

Current concepts in synchronization of estrus and ovulation of dairy cows

C. S. Whisnant¹, S. P. Washburn, and P. W. Farin

Departments of Animal Science and Food Animal and Equine Medicine, North Carolina State University, Raleigh 27695

Abstract

Estrus synchronization has become a routine reproductive management tool for dairy producers. Reproductive performance has decreased over the last 25 yr on dairy farms, and the lack of estrus detection or inaccurate detection are major reasons for increased numbers of days open for the average cow. Estrus synchronization methods that control both the function of the corpus luteum and the development of follicles with viable oocytes are critical to efficacy. This article reviews some of the synchronization methods used in dairy cattle, especially the recent protocols involving the use of GnRH. Conception and pregnancy rates from a series of studies using the Ovsynch protocol averaged 34.9 and 34.9%, respectively. Conception and pregnancy rates from breeding at detected estrus after PGF_{2α} averaged 37.7 and 19.8%, respectively. Because pregnancy rates are typically below 40%, possible methods for early identification of open cows and resynchronization will be discussed.

Key Words: Dairy Cows, Estrus, Ovulation

Introduction

Reproductive performance has been declining in dairy cows with increasing number of days open and decreased conception rates over the last 25 yr (Silvia, 1998). One major problem is lack of accurate estrus detection. Several studies have estimated that ≤ 50% of the cows are detected in estrus (Barr, 1975; Stevenson and Britt, 1977; Heersche and Nebel, 1994), and milk progesterone assays indicate that between 5 and 25% of cows inseminated are not in estrus (Reimers et al., 1985; Nebel et al., 1987), with some herds having more than a 30% error rate. Increasing herd size and other factors have contributed to the difficulty with accurate estrus detection. Use of bovine somatotropin (bST) may contribute to this decline in reproductive efficiency. First, fewer cows treated with bST are detected in estrus after prostaglandin F_{2α} (PGF_{2α}) compared with cows not receiving bST (Kirby et al. 1997). Regardless of the causes of the estrus detection problem, it has led to an increased interest in synchronization of estrus and ovulation. This paper will address the recent literature concerning synchronization of estrus and ovulation in lactating dairy cows. Dairy heifers will not be considered except when included in the same study with lactating cows. For the purpose of this review, conception rates will refer to the percentage of cows that became pregnant among those cows actually inseminated and pregnancy rate will represent the percentage of all cows in a group that became pregnant. Pregnancies are based on diagnosis by palpation and(or) ultrasonography per rectum. Thus, pregnancy rate is a product of heat detection efficiency and conception rates except in protocols using fixed time insemination for all cows where conception and pregnancy rates are synonymous.

Discussion

Prostaglandin F_{2α}

For some years, PGF_{2α} and its analogs were the only drugs approved for use in lactating cows. A number of different protocols have been developed to use PGF in breeding programs or schedules. These variations include time intervals between PGF injections and methods to identify the subpopulation of cows responsive to PGF. Rosenberg et al. (1990) and Folman et al. (1990) compared estrus synchronization and pregnancy rates in groups of lactating cows given two injections of PGF either 11 or 14 d apart and inseminated according to detected estrus following the second injection. Estrus occurred earlier after the second PGF injection when it was given 11 d after the first, but a similar percentage of animals were detected in estrus for each group (Rosenberg et al., 1990). Pregnancy rates were greater for cows receiving PGF injections 14 d apart (Folman et al., 1990). The 14-d treatment regimen also has the advantage of being easier for producers to schedule and has become a standard protocol used in reproductive management. Treatment with PGF at 14-d intervals may affect the duration of subsequent estrous cycles. Morbeck et al. (1991) compared the length of the cycle following two injections of PGF to that of a spontaneous cycle in a group of nonlactating dairy cows. The mean length of the induced cycles was 1.5 d longer than the length of the spontaneous cycles. If progesterone concentrations were less than 1 ng/mL at the time of the first injection, cycle length was increased by 3.4 d compared with cows that had greater than 1 ng/mL of serum progesterone.

