Current Concepts for Estrus Synchronization and Timed Insemination

W.W. Thatcher, MS, PhD[1]; D.J. Patterson, MS, PhD[3]; F. Moreira, DVM, MS, PhD[1]; M. Pancarci, DVM, MS[1]; E.R. Jordan, MS, PhD[4]; C.A. Risco, DVM[2]
[1]*Department of Animal Sciences, IFAS*
[2]*Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32611-0920*
[3]*Department of Animal Science, University of Missouri, Columbia, MO, 65211*
[4]*Department of Animal Science, Texas A&M University, Dallas, 7252-6599, TX*

Introduction

The cattle industries have a wide range of reproductive technologies that are available to producers and veterinarians to program reproductive management with the use of estrus synchronization and timed insemination protocols. It is of paramount importance that users of this technology have a thorough understanding of the systems as to how they regulate pituitary, ovarian and uterine function. Major advancements have come about because we now understand how to control and coordinate ovarian follicular, corpus luteum and uterine functions in a manner that is conducive to normal fertility in cattle under different physiological conditions i.e., non-lactation, lactation, anestrus, intensive and extensive management conditions etc. At this meeting in 1998, the various practical systems to control the estrous cycle in dairy animals were reviewed.[30] Objective of this presentation is to provide an update of current reproductive strategies for synchronization and timed insemination in dairy and beef cattle. The focus will be with the use of those pharmacological drugs available to the cattle industries in the United States. Effective estrus synchronization programs provide a number of advantages: cows or heifers are in estrus at a predicted time which facilitates AI, and embryo transfer; time and labor expense for detection of estrus are reduced; AI becomes more practical under extensive conditions; precise control of ovulation permits a timed insemination without the need for detection of estrus; and specific timed treatments to improve embryo survival can be implemented effectively.

The following protocols and terms referred to throughout this manuscript are defined as follows:

Protocols:

PG: Prostaglandin F_2a (PG; Lutalyse, Estrumate, ProstaMate).

GnRH-PG: Gonadotropin-releasing hormone injection (Cystorelin, Factrel, Fertagyl) followed in 7 days with an injection of PG; Select Synch.

GnRH-PG-GnRH: Gonadotropin-releasing hormone injection followed in 7 days with an injection of PG and a GnRH injection give 48 h after PG; Ovsynch.

PG-PG-GnRH-PG-GnRH: Two injections of PG are given 14 days apart with the first injection of the Ovsynch given 12 to 14 days after the second PG.; Pre-synch-Ovsynch.

GnRH-PG-ECP: Gonadotropin-releasing hormone injection followed in 7 days with an injection of PG and an ECP injection given 24 h after PG; Heatsynch.

MGA-PG: Melengestrol acetate (MGA; 0.5 mg/hd/day) is fed for a period of 14 days with PG administered 17 or 19 days after MGA withdrawal.

7-11 Synch: MGA is fed for 7 days, PG is administered on the last day MGA is fed,
GnRH is administered 4 days after the cessation of MGA, and a second injection of
PG is administered 11 days after MGA withdrawal.

Terms:

Estrus response: The number of females that exhibit estrus during a synchronized period.

Synchronized period: The period of time during which estrus is expressed after treatment.

Synchronized conception rate: The proportion of females that becomes pregnant of those exhibiting estrus and inseminated during the synchronized period.

Synchronized pregnancy rate: The proportion of females that become pregnant of the total number treated.

The goal for a successful estrus synchronization program is precise control of estrus, which will allow fixed-time AI without the need for estrus detection. However, this needs to be coupled with high fertility at the synchronized estrus or ovulation. The target populations are dairy cattle, in which herd pregnancy rates are often times low due to poor heat detection, low conception rates and occurrence of anestrus, and beef cattle that have a high incidence of anestrus at the designated time of breeding. Strategies for ovulation control have been based on controlling life span of the CL with prostaglandins, induction of ovulation with GnRH, or prevention of estrus using progestagen treatments. As our knowledge regarding control of the estrous cycle has been expanded, appropriate combinations of these approaches that are physiological have been successful. Prostaglandins alone do not provide acceptable synchrony, because the time of ovulation depends on the stage of development of the dominant follicle at the time of prostaglandin-induced regression of the CL. This problem has been resolved partially with the injection of GnRH that causes ovulation of the dominant follicle at certain stages of the cycle and recruitment of a new dominant follicle. Prostaglandin is injected 7 days after GnRH to induce CL regression at a time a new dominant follicle is present that induces estrus 48 to 72 h later. Thus this system has synchronized follicle development with CL regression. This system can be expanded further with a second GnRH injection given 48 h after prostaglandin injection to synchronize precisely the time of ovulation of the dominant follicle. Precision of follicle recruitment following the first injection of GnRH is dependent upon successfully turning over a dominant follicle. Thus the concept of pre-synchronization was introduced to enhance the probability of having a dominant follicle ($\geq$ 10mm) that will be induced to ovulate to the first GnRH injection and the assurance that a CL will be present throughout the synchronization period (i.e., a CL will not regress prior to the time that prostaglandin is injected).

Progestogens have been utilized in several ways as part of a synchronization program. Long-term treatment with a progestogen (e.g, 14 days) permits spontaneous CL regression to occur followed by withdrawal of the progestogen to allow a synchronized estrus. Such programs resulted in excellent synchrony of estrus but poor conception or pregnancy rates because of aged-persistent follicles of low fertility. However, progestogen treatments could be used to pre-synchronize estrus and allow for subsequent implementation of a synchronization program that would benefit from the staged presence of a new dominant follicle and CL. Alternatively, progestogen treatment could be given concurrently with GnRH and PG treatments to insure progestogen exposure, prevent premature occurrence of estrus, and enhance cyclic responses in anestrous animals. At the present time, MGA, an oral progestin, has recently received clearance from the FDA for use in reproductive classes of beef and dairy females. No other progestin (implant [e.g., Norgestomet] or intravaginal insert [CIDR or PRID] is approved currently (June, 2001) for use in the United States.

