	54,4 (250)	59,7 (285)	<0,001	0,29
Cows without CL	75,8 (66)	81,6 (87)	81,6 (38)	0,34	0,99
Pregnant					
day 38	33,5 (412)	40,5 (410)	39,8 (392)	0,02	0,60
day 66	30,2 (410)	36,4 (409)	36,2 (392)	0,04	0,70
Pregnancy loss					
day 38 to 66	8,8 (136)	9,7 (165)	9,0 (156)	0,88	0,85

[1] Adapted from Galvão et ál. (30, 31).

[2] Control = two injections of PGF_2 at 37 and 51 DIM, then enrolled in the timed AI 14 d later; PShort = two injections of PGF_2 at 40 and 54 DIM, then enrolled in the timed AI 11 d later; PShortG = same as PShort, but with an injection of GnRH 7 d before the first GnRH of the timed AI.

[3] Interval = contrast for the effect of 14 vs. 11 d interval (Control vs. PShort + PShortG); GnRH = contrast for the effect of GnRH 7 d before initiation of timed AI (PShort vs. PShortG).

[4] Ovulation to GnRH was evaluated in cows with or without a CL on the day of treatment.

ended up receiving their first AI early in the postpartum period. Studies have demonstrated that response to timed AI programs improves as the lactation progresses up to 70 to 90 days postpartum (19, 6), and we have demonstrated that cows inseminated at estrus following the presynchronization have smaller conception rate than those inseminated after the completion of the entire program (presynchronized timed AI), 3 weeks later (10). However, insemination of cows at estrus during the presynchronization reduces the interval to first AI and costs associated with hormones and labor.

In an attempt to evaluate whether cows should be inseminated following presynchronization or subjected to timed AI, Chebel et ál. (9, 10) assigned 1,019 Holstein cows to a presynchronization with PGF_{2a} (CON) or PGF_{2a} and CIDR (CTAI and CED). All cows received 2 injections of PGF_{2a} on days 35 ± 7 and 49 ± 7 after calving. Cows in CTAI and CED received a CIDR on d 42 ± 7. After the second PGF_{2a} and CIDR removal on day 49 ± 7, cows were observed for estrus, but only CON and CED were inseminated. On day 62 ± 7 CON and CED cows not inseminated in estrus and all CTAI began the Ovsynch and were timed AI on day 72 ± 7. Cows in CON and CED had smaller conception rates that CTAI on d 31 after the first AI, however, because they were inseminated on average 2 weeks earlier, median days open for the first 300 d postpartum were similar between CED and CTAI (Figure 3).

These results suggest that insemination of cows after the second PGF_{2a} of the presynchronization results in smaller conception rate, but because cows are inseminated earlier, days open are not affected. This gives flexibility to producers that might decide to inseminate cows that display estrus after the second $PGF_{2\alpha}$ of the presynchronization, or inseminate all cows at timed AI. The first will reduce costs with treatments, but the latter will optimize first service conception rates, with both resulting in similar time to pregnancy.

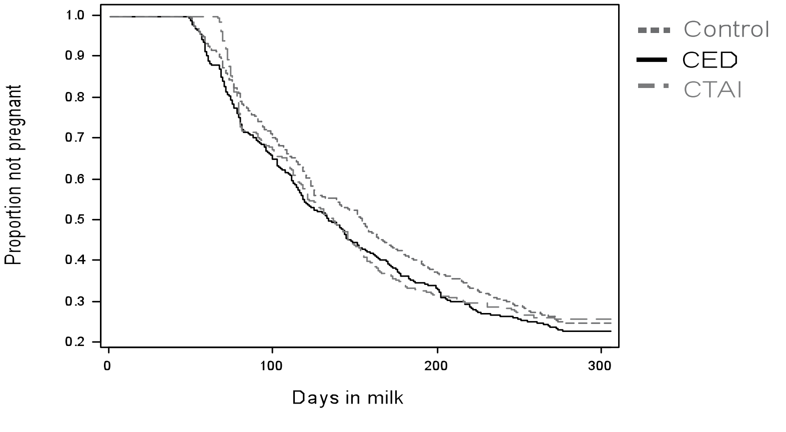

Figure 3. Survival curves for time to pregnancy in cows subjected to different insemination protocols for first AI. Median days open were 154, 133, and 136 for control, CED, and CTAI, respectively.

It is important to emphasize that in order for systematic breeding programs to work, there must be high compliance at every step of the program. Each individual farm has to develop a system to assure that cows receive the correct hormonal treatment on the correct day. Failure in complying with the programs can result in reduced insemination rate and conception rate. Because some programs require handling of cows multiple times to administer hormonal treatments, it is important that they tailored to the needs of the farm as long as critical steps are not ignored.

PROGRAMS FOR RESYNCHRONIZATION OF NONPREGNANT COWS

Resynchronization of nonpregnant cows is required if optimum pregnancy rates 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 reinseminated as quickly as possible.