Attempts have been made to develop management practices to identify cows that should respond to PGF and avoid treating cows that will not respond, such as those without a functioning corpus luteum (CL). Stevenson and Pursley

(1995) used milk progesterone tests to identify cows with elevated concentrations of progesterone that subsequently were treated with PGF. Reproductive performance of these cows was compared with cows submitted for AI based on twice-daily estrus detection. The use of milk progesterone tests decreased days to first AI, calving intervals, and cost per pregnancy, but the cost was greater than a blind weekly injection of PGF to all noninseminated animals. Similar results were found when transrectal palpation was used to identify cows for PGF treatment (Plunkett et al., 1984). Kristula et al. (1992) found that weekly PGF injections followed by standard estrus detection decreased days to first insemination and increased pregnancy rate by 30% over the first 90 d postpartum compared with transrectal palpation followed by PGF administration to cows identified as having a palpable CL. Costs per pregnancy were lower for the weekly PGF injection method as well (Kristula et al., 1992).

Timed insemination has been tried with PGF, but conception rates were consistently lower than with breeding after estrus detection (Lucy et al., 1986; Stevenson et al., 1987; Archbald et al., 1992). Differences in follicular development at time of CL regression due to the wavelike nature of follicular development result in varying times to estrus and ovulation (Thatcher et al., 1996). Treatment with gonadotropin-releasing hormone (GnRH) at 72 h after PGF improved conception rates to a timed insemination at 80 h following PGF (41 vs 33%) (Lucy et al., 1986), but conception rates were still lower than with breeding after detected estrus (51 vs 41%). Stevenson et al. (1987) found a 23% conception rate following a single insemination 80 h after PGF compared with 54% for insemination 12 h after detected estrus. Giving two inseminations at 72 and 96 h after insemination produced conception rates of 30% (Stevenson et al., 1987). Archbald et al. (1992) compared pregnancy rates in six groups of dairy cows. All cows had palpable corpora lutea and serum progesterone concentrations above 1 ng/mL at the time of PGF treatment. Group 5 was bred 12 h after detected estrus following PGF and Group 6 was inseminated 12 h after a spontaneous estrus. Groups 1 through 4 were bred to a single insemination between 72 and 80 h after PGF and given no additional treatment, GnRH, PGF, or GnRH and PGF at breeding, respectively. Cows receiving GnRH alone had the highest pregnancy rates of the timed insemination groups and were not different from Groups 5 and 6 (Archbald et al., 1992).

Progesterone and Analogs

Progesterone or its analogs can be used to synchronize estrus in cows. Although these methods have not been approved for use in lactating dairy cows in the United States, research on their use has been done and they are approved in other countries. Two devices have been developed, a progesterone-releasing intravaginal device (PRID; CEVA, Paris, France) and a controlled internal drug-releasing device (CIDR; InterAg, Hamilton, NZ). The PRID contains 1.55 g of progesterone in a silicone elastomer coil along

with 10 mg estradiol benzoate (EB) in a gelatin capsule, and the CIDR contains 1.9 g of progesterone in a silastic elastomer. The CIDR-B also has a capsule containing 10 mg of EB attached to the silastic elastomer. Degree of synchrony increases with the length of time the CIDR are in a group of cows, but fertility decreases with increased length of progesterone treatment (Macmillan and Peterson, 1993). Tjondronegro et al. (1987) reported that treatment with a PRID for 12 d resulted in more cows displaying estrus and a greater degree of synchrony than cows treated with a CIDR for 9 or 12 d. Most cows that did not display estrus had elevated milk progesterone concentrations during the days after CIDR removal, indicating a functional CL and the need for a luteolytic agent in the synchronization method. The CIDR had a better retention rate within the vagina (100%) than did the PRID (89.4%) (Tjondronegro et al., 1987).