Estradiol benzoate and estradiol valerate have been used to induce follicle turnover with or without injection of progesterone. Injection of the estrogens reduces FSH secretion such that FSH dependent follicles (follicles undergoing recruitment e.g., 2 to 8 mm) will undergo regression, whereas the injection of progesterone will induce turnover of LH–dependent follicles (e.g., $\geq$ 10 mm). Thus, estrogens and progestogens given concurrently will induce follicle turnover and new wave emergence regardless of what stage of the follicular wave and/or stage of the estrous cycle. In contrast, GnRH will only induce follicle turnover in follicles $\geq$ 10 mm. However, these estrogens are not approved in the United States for cattle. An alternative use of estrogens is their ability to induce a preovulatory surge of LH as part of an ovulatory control system.

With this background, systems that are used currently in dairy and beef cattle will be described.

Dairy Cattle

GnRH-PG-GnRH: Ovsynch. The Ovsynch program synchronizes ovulation and permits a timed insemination (TAI).[22] Pregnancy rates are normal following a timed insemination.[25] However, the challenge is to further optimize this system. The Ovsynch protocol is idealized in Figure 1. In this example, concentrations of progesterone are monitored to document presence of a CL (the CL produces progesterone), and an idealized description of follicle development is presented. In this example, the cow is injected with GnRH (Monday, 5:00 PM) on day 5 of the estrous cycle. At this time, the cow has a dominant and healthy follicle that ovulates in response to the GnRH-induced release of LH; furthermore, the increase in FSH induced by the GnRH injection induces recruitment of a new pool of follicles in approximately 2 days (day 7) and one of the follicles is selected to become the dominant follicle.[16] On day 12 of the cycle (7 days after the injection of GnRH), PG is injected (e.g., Monday, 5:00 PM) to regress both the original CL present at day 5 of the cycle and a newly formed CL that was induced by the injection of GnRH on day 5 of the cycle. The decrease in progesterone associated with regression of the CL accelerates growth of the newly recruited dominant follicle and a second injection of GnRH is made 2 days after the injection of PG (e.g., Wednesday, 5:00 PM). The second injection of GnRH induces ovulation 24 to 32 hours later.[21] Knowing that ovulation will occur at this time, the timed insemination is given at approximately 16 hours after the injection of GnRH (e.g., 9:00 AM on Thursday). This permits sufficient time for sperm to develop the capacity to fertilize the egg so that when it ovulates, a fertile population of sperm is present to carry out fertilization or initiate a pregnancy. This is an idealized scenario and the timing of injections is con-

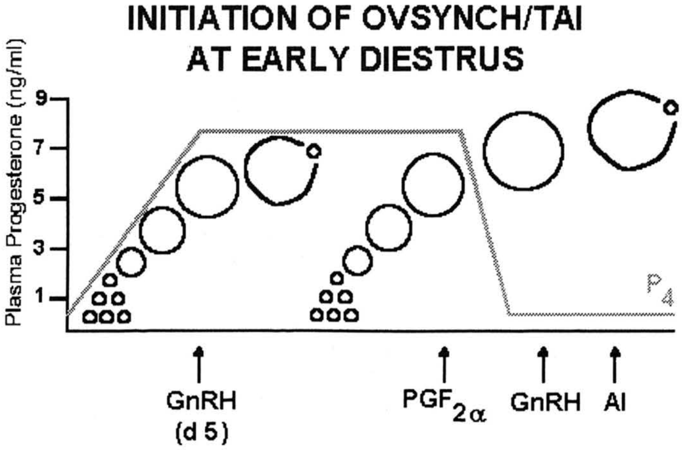

Figure 1. Plasma progesterone and follicle dynamics during an Ovsynch/TAI program started on day 5 of the estrous cycle.

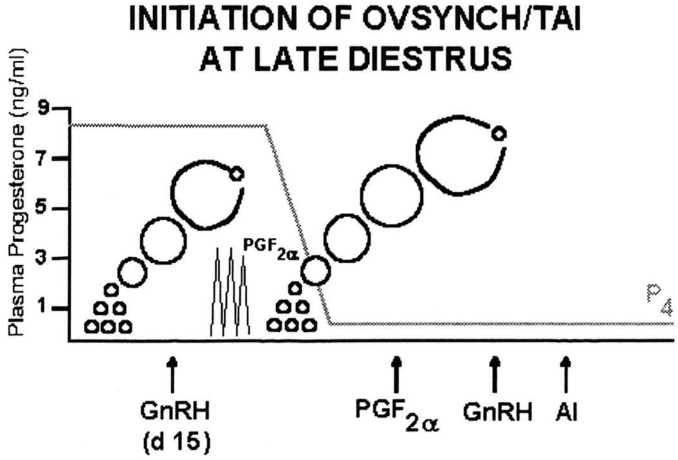

Figure 2. Plasma progesterone and follicle dynamics during an Ovsynch/TAI program started on Day 15 of the estrous cycle.

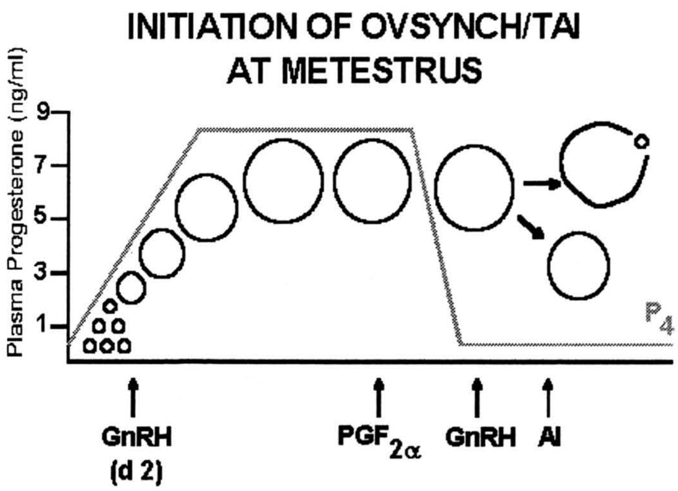

Figure 3. Plasma progesterone and follicle dynamics during an Ovsynch/TAI program started on Day 15 of the estrous cycle.