Protocols for resynchronizing cows may rely on increasing display of estrus for insemination of cows upon detection of a synchronized estrus, or they may utilize methods to control ovulation such that nonpregnant cows are enrolled in a timed AI program.

The use of CIDR devices was first introduced in New Zealand as a tool to deliver progesterone for estrous synchronization and induction of cyclicity in cattle. Resynchronization of lactating dairy cows with CIDR can be achieved by insertion of the device on d 14 after AI and removal 7 days later. This protocol resulted in increased return to estrus in nonpregnant cows in some (32), but not all studies (10), with mixed results on conception rates. In herds capable of reinseminating more than 55% of the nonpregnant cows prior to pregnancy diagnosis, it is unlikely that the use of the CIDR will improve reinsemination rates and reduce time to pregnancy.

Another strategy is to initiate the synchronization program before diagnosis of nonpregnancy. Treatment with GnRH 7 d prior to pregnancy diagnosis allows for faster reinsemination of nonpregnant cows when no estrous detection is implemented. Chebel et ál. (33) evaluated conception rates in 585 Holstein cows on two dairy farms assigned to resynchronization with GnRH 7 d prior to pregnancy diagnosis or at the moment of diagnosis of nonpregnancy. Nonpregnant cows were time-inseminated following the Ovsynch program. Cows that displayed estrus during the study were inseminated in the same day. The authors demonstrated that treatment with GnRH on d 21 after the pre-enrollment AI reduced detection of estrus in the next 7 d, but allowed for reinsemination of nonpregnant cows within 3 days of nonpregancy diagnosis with no effect on conception rates during the pre-enrollment or resynchronized AI. Fricke et ál. (34) demonstrated that resynchronization of cows of unknown pregnancy status with GnRH 7 d prior to pregnancy diagnosis should not begin on day 19 after the initial AI. In that study, resynchronized pregnancy rates were smaller for cows initiating the timed AI on d 19 than 26 after the initial AI.

Recently, Galvão et ál. (31) evaluated different methods to resynchronize estrus and ovulation in dairy cows with use of CIDR, ECP and GnRH given 7 d prior to diagnosis of nopregnancy. Holstein cows were assigned to 1 of 3 treatments, Control (n = 167), resynchronization with a timed AI protocol upon diagnosis of nonpregnancy on d 31 after preenrollment AI, CIDR-G (n = 159), use of an intravaginal progesterone insert from d 14 to 21 after AI, with AI at estrus from d 21 to 24 and initiation of a timed AI protocol on d 24 after AI in cows not reinseminated; and CIDR-G + ECP (n = 169), same treatment as CIDR-G but with an injection of 1 mg of estradiol cypionate at the time of progesterone insert removal. Cows were continuously reenrolled in the same treatment until diagnosed pregnant, which resulted in a total of 1,148 AI. Conception rates for the pre-enrollment and resynchronized AI were similar among treatments. More importantly, the interval from study enrollment to pregnancy was not different among treatments, which indicate that reproductive performance of dairy cows did not differ among the 3 resynchronization treatments evaluated. Lack of positive effects from the more aggressive resynchronization programs were probably the result of the high estrous detection rate (60%) in Control cows. Therefore, it is likely that methods to expedite reinsemination of nonpregnant cows will only improve reproductive performance in herds with poor estrous detection.

ADVANTAGES OF SYSTEMATIC BREEDING PROGRAMS AND CONCLUSING REMARKS

The reproductive program on a dairy herd should be simple to implement, robust, and result in predictable reproductive performance. Most farms, particularly large dairy herds, avoid complicated programs that require several handlings of cows to synchronize estrus or ovulation and deliver the AI. Producers are reluctant to handling cows more than once a day because of the perceived loss in production due to disturbances of normal cow activities. On the other hand, programs that are not properly implemented, in which convenience prevails over biology, can lead to mediocre reproductive results. As herd size increases and more cows are confined to concrete, the efficiency of estrous detection declines. Furthermore, as we select animals to greater milk production, their ability to display signs of estrus is reduced (3, 4). These combined effects of environment and animal biology limit our ability to detect estrus and inseminate cows. This has forced producers to implement ovulation synchronization programs to assist in alleviating problems with suboptimal estrous detection.

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 21-d estrous detection rate is high (> 60%) implementation of timed AI protocols is expected to have little if any impact on reproductive efficiency, except during the first postpartum AI. Because pregnancy rate at first postpartum AI explains most of the variation in the calving interval, implementation of controlled breeding programs is expected to have the greatest impact during the first postpartum AI, when the entire herd is eligible to be inseminated. However, protocols that maximize returns to estrus and re-insemination of nonpregnant cows should optimize pregnancy rate and overall reproductive efficiency. Combining sequential injections of $PGF_{2\alpha}$ with timed AI programs, such as in the Presynch/Ovsynch program, gives produces the flexibility to inseminate cows at estrus, but it assures that all cows will be first inseminated at a given day postpartum. Furthermore, by presynchronizing the estrous cycle of cows, response to timed AI is optimized.

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