Many studies have used injections of PGF_{2α} or estrogen or both in conjunction with a progesterone device. Gyawu et al. (1991) compared the effectiveness of 12-d treatment with a PRID containing EB with treatment using PRID-EB with cloprostenol, a PGF_{2α} analogue (given on d 11 of PRID-EB treatment and with a third group of cows that received two injections of cloprostenol 11 d apart. Insemination was done 48 and 72 h after PRID-EB removal or 72 and 96 h after the second cloprostenol injection for Group 3. The PRID-EB plus cloprostenol treatment resulted in significantly higher synchrony of ovulation and pregnancy rates compared with the other groups (Gyawu et al., 1991). These researchers concluded that cloprostenol increased effectiveness because EB was not effective in inducing luteolysis. Cows that were between d 0 and 10 of their estrous cycle at the initiation of treatment had significantly improved pregnancy rates compared with those beyond d 10 of their cycle. These and other results indicate that synchrony of estrus following the use of progestins alone is not sufficient for success with a fixed time insemination. Martinez et al. (1998) administered CIDR to dairy heifers for 7 d, and heifers received an injection of cloprostenol on the day of CIDR removal. Heifers then received either 1 mg of EB on the day after CIDR removal and were inseminated 30 h later (Group 1) or were inseminated at the same time as Group 1 and given an injection of GnRH (100 μg) at time of insemination (Group 2) or inseminated 12 h after detected estrus (Group 3). Estrus detection rates were higher in Groups 1 and 3, with pregnancy rates highest in Group 1. The treatment for Group 1 was repeated in another experiment using both heifers and lactating cows, which compared 1 mg of EB with 5 mg of estradiol. Both estrogen treatments were equally effective (60% pregnancy rates), and there were no differences between heifers and cows (Martinez et al., 1998).

Another variation has been to use progestins to enhance the response to PGF treatment. Xu and Burton (1998) treated cows with two injections of PGF 13 d apart. Half of the cows had a CIDR without EB inserted for 5 d before the second PGF injection. This protocol increased the estrous response and conception rate to AI. Insertion of a used

CIDR during d 16 to 21 after breeding to resynchronize estrus in nonpregnant cows did not affect conception rates to first or second AI (Xu and Burton 1998). Smith and Stevenson (1995) compared pregnancy rates in cows receiving two PGF injections 14 d apart with those in cows given either norgestomet or a PRID that contained no estrogen between PGF injections. The progestins were administered for 7 d beginning on d 8 after the first PGF, and the second PGF injection was given either 1 or 6 d after progestin administration. Pregnancy rates were greater in cattle with a CL present 14 d after the first PGF injection. Exogenous progestins did not enhance fertility when a CL was present and reduced fertility in cows without a CL. The reduced fertility was associated with increased diameter of the ovulatory follicle and serum estradiol concentrations (Smith and Stevenson, 1995). Further research is needed with progestin devices to determine if fixed time insemination can be made practical.

Use of GnRH

Research into the control of follicular waves led to the observation that GnRH or an analogue could be used to cause ovulation or luteinization of dominant bovine follicles (Macmillan et al., 1985; Thatcher et al., 1989; see review by Twagiramungu et al., 1995). Attempts have been made to incorporate GnRH within a two injection PGF program. Stevenson et al. (1996) gave cows receiving two injections of PGF 14 d apart an injection of GnRH 7 d before the second PGF treatment. Inclusion of a GnRH injection reduced conception rates compared with cows receiving only the two injections of PGF. In contrast, LeBlanc et al. (1998) reported that a GnRH injection given 7 d before the second PGF injection had no effect on the estrus response or fertility of lactating cows.

Pursley et al. (1997b) found that 85% of the cows treated with GnRH (100 µg) ovulated, and 100% started a new follicular wave. With follicle development synchronized, luteolysis with PGF_{2α} results in a tighter synchrony of estrus. Cows treated with this method ovulated between 84 to 120 h after PGF injection (Pursley et al., 1995). Thatcher et al. (1989) injected GnRH followed 7 d later by PGF and bred heifers 12 h after detected estrus. Conception and pregnancy rates were similar to heifers bred after two injections of PGF 14 d apart.

Furthermore, a method has been developed which allows the use of timed insemination by adding a second injection of GnRH after PGF (Pursley et al., 1995; Schmitt et al., 1996; Stevenson et al., 1996). The most commonly used method consists of GnRH (100 µg), followed 7 d later by PGF (25 mg Lutalyse; Pharmacia & Upjohn, Kalamazoo, MI) followed 48 h later by a second injection of GnRH (Ovsynch). Different intervals between PGF and the second GnRH injection and between the second GnRH and insemination have been examined (Pursley et al., 1995; Stevenson et al., 1996). Pursley et al. (1995) gave GnRH followed 7, 8, or 9 d later with PGF. A second injection of GnRH was