sidered critical to the success of the program. If an interval of less then 7 days is used between GnRH and PG injection, the ability to effectively regress a newly developed CL is reduced. If the second injection of GnRH is delayed to longer than 48 hours or 2 days, then more cows are detected in heat prior to injection of GnRH, cows become asynchronized, and the timing of insemination is not correct. It is essential that producers not alter the protocol. One commonly asked question is, can cows be inseminated at the time of the GnRH injection or at 24 h after the injection of GnRH to make the insemination process more convenient? Pregnancy rates will be lowered at the 24 h insemination, and optimal insemination times appear to be between 12-18 hours after the GnRH injection.[23]

Success of the program is dependent on whether lactating dairy cows are cycling as well as stage of the estrous cycle at the time the Ovsynch protocol is initiated in cycling cows. Clearly, if cows in the group are not cycling then it is a given that pregnancy rates of the group will be somewhat less even though the Ovsynch protocol itself may induce some cows to begin to cycle and perhaps conceive. Figures 2 and 3 provide examples as to specific stages of the estrous cycle that lead to lower pregnancy rates when the Ovsynch protocol is initiated. In Figure 2, a cow initiates the program at day 15 of the estrous cycle when a normal second wave follicle is under development. This follicle may or may not ovulate depending upon how mature it is. In many instances the second wave follicle is too small to ovulate and a new CL does not develop. At 2 to 4 days after the injection of GnRH, the cow spontaneously induces regression of the CL by releasing PG from the uterus. Thus, at the time of the PG injection given 7 days after the GnRH injection, the cow has already regressed the CL and may even be in heat.[16] Such cows will be asynchronized in that they will ovulate prematurely; if we continue the protocol then insemination is made too late and the cow does not conceive. A second problematic stage of the cycle is in the early phases of the estrous cycle (e.g., day 2) as demonstrated in Figure 3. In this scenario, the cow already has been in heat, ovulated and is recruiting a new dominant follicle. This is a small follicle and the injection of GnRH on day 2 does not alter development of the dominant follicle and does not recruit a new dominant follicle.[16] As a consequence, at the time of the second GnRH injection, the dominant follicle is considered aged and has expressed dominance for 5 days or longer. Follicles that have periods of dominance longer than 5 days[2] or cows that initiate the Ovsynch program in early stages of the estrous cycle are less fertile.[16,31] Follicles may ovulate but oocytes are less fertile, or some cows may fail to ovulate their follicle in response to the second injection of GnRH. We can project what the success rate of the Ovsynch program will be in an idealized situation in which all cows are cycling and are at random stages of the estrous cycle when the program is initiated (Table 1). Assuming a 20-day estrous cycle, we would expect 5% of the cows to be at each day of the estrous cycle. Thus, for a group of 100 cows, the percent of cows in early stages of the cycle (problematic), early diestrus, late diestrus (problematic) and proestrus are depicted in Table 1 with expected pregnancy rates at each stage for the reasons described above. An expected overall pregnancy rate of 36% is anticipated. However, it is possible to manipulate the estrous cycle of cows such that they are in the ideal stage of the estrous cycle when the Ovsynch program is initiated. One idealized scenario is to inject all cows twice with PG at an interval of 14 days and to initiate the first GnRH injection of the Ovsynch program on day 12 after the second injection of PG. Such an idealized manipulation is demonstrated in Table 2. If all cows were cycling, we would expect 90% of the cows to be in the ideal stage of the estrous cycle, between 5 to 10 days, when the Ovsynch program is initiated 12 days after the second injection of PG. With this scenario, an expected pregnancy rate to the Ovsynch program is 48%.

Table 1. Herd distribution, expected pregnancy rates (PR), and pregnancies (Preg) in a 100 cyclic cow herd initiating the Ovsynch/TAI protocol at random stages of the estrous cycle.

Day of the cycle	Herd distribution	Expected PR	Preg in a 100 cow herd
1 to 4	20%	20%	4
5 to 12	40%	50%	20
13 to 15	25%	20%	5
18 to 20	15%	50%	7
Total	100%	---	36%

Table 2. Herd distribution, expected pregnancy rates (PR), and pregnancies (Preg) in a 100 cyclic cow herd after pre-synchronization through two PG injections 14 days apart (the 2nd PG is given 12 days prior to initiation of the Ovsynch protocol).

Day of the cycle	Herd distribution	Expected PR	Preg in a 100 cow herd
5 to 10	90%	50%	45
13 to 17	5%	20%	1
18 to 20	5%	50%	2
Total	100%	---	48%

Such a proposed treatment program prior to implementation of the Ovsynch program is called pre-synchronization with a standard protocol that is practiced in the industry. It is imperative that the producer and veterinarian have a thorough understanding of the principles of ovarian manipulation in order to understand how the system functions when they make herd management decisions as to how to implement the program.

PG-PG-GnRH-PG-GnRH: Pre-synch-Ovsynch. In a recent study[17] a total of 543 cows were assigned randomly to an experiment in which half of the cows received the pre-synchronization program. The pre-synchronization program was initiated on a weekly basis such that cows 34 to 40 days postpartum (37± 3 days) received an injection of PG and this was followed 14 days later (51 ±3 days) with a second PG injection. In contrast, control cows (no pre-synchronization) did not receive the two injections of PG. The rationale for the pre-synchronization program is described above and potential impact on pregnancy rates presented in Table 2. On day 63 ± 3 days, the first injection of GnRH of the Ovsynch program was initiated, and this was 12 days after the second injection of PG of the pre-synchronization program. The pre-synchronization program will place cows between days 5 to 10 of the cycle at the time of the GnRH injection depending upon what day they expressed estrus after the second injection of PG. Days 5 to 10 of the cycle are considered an optimal time to begin the Ovsynch program as discussed above. On day 7 following the first GnRH injection (70 ± 3 days postpartum), all cows receive an injection of PG and receive the preovulatory injection of GnRH on day 72 ± 3 days postpartum. All cows were time inseminated at day 73 ± 3 days postpartum. Relative comparisons of progesterone concentrations in plasma allow us to determine if cows are cycling (samples on days 51 and 63), stage of the cycle at the beginning of the Ovsynch program (samples on days 63 and 70), whether CL regression was successfully induced (samples on days 70 and 72) and whether cows had a synchronized ovulation (samples on days 72 and 79). All cows were examined by ultrasonography for pregnancy on day 32 after timed insemination and pregnant cows re-examined for pregnancy by rectal palpation on day 74 after timed insemination. This allowed us to characterize fetal losses between 32 and 74 days of pregnancy. All cows diagnosed open at day 32 after the first timed insemination were injected with GnRH and the Ovsynch program repeated with second insemination occurring at 115 days of lactation.