administered at the same time in all groups; that is 48, 24, or 0 h after PGF. Conception rates were 54, 46, and 11%, respectively (Pursley et al., 1995). In another study, the second GnRH injection was administered 30 to 32 h after PGF and breeding occurred 18 to 19 h after GnRH (Stevenson et al., 1996). Conception rates were lower in the time-inseminated group compared with cows treated with two injections of PGF 14 d apart and bred after detected estrus. However, pregnancy rates were higher for the GnRH treated cows because only 53% of PGF treated cows were detected in estrus (Stevenson et al., 1996). Burke et al. (1996) compared the response to GnRH followed 7 d later by PGF and breeding 12 h after detected estrus with the conception and pregnancy rates after a timed insemination performed 16 h after a second GnRH injection. Conception rates were lower for the timed insemination (26.5 vs 41.5%); however pregnancy rates were similar because fewer cows given PGF were inseminated. Both heifers and cows were used in the study of Stevenson et al. (1996) and pregnancy rates were similar for GnRH-treated cows and heifers, but pregnancy rates were significantly lower in GnRH-treated heifers than PGF-treated heifers. Pursley et al. (1997b) also found that heifers treated with GnRH-PGF-GnRH had lower pregnancy rates than heifers treated with two injections of PGF. Only 54% of heifers ovulated in response to the first GnRH injection compared with 85% of cows (Pursley et al., 1997b). It has been speculated that heifers may have more estrous cycles with three waves of follicular development (Pursley et al., 1997b). Conception rates were similar among cows treated with the Ovsynch method and bred at a fixed time compared with cows given the two-injection PGF method and bred 12 h after detected in estrus. The second injection of GnRH was given at 30 to 36 h after PGF in this study (Pursley et al., 1997b).

Schmitt et al. (1996) examined the timing of the second injection of GnRH comparing 24 and 48 h after PGF, and also comparing hCG 48 h after PGF with GnRH at 48 h after PGF in dairy heifers. Results indicated that GnRH 48 h after PGF gave a better response with timed insemination than 24 h and that there was no difference between GnRH- and hCG-treated groups (Schmitt et al., 1996).

Timing of the PGF injection was examined by Pursley et al. (1995). Cows received the PGF injection 48, 24, or 0 h before the second GnRH injection, which was 7, 8, or 9 d after the first GnRH injection, respectively. Cows in the first two groups had higher conception rates than cows given PGF 0 h before the second GnRH (Pursley et al., 1995). Peters and Pursley (1999) administered both the PGF and the second GnRH injections 7 d after the first GnRH injection and bred cow 16 h later. Pregnancy rates were decreased (14.7% vs 31.3%) for this protocol compared with the standard Ovsynch timing.

The effect of time of insemination was tested by Pursley et al. (1998). In that study the second GnRH injection was administered 48 h after PGF and cows were inseminated at 0, 8, 16, 24, and 32 h after GnRH. Ovulation occurs between 24 and 32 h after the second GnRH with the Ovsynch system (Pursley et al., 1995). Pregnancy rates, as assessed

by ultrasonography between d 25 and 35 after insemination were similar for 8-, 16-, and 24-h groups but were lower in the 0- and 32-h groups. Based on calving rate, there was no difference between 0-, 8-, 16-, and 24-h insemination groups because the 0-h group had decreased pregnancy loss between ultrasound diagnosis and calving. However, the 32-h group had a lower calving rate (Pursley et al., 1998). Data from postpartum beef cows suggests that insemination at 0 and 24 h after the second GnRH produce equivalent pregnancy rates (Geary and Whittier, 1997).