Plasma progesterone in two samples collected 12 days apart (on days 51 and 63 postpartum) identified what cows were anestrus when the Ovsynch program was initiated. If cows had progesterone ≤1ng/ml in both samples they were considered to be anestrus. It was important to determine which cows are cycling since pre-synchronization treatment effect would not be evaluated properly in cows that are not cycling. Furthermore, this assessment of anestrous status will allow us to document the frequency of this condition and its impact on reproductive performance of the herd. It is interesting that 23.4% of the cows were anestrus or had not started to cycle by 63 days postpartum. Not surprising is the observation that the frequency of anestrus was greater for primiparous or first-calf heifers (35.6%) than multiparous cows (16.9%). The occurrence of anestrus decreased as body condition scores recorded at initiation of the Ovsynch improved. Therefore, body condition may be used to some degree to estimate the relative nutritional status of lactating dairy cows, and its impact on the frequency of anestrous cows at initiation of a reproductive management system. As anticipated, anestrous cows did not perform as well as cyclic cows in terms of pregnancy rates to the first-service Ovsynch protocol. Pregnancy rate at 74 days after insemination was only 22.4% for anestrous cows, which was lower than the 41.7% pregnancy rate at 74 days after insemination for cyclic cows. Postpartum management of lactating dairy cows is of extreme importance and will greatly affect reproductive performance. Efforts to maximize cow health, comfort, and nutritional status following parturition (e.g., enhance dry matter intake) will be reflected later in the lactation in terms of a higher incidence of cycling cows and improved reproductive performance.

Since anestrus had such a highly significant affect on pregnancy rates, reproductive performance was examined only in cyclic cows. Increased pregnancy rates were detected when cows were pre-synchronized (52.3%) compared to cows not pre-synchronized (31.1%). The reason for increased pregnancy rates to the Ovsynch protocol in cows pre-synchronized was related to the frequency of cows initiating the synchronization protocol at favorable stages of the estrous cycle. As indicated above, it was hypothesized that pregnancy rates to the Ovsynch protocol would be increased if cows received the first GnRH injection between days 5 to 10 of the cycle, and that pre-synchronization would synchronize approximately 90% of the cycling cows such that these cows would be between days 5 and 10 of the cycle when the Ovsynch protocol was initiated. Cows with high plasma progesterone (> 1.0 ng/ml) at both day 63 and day 73 (i.e., HIGH-HIGH cows) probably initiated the Ovsynch protocol at the optimal stage of the estrous cycle. Results from the frequency of cyclic cows classified as HIGH-HIGH indicated that approximately 87.4% of pre-synchronized cows were classified as HIGH-HIGH versus 71.7% of cows not pre-synchronized were HIGH-HIGH cows. Therefore, we were successful in programming the cows to be in the optimal stage of the cycle to begin the Ovsynch program and this resulted in a greater synchronized pregnancy rate. A single PG injection at 12 days prior to initiating the Ovsynch protocol increased synchronized pregnancy rate in multiparous cows. This was due to an increase in cows being in early diestrus at onset of the Ovsynch protocol.[5]

Ovsynch in Lactating Dairy Cows During Periods of Heat Stress:

Pregnancy rate, which is a product of estrus detection and conception rates, is reduced during seasonal periods of heat stress. Heat stress reduces plasma estradiol during proestrus and lowers estrus detection rates.[10] Conception rates also are reduced during heat stress due to elevations in body temperature that result in early embryonic death.[24] De la Sota *et al*[7] compared the efficiency of a reproductive management program involving the Ovsynch program with a typical farm management program involving PG treatment alone in which cows were inseminated at detected estrus, under heat stress conditions in Florida. The hypothesis was that because Ovsynch program increases estrus detection rate to 100% (all cows are inseminated), the pregnancy rate should increase. The study[7] was conducted from May to September with primiparous (n=133) and multiparous (n = 171) lactating Holstein cows. At 30 ± 3 days postpartum, all cows were injected with PG to regress any existing CL. The VWP was set at 60 d postpartum. Timed inseminated cows (n= 148) were synchronized using the Ovsynch protocol shown in Figure 1. Cows in the control group (n= 156) were injected with PG at 57±3 days postpartum and inseminated when detected in estrus. All cows in both groups were re-inseminated at subsequent detected estruses. First inseminations occurred from May through September, 1995. Pregnancy rate was greater for Ovsynch- managed cows than cows of the control group (13.9 vs 4.8%, $P < .05$). The proportion of cows detected in estrus and inseminated during days 1 to 6 after injection of PG was only 18.1% for control cows, compared to an insemination rate of 100% for Ovsynch cows. The interval between PG injection and insemination was 35.5 days for control cows compared with only 3.0 days for Ovsynch cows ($P < .05$). Likewise, number of days postpartum to the first insemination was less in Ovsynch than in control cows (58.7 vs 91.0 days, $P < .05$). The longer interval from PG injection to insemination for the control group reflects summer time reduction in detection of heats that was eliminated with Ovsynch. Conception rate for control cows detected in estrus and inseminated was greater (25.9%) than cows of the Ovsynch group (13.2%, $P < .05$). However, this increase in conception rate is outweighed by the 100% submission rate for insemination of the Ovsynch group. The overall pregnancy rate by 120 days postpartum was greater for

Ovsynch cows when compared with control (27.0 vs 16.5%, $P < .05$). Number of days open for cows that conceived by 120 days postpartum was 12.4 days less for Ovsynch compared with control-managed cows (77.6 vs 90.0, $P < .05$).

To achieve the greater pregnancy rate at first service with Ovsynch (13.9 vs 4.8%; $P < .05$), an appreciably greater number of services was made compared to the control group. This contributed to a greater number of services per conception by 120 days postpartum for the Ovsynch group (1.63 vs 1.27; $P < .05$). However, by 365 days postpartum number of services per conception was the same (3.76 Ovsynch and 3.52 control). A greater number or proportion of control cows needed to be inseminated through the remaining 365-day period. This led to an equal number of services per conception and a smaller proportion of cows pregnant by 365 days. Furthermore, the total number of services per cow (pregnant and open) through 365 days did not differ (3.87 Ovsynch vs 3.72 control).

As expected pregnancy rate was significantly higher for the Ovsynch group because of the higher number of cows inseminated. The Ovsynch management program will not protect the embryo from temperature induced embryonic death, but limitations induced by heat stress on detection of estrus are eliminated. Arechiga and coworkers[1] also demonstrated that the Ovsynch program increased the cumulative pregnancy rate of lactating Holstein cows during summer. However, pregnancy rate at first service was not increased. A sustained advantage in cumulative pregnancy rate observed by De la Sota[7] (Figure 4) and in the experiments of Arechiga *et al*[1] during summer periods of heat stress indicates that the Ovsynch protocol may have

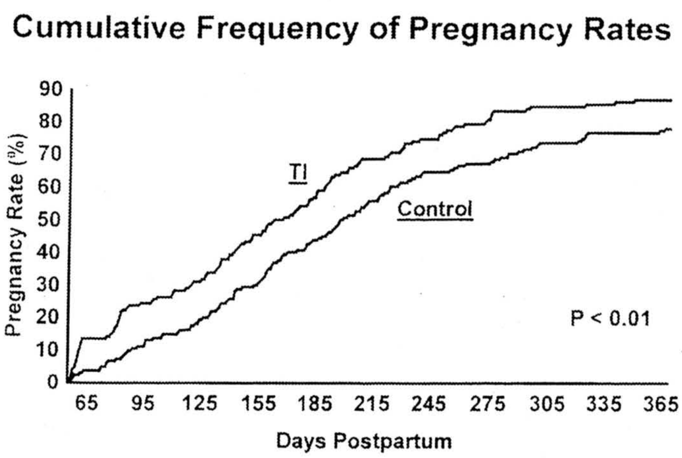

Figure 4. Cumulative frequency of pregnancy rates (the occurrence of pregnancy for experimental cows was cumulated throughout a 365 day period) in lactating dairy cows that received their first postpartum service in summer as an Ovsynch or at a detected estrus in control cows that received a single injection of PG.

some beneficial carryover effect to improve pregnancy rates. Perhaps sequential injections of GnRH stimulated turnover of ovarian follicles that were damaged by heat stress such that subsequent follicles destined for ovulation produced more fertile oocytes.

A recent report[6] indicated that Ovsynch increased 27-30 day pregnancy rates compared to cows inseminated at detected estrus in response to a Select Synch program. However, this advantage was lost by 40 to 50 days of pregnancy due to a trend for higher embryo death losses. This later stage death loss of embryos during seasonal periods of heat stress warrants further investigation.

GnRH-PG-ECP: Heatsynch. One of the uses of exogenous estradiol as part of estrus synchronization systems is based on estradiol's ability to induce a LH surge by stimulating hypothalamic secretion of GnRH. Estradiol also increases pituitary sensitivity to GnRH, apparently by increasing the number of GnRH receptors within the pituitary. Estradiol treatments during late diestrus and proestrus (low progesterone environment) will induce preovulatory surges of LH and FSH.[12,29] The preovulatory surge induced by an injection of estradiol at proestrus lasts for approximately 10 h,[27] which is similar to the spontaneous surge. Indeed these LH surges are of a greater duration than that induced by GnRH. We have conducted a series of experiments to evaluate whether estradiol cypionate could be used as part of a timed insemination program in dairy animals to replace the second GnRH injection of an Ovsynch program.

Estradiol cypionate (ECP), an esterified form of estradiol-17β, is approved for use in lactating dairy cows in the United States. Recent results utilizing dairy heifers indicated that ECP can replace the second GnRH injection in TAI to successfully induce ovulation, when administered 24 h after the injection of PG.[15] Since it was determined that dairy heifers and nonlactating dairy cows ovulated 62 and 60 h after ECP injection, respectively, artificial insemination was scheduled 48 h after ECP injection. The use of ECP within the Ovsynch protocol may offer dairy producers an alternative, cost efficient reproductive management system if effective in lactating dairy cows. Several experiments were conducted in lactating dairy cows with the following objectives: 1) to determine whether ECP can replace the second GnRH injection of a TAI protocol by evaluating pregnancy rate to first service in lactating dairy cows, and 2) to characterize the time of induced estrus and ovulation after injection of ECP in lactating dairy cows during proestrus.[18]

Experiment 1 evaluated pregnancy rates when estradiol cypionate (ECP) was used to induce ovulation as part of a timed artificial insemination (TAI) protocol in comparison to Ovsynch for lactating dairy cows in Florida (n=371) and Texas (n=321). Cows were pre-synchronized with two injections of PG given 14 d apart with TAI protocols beginning 14 d after the second injection of PG. The TAI protocols consisted of an injection of GnRH followed by PG 7d later. Cows were injected either with GnRH (Treatment I, Ovsynch) at 48 h after PG and inseminated 16-24 h later or with ECP (1 mg, IM) at 24 h after PG, (Treatment II; Heatsynch) and inseminated 48 h later. In Florida, pregnancy rates after TAI were 37.1 ± 5.8% for Ovsynch compared to 35.1 ± 5.0% for Heatsynch (Table 3). Pregnancy rate to first insemination was higher for primiparous (47.1%) cows than multiparous (25.0%) cows (P < 0.01). Primiparous cows by design were not inseminated until 100 days postpartum versus 78 days postpartum for multiparous cows. There was a tendency for a parity by treatment interaction (P < 0.10) in which pregnancy rates did not differ between Ovsynch and Heatsynch in primiparous cows, but pregnancy rate tended to be lower for the Heatsynch group in multiparous cows (Table 3). It is important to recognize that any difference in parity of the Florida study is confounded with differences in days postpartum for TAI (primiparous, 100 days versus multiparous, 78 days).