Pursley et al. (1997a) compared treatment with Ovsynch with regular PGF injections followed by estrus detection as means of reproductive management. The voluntary waiting period was 50 d postpartum for both groups and cows bred were checked for pregnancy via ultrasonography 32 to 38 d after breeding and started again on the same protocol used for that animal previously. Conception rates for first service and days open were reduced for the Ovsynch group. Sixty and 100-d pregnancy rates were higher for the Ovsynch group (37 and 53%, respectively) than for the PGF group (5 and 35%, respectively) (Pursley et al., 1997a). Momcilovic et al. (1998) compared Ovsynch with two injections of PGF 14 d apart, a single injection of PGF, and cows bred after being seen in spontaneous estrus. Cows were treated such that all cows received PGF on the same day. Ovsynch-treated cows had higher pregnancy rates and fewer days open than other groups (33% and 107 d open for Ovsynch vs 11% and 129 d for two injections of PGF, 6% and 116 d for untreated, and 12% and 122 d for the single injection PGF $P < .01$). There was no effect of body condition score or lactation number, but there was a time-of-year interaction with lower pregnancy rates from March to October (Momcilovic et al., 1998). Vasconcelos et al. (1997a) found that pregnancy rates were lowered in cows bred when the temperature exceeded 32°C. Britt and Gaska (1998) compared the Ovsynch method with two injections of PGF 14 d apart and found that conception rates were not different, but pregnancy rates were increased and services per conception decreased in the Ovsynch group. Estrus detection efficiency was at 58% for the PGF-treated cows in this study. Using a \$1.00/d cost for days open and labor costs of \$10, the Ovsynch program was significantly less expensive than the two-injection PGF method (Britt and Gaska, 1998). Jobst et al. (1999) found conception and pregnancy rates lower and costs per pregnancy higher for Ovsynch than 14-d PGF and cautioned that individual herd estrus detection efficiency would determine the best management practice. Stevenson et al. (1997) found that, although conception rates after detected estrus were greater than those after fixed-time AI with Ovsynch, pregnancy rates were higher for the Ovsynch program because of estrus detection problems. Percentage of treated cows detected in estrus with the 14-d PGF protocol and GnRH followed 7 d later with PGF were 45.5 and 64.1%, respectively. The advantage for Ovsynch seems to be in herds where estrus detection is a problem. Costs for Ovsynch would be lowered by using one-half of the dose of GnRH. Fricke et al. (1998) reported

that the dose of GnRH could be reduced from 100 µg to 50 µg at both injections without affecting pregnancy rates. The time of the cycle when the Ovsynch protocol is started may affect pregnancy rates. Vasconcelos et al. (1997b) reported that pregnancy rates were higher for cows that were started on d 5 to 12 of the estrous cycle compared with d 1 to 4 or 13 to 21. Conception and pregnancy rates from several studies using the Ovsynch protocol and comparative rates from cows treated with 14-d PGF and bred after detected estrus are summarized in Table 1. Conception rates although variable tended to be slightly less with Ovsynch but pregnancy rates were generally higher with Ovsynch than after PGF with estrus detection rates being the determining factor. In Table 2, results are presented from some variations of the standard Ovsynch protocol of GnRH, 7 d later PGF followed 48 h later with a second injection of GnRH.

Collectively, the results in Table 1 and other studies mentioned above seem to indicate the Ovsynch method results in acceptable pregnancy rates following fixed time insemination under most conditions. Still, 60% or more of the cows will not conceive to the timed insemination and must be dealt with subsequently. Chenault (1992) stated that "cows that fail to conceive are not addressed adequately by these programs." This review was dealing with PGF and progesterone-based methods, but the statement applies also to the Ovsynch method. Further development of methods that can become a part of routine herd management are needed and early pregnancy detection must be a part of these methods to identify cows for retreatment. Pursley et al. (1997a) stated that "because less than one-half of the cows become pregnant, it is imperative to identify open cows as early as possible." Effective pregnancy diagnosis using ultrasound with a high degree of accuracy seems to be possible by 25 d after insemination in cows (Unpublished observations of authors).

Resynchronization of cows that have gone through the Ovsynch protocol has been attempted (Moreira et al., 1997). Cows were given GnRH on d 20 after breeding and examined for pregnancy via ultrasound on d 27 after breeding. Open cows were given PGF at this time and were inseminated after estrus or given GnRH 48 h later and bred 16 h after GnRH. Pregnancy rate at a second ultrasound for the resynchronized cows was 33% (Moreira et al., 1997), which was similar to pregnancy rates after the first insemination. With these concerns in mind, we developed a similar protocol to that of Moreira et al. (1997) to allow for the earliest identification and rebreeding of those cows that failed to become pregnant to the timed insemination. In this protocol, lactating cows (40 to 350 d postpartum) went through the standard Ovsynch injections and were bred 16 to 24 h after the second GnRH treatment. Eighteen d after breeding cows in the early rebreeding group (OS+) received an injection of GnRH. Seven d later, cows were checked for pregnancy via rectal ultrasonography using a 7.5-MHz probe. Open cows were injected with PGF at this time and 2 d later received another injection of GnRH and were inseminated 0 to 16 h after GnRH. Pregnancy rates for cows undergoing this protocol were compared with a group

of cows receiving the standard Ovsynch (OS) with no additional treatment after breeding and with cows treated with two injections of PGF and bred after detected estrus. Cows that did not conceive to the first insemination in the PGF and OS groups were bred after being detected in estrus or treated according to the herd manager's direction after being diagnosed nonpregnant at the normal herd pregnancy check. Pregnancy rates to the first insemination were 32.5, 31.7, and 25%, respectively, for the OS+, OS, and PGF groups. It was possible to diagnose pregnancy at d 25 after breeding and nonpregnant cows rebred after PGF on d 25 and GnRH 2 d later had a pregnancy rate of 30.4% to that insemination. A sample schedule showing dates of injections, inseminations and ultrasonography is shown in Table 3.