In Texas, pregnancy rates were 28.2 ± 3.6 % for Ovsynch and 29.0 ± 3.5 % for Heatsynch. Overall pregnancy rates did not differ between Ovsynch and Heatsynch treatments. No difference in pregnancy rate was detected between primiparous (40.6%; n=105) and multiparous (34.7%; n=216) cows that were inseminated at the same days postpartum (i.e., 73 days). An interaction between treatment and whether cows were in estrus at TAI (P<0.05) was detected in which cows in estrus had a higher pregnancy rate in the Heatsynch group; whereas, pregnancy rates were comparable in estrus and non-estrus cows of the Ovsynch group (Figure 5). Occurrence of estrus on the day of TAI was greater in the Heatsynch group (65.2%>17.8%, P<0.01). Since no difference was detected in pregnancy rate due to parity, it is possible that a delay in TAI for first calf heifers may have improved fertility in the Florida study.

In Experiment 2, estrus (monitored by the Heat Watch system) and ovulation times were determined in lactating dairy cows submitted to the Heatsynch protocol.[18] Frequencies of detected estrus and ovulation after ECP were 75.7% (28/37) and 86.5% (32/37), respectively. Mean intervals to ovulation were 55.4 ± 2.7 h (n=32) after ECP and 27.5 ± 1.1 h (n=27) after onset of estrus. Estrus occurred at 29.0 ± 1.8 h (n=28) after ECP. It is recommended that any cow detected in estrus by 24 h after ECP injection be inseminated at 24 h, and all remaining cows be inseminated at 48 h, since 75% (n=24/32) of the ovulations occurred between ≥48 h to ≤72 h after ECP. Synchronization of ovulation and fertility results indicated that ECP could be utilized to induce ovulation for a timed insemination. Based on synchronization of ovulation and pregnancy rates, ECP can be utilized to induce ovulation in place of GnRH for a timed insemination. Timing of ECP injection to induce ovulation for a timed insemination differs compared to the use of GnRH. Greater uterine tone, ease of insemination and occurrence of estrus improve acceptance by the inseminator. On the other hand, in facilities with poor footing the reduced estrous expression following GnRH may be preferred.

Beef Cattle

Effective estrus synchronization programs in beef cattle offer the following advantages: 1) cows or heifers are in estrus at a predicted time which facilitates AI, embryo transfer, or other assisted reproductive techniques; 2) the time required for detection of estrus is reduced thus decreasing labor expense associated with estrus detection; 3) cycling females will conceive earlier

Table 3. Pregnancy rates of primiparous and multiparous cows at 46 ± 3 d after insemination (LSM ± SE; Florida site).

Parity	Ovsynch Group (n=179)	Heatsynch Group (n=192)	Overall (n=371)
Primiparous (n=201)	43.5 ± 6.9 (n=100)	50.7 ± 6.3 (n=101)	47.1 ± 5.6
Multiparous (n=170)	30.6 ± 7.2 (n=79)	19.4 ± 6.5 (n=91)	25.0 ± 5.7
Overall (n=371)	37.1 ± 5.8	35.1 ± 5.0	39.6 ± 2.5[a,b]

[a,b]Treatment x Parity Interaction, P<.10; Parity, P<0.01

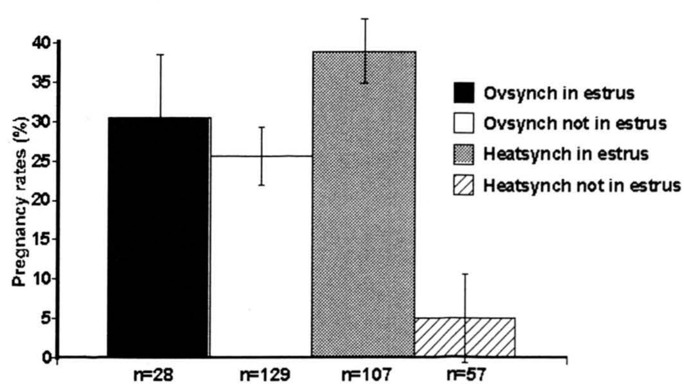

Figure 5. Pregnancy rates at 37 days after TAI (LSM ± SE) in lactating dairy cows that were in estrus or not in estrus at the time of insemination following Ovsynch and Heatsynch treatments.

during the breeding period; and 4) AI becomes more practical.[11]

Timed Insemination Programs involving GnRH and PG. Protocols for fixed-time insemination such as Ovsynch and variations of the Ovsynch protocol (CO-Synch and Select Synch) were tested in postpartum beef cows (Figure 6). It is important to understand that treatment variations of Ovsynch currently being used in postpartum beef cows have not undergone the same validation process that Ovsynch underwent in lactating dairy cows.

As a review and using Ovsynch as a reference point, the protocols for beef cattle (Figure 6) are:

- *Ovsynch.* At day 0, a GnRH injection is given to stimulate endogenous lutenizing hormone (LH). The LH release causes ovulation of a follicle and subsequent formation of a corpus luteum on the ovary if injected at the appropriate stage of the cycle when there is a potential ovulatory dominant follicle. After ovulation, the ovary begins to mature another dominant follicle. At day 7, a PG injection is given to regress the corpus luteum. Approximately 48 hours later, the newly recruited dominant follicle will have reached maturity; so another GnRH injection is given to cause ovulation. Timed insemination at 16 hours after the second GnRH injection produces the best pregnancy rates. In noncycling cows, close to 50% became pregnant on the Ovsynch program.
- *Cosynch.* A modification of the Ovsynch timed-insemination program, it implements timed AI at the time the final GnRH injection is given. This reduces cattle handling to three times.
- *Select Synch.* A modified Ovsynch program, Select Synch calls for heat detection rather than timed-AI. At day 0, a GnRH injection is given to stimulate ovulation and create new follicle growth and PG is given 7 days later to regress the corpus luteum. The follicle is allowed to ovulate naturally therefore no second GnRH injection is given. Heat detection is used to determine estrus, with insemination occurring 12 hours after detection. In certain dairy cow studies,[4,6] this program was the control group to evaluate pregnancy rates of the Ovsynch program. The majority of cycling cows come into estrus 36-71 hours after PG injection with Select Synch. Without a pre-synchronization program, some cows will be in heat a day or so prior to PG injection and they should be inseminated and not given the PG injection.

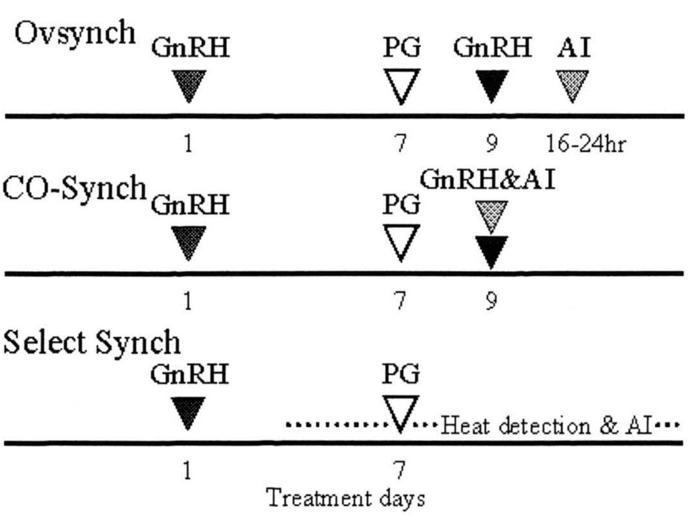

Figure 6. Methods currently being used used to synchronize ovulation in postpartum beef cows: Ovsynch, CO-Synch and Select Synch.

Select Synch and Cosynch protocols have similar effects on pregnancy rates in noncycling cows (29.0% and 28.4%, respectively). However, 60% of cycling cows on the Select Synch protocol became pregnant, while about 40.0% became pregnant on the Cosynch program. Thus, the Select Synch program seems to work best on cycling cows.[28]

In a comparison between Ovsynch and Cosynch,[9] beef cows (n =473) stratified by breed, postpartum interval, age, and AI sire at two locations were randomly allotted to one of four treatments for synchronization of ovulation. Ovulation synchronization protocols included the Ovsynch protocol with (n =114) or without (n =123) 48-h calf removal from d 7 to 9 (d 0 =1st GnRH injection) or the Cosynch protocol with (n =119) or without (n =117) 48 h calf removal from d 7 to 9. Blood samples were collected twice at a 10 d interval just prior to the first GnRH injection for analysis of serum progesterone. Cows with at least one serum progesterone concentration greater than 1 ng/mL were considered to be cyclic at the time of treatment. Pregnancy rates of cows that received the Cosynch +calf removal, Ovsynch +calf removal, Cosynch, or Ov-synch protocol (Figure 7) were not different (*P* =0.50). Pregnancy rates were not different (*P* =0.80) between Cosynch- and Ovsynch-treated cows as a main effect; however, both cyclic status (cyclic versus anestrus) and 48-h calf removal (removal versus no removal) affected pregnancy rates. Pregnancy rates of cyclic cows (66%) were greater (*P* =0.01) than those of anestrous cows (53%), regardless of which synchronization protocol was used. When data were pooled across synchronization protocol, pregnancy rates of cows with 48-h calf removal (62%) were greater (*P* =0.09) than pregnancy rates of cows without calf removal (53%). The Ovsynch and Cosynch +calf removal protocol induced a fertile ovulation in cyclic and anestrous cows, required handling cattle just three times, resulted in high pregnancy rates from timed insemination, and should be a useful program for synchronization of ovulation in beef

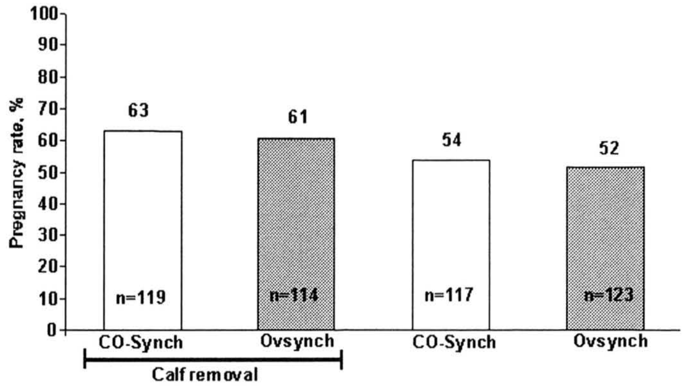

Figure 7. Pregnancy rates of cows that received the CO-Synch or Ovsynch protocol for synchronization of ovulation with or without 48-h calf removal (P=0.50). Adapted from Geary *et al*, *J Anim Sci* 79:1-4, 2001.

cows. Pregnancy rates reported are excellent compared to those of lactating dairy cows. It should be noted that bulls were placed with cows 5 days following timed insemination with pregnancy rates conducted at 60 and 90 days after timed insemination. No attempt was made to determine progeny paternity. Furthermore, the incidence of short cycles among anestrous cows is not known as to whether on return to estrus these cows became pregnant. Nevertheless, overall pregnancy rates to the program are impressive under field conditions. In cattle with Bos Indicus breeding, pregnancy rates were greater for Cosynch compared to Select Synch in postpartum and lactating cows.[14]

Programs Involving the use of MGA

Feeding MGA for 14 days followed by PG injection 17 days after MGA feeding (MGA-PG protocol) is an effective method of estrous cycle control in heifers.[3,19] More recently, an increase in estrus response, synchronized conception and pregnancy rates, and fecundity in the postpartum cow was reported among cows treated with the MGA-PG protocol when compared with PG alone.[8,20] The MGA-PG protocol avoids problems with reduced conception encountered with inseminations at the immediate estrus following MGA feeding and offers advantages compared with untreated controls.[3,19] The disadvantages of the MGA-PG system include: a) anestrous cows that experience a short luteal phase after the period of MGA feeding which in some cases necessitates a second PG injection; b) the potential for an increased incidence of twinning, which is undesirable from a management viewpoint in many beef production systems; and c) the length of the treatment period.