Implications

Development of a practical method for fixed time insemination offers significant advantages to dairy producers if conception rates are close to those seen after breeding at detected estrus. The Ovsynch program seems to reach that level of success under most management conditions. Further research is needed to identify cows that fail to conceive as early as possible so that they can be included in a resynchronization program. Development of routine methods based on the use of prostaglandin $F_{2\alpha}$ and gonadotropin-releasing hormone for resynchronization should help dairy producers improve reproductive performance. Alternatively, improved estrus detection would increase effectiveness of other reproductive protocols.

Literature Cited

- Archbald, L. F., T. Tran, R. Massey, and E. Klapstein. 1992. Conception rates in dairy cows after timed-insemination and simultaneous treatment with gonadotropin releasing hormone and/or prostaglandin F_2 alpha. *Theriogenology* 37:723-731.
- Barr, H. L. 1975. Influence of estrous detection on days open in dairy herds. *J. Dairy Sci.* 58:246-247.
- Britt, J. S., and J. Gaska. 1998. Comparison of two estrus synchronization programs in a large confinement-housed dairy herd. *J. Am. Vet. Med. Assoc.* 212:210-212.
- Burke, J. M., R. L. De La Sota, C. A. Risco, C. R. Staples, E. J.-P. Schmitt and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385-1393.
- Chenault, J. R. 1992. Pharmaceutical control of estrous cycles. In: H. H. Van Horn and C. J. Wilcox (Ed.) *Large Dairy Herd Management*. pp 153-163. American Dairy Science Association, Champaign, IL.
- Folman, Y., M. Kaim, Z. Herz, and M. Rosenberg. 1990. Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. *J. Dairy Sci.* 73:2817-2825.
- Fricke, P. M., J. N. Guenther, and M. C. Wiltbank. 1998. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. *Theriogenology* 50:1275-1284.
- Geary, T. W., and J. C. Whittier. 1997. Modification of the Ovsynch estrous synchronization protocol for use in beef cows. *J. Anim. Sci.* 75(Suppl. 1):236 (Abstr.).
- Gyawu, P., M. J. Ducker, G. S. Pope, R. W. Saunders, and G. D. A. Wilson. 1991. The value of progesterone, oestradiol benzoate and cloprostenol in controlling the timing of oestrus and ovulation in dairy cows and allowing successful fixed time insemination. *Br. Vet. J.* 147:171-182.
- Heersche, Jr., G., and R. L. Nebel. 1994. Measuring efficiency and accuracy of detection of estrus. *J. Dairy Sci.* 77:2754-2761.
- Jobst, S. M., R. L. Nebel, M. L. McGilliard, and K. D. Pelzer. 1999. Evaluation of reproductive performance in lactating dairy cows using three systematic breeding protocols: 14-d PGF, timed AI, and GnRH-PGF. *J. Dairy Sci.* 82(Suppl. 1):57 (Abstr.).
- Kirby, C. J., S. J. Wilson, and M. C. Lucy. 1997. Response of dairy cows treated with bovine somatotropin to a luteolytic dose of prostaglandin $F_{2\alpha}$. *J. Dairy Sci.* 80:286-294.
- Kristula, M., R. Bartholomew, D. Galligan, and C. Uhlinger. 1992. Effects of a prostaglandin $F_{2\alpha}$ synchronization program in lactating dairy cattle. *J. Dairy Sci.* 75:2713-2718.
- Larson, L. L., and P. J. H. Ball. Regulation of estrous cycles in dairy cattle: A review. *Theriogenology* 38:255-267.
- LeBlanc, S. J., K. E. Leslie, H. J. Ceelen, D. F. Kelton, and G. P. Keefe. 1998. Measures of estrus detection and pregnancy in dairy cows after administration of gonadotropin-releasing hormone within an estrus synchronization program based on prostaglandin $F_{2\alpha}$. *J. Dairy Sci.* 81:375-381.
- Lucy, M. C., J. S. Stevenson, and E. P. Call. 1986. Controlling first service and calving interval by prostaglandin $F_{2\alpha}$, gonadotropin releasing hormone, and timed insemination. *J. Dairy Sci.* 69:2186-2194.
- Macmillan, K. L., A. M. Day, V. K. Taufa, M. Gibb, and M. G. Pearce. 1985. Effects of an agonist of gonadotropin-releasing hormone in cattle. I. Hormone concentrations and oestrous cycle length. *Anim. Reprod. Sci.* 8:203-212.
- Macmillan, K. L. and A. J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronization, increasing pregnancy rate and the treatment of postpartum anoestrus. *Anim. Reprod. Sci.* 33:1-25.
- Martinez, M. F., J. P. Kastelic, G. P. Adams, E. Janzen, W. Olson, and R. J. Mapletoft. 1998. Alternative methods of synchronizing estrus and ovulation for fixed time insemination in cattle. *Theriogenology* :350 (Abstr.).
- Morbeck, D. E., H. D. Tyler, and J. H. Britt. 1991. Duration of estrous cycles subsequent to two injections of prostaglandin $F_{2\alpha}$ at a 14 day interval in nonlactating Holstein cows. *J. Dairy Sci.* 74: 2342-2346.
- Momcilovic, D., L.F. Archbald, A. Walters, T. Tran, D. Kelbert, C. Risco, and W. W. Thatcher. 1998. Reproductive performance of lactating dairy cows treated with gonadotropin releasing hormone and/or prostaglandin $F_{2\alpha}$ for synchronization of estrus and ovulation. *Theriogenology* 50:1131-1139.
- Moreira, F., C. Risco, M. F. A. Pires, J. D. Ambrose, M. Drost, and W. W. Thatcher. 1997. Use of timed insemination in lactating dairy cows receiving bovine somatotropin. *J. Dairy Sci.* 80: (Suppl. 1):239 (Abstr.).
- Nebel, R. L., W. D. Whittier, B. G. Cassell, and J. H. Britt. 1987. Comparison of on-farm and laboratory milk progesterone assays for identifying errors in detection of estrus and diagnosis of pregnancy. *J. Dairy Sci.* 70:1471-1476.
- Peters, M. W., and J. R. Pursley. 1999. Failure to achieve adequate pregnancy rates per AI after treatment with a modified version of Ovsynch. *J. Dairy Sci.* 82(Suppl. 1):100 (Abstr.).