Patterson and colleagues have focused research efforts on the development of effective and economical progestin (MGA) based estrus synchronization programs for peripubertal heifers and postpartum suckled beef cows. Recently, an estrus synchronization protocol for beef cattle was designed to: 1) shorten the feeding period of MGA without compromising fertility, and 2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development (Figure 8).[13] This new treatment, 7-11 Synch (see Introduction for description of protocol), was compared with the GnRH-PG protocol. In essence, the protocol of MGA feeding and PG has pre-synchronized follicle development such that the subsequent GnRH injection induces ovulation, CL development and follicle emergence so that PG 7 days later induces CL regression coincidentally with development of the synchronized dominant follicle (Figure 8). Synchrony of estrus during the 24-hour peak response period (42 to 66-hour) was significantly higher among 7-11 Synch treated cows (Figure 9). Furthermore, the distribution of estrus was reduced from 144 hours for GnRH-PG treated cows to 60 hours for cows assigned to the 7-11 Synch treatment (Figure 9).[13] The 7-11 Synch protocol resulted in a higher degree of estrus synchrony (91% vs 69%) and greater AI pregnancy rate during a 24-hour peak response period compared to the GnRH-PG protocol (69% and 47%, respectively).

These studies with MGA demonstrate significant improvements in specific reproductive endpoints among cows that received MGA prior to the administration of PG compared with cows that received PG only, including increased estrus response and improved synchronized conception and pregnancy rates. More importantly recent studies observed a significant improvement in synchrony of estrus without compromising fertility in postpartum beef cows and beef heifers that were pretreated for a short period with MGA prior to GnRH and PG. Patterson and his colleagues propose that progestin (MGA) treatment prior to the GnRH-PG estrus synchronization protocol will successfully: 1) induce ovulation in anestrous postpartum beef cows and peripubertal beef heifers; 2) reduce the incidence of a short luteal phase among anestrous cows induced to ovulate; 3) increase estrus response, synchronized conception and pregnancy

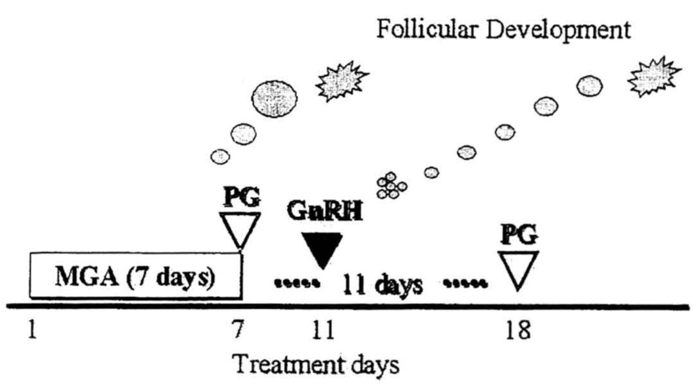

Figure 8. Illustration of the treatment schedule and events associated with the 7-11 Synch protocol (Kojima *et al*, 2000).

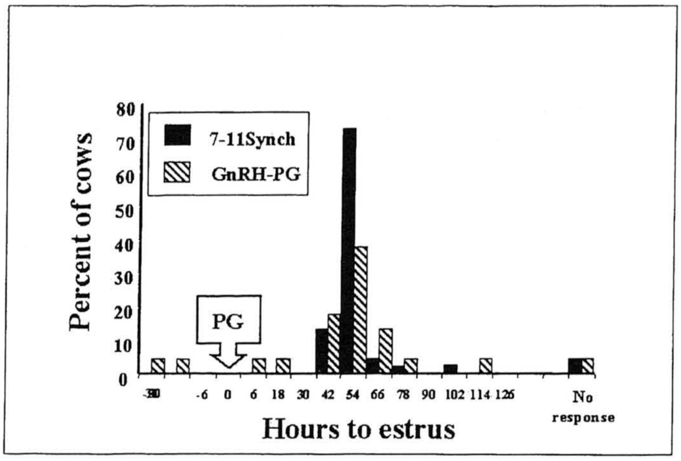

Figure 9. Estrus response of cows treated with the 7-11 Synch or GnRH-PG protocols (Kojima *et al*, 2000).

rate; and 4) increase the likelihood of successful fixed-time insemination. Current studies indicate that new methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers in which the GnRH-PG protocol is preceded by a progestin offer significant potential to more effectively synchronize estrus with resulting high fertility.

Perspective

A vast array of options has been developed for the reproductive management of cattle. Such management systems have been fine-tuned to result in maximum pregnancy rates and increase the overall reproductive efficiency of the herd unit. As observed in the experiments described above, pregnancy rates as great or greater than 50% to a single AI service were achieved in both dairy and beef production systems. It is important to emphasize, that as reproductive systems become more efficient and incorporate several levels for the control of reproductive processes, producers and veterinarians need to have thorough understanding of the technology.

It is important for producers to realize that such reproductive management systems cannot solve all reproductive problems *per se*. For instance, incidence of cows in anestrus greatly reduces the reproductive efficiency in both dairy and beef herds and will not be resolved solely by synchronization systems. Providing optimal nutritional management, maximizing cow comfort, and maintaining a good herd health program are all pre-requisites for the success of any reproductive program.

To date, management of dairy cows has been driven by the necessity to maximize milk production with a high overall success. With the advent of new technologies to precisely manipulate reproductive function in lactating dairy cows, dairy producers are presented with a new opportunity. Coordination of management strategies to maximize <u>both</u> milk production and reproductive performance may optimize the economic return of dairy herds, and allow for the industry to take complete advantage of the genetic potential to improve milk production through artificial insemination. Further research is necessary to fine tune management systems that are both practical and able to fulfill this objective. Producers can look forward to new strategies to reduce anestrus, synchronize return services and enhance embryo survival. Many of the advancements made in the dairy area have benefited the development of beef reproductive management programs and vice versa. The cattle industries can expect additional advancements with the exciting research approaches now underway.

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