- Plunkett, S. S., J. S. Stevenson, and E. P. Call. 1984. Prostaglandin F₂ for lactating dairy cows with a palpable corpus luteum but unobserved estrus. *J. Dairy Sci.* 67:380-387.
- Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80:301-306.
- Pursley J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF₂ α and GnRH. *Theriogenology* 44:915-923.
- Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization in lactating dairy cows. *J. Dairy Sci.* 81:2139-2144.
- Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H.A. Garverick, and L. L. Anderson. 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or estrus. *J. Dairy Sci.* 80:295-300.
- Reimers, T. D., R. D. Smith, and S. K. Newman. 1985. Management factors affecting reproductive performance of dairy cows in the northeastern United States. *J. Dairy Sci.* 68:963-972.
- Rosenberg, M., M. Kaim, Z. Herz, and Y. Folman. 1990. Comparison of methods for the synchronization of estrous cycles in dairy cows. 1. Effects on plasma progesterone and manifestation of estrus. *J. Dairy Sci.* 73:2870-2816.
- Schmitt, E. J.-P., T. Diaz, M. Drost, and W. W. Thatcher. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J. Anim. Sci.* 74:1084-1091.
- Silvia, W. J. 1998. Changes in reproductive performance of Holstein dairy cows in Kentucky from 1972 to 1996. *J. Anim. Sci.* 76(Suppl.1):244 (Abstr.).
- Smith, M. W., and J. S. Stevenson. 1995. Fate of the dominant follicle, embryonal survival, and pregnancy rates in dairy cattle treated with prostaglandin F_{2 α} and progestins in the absence or presence of a functional corpus luteum. *J. Anim. Sci.* 73:3743-3751.
- Stevenson, J. S., and J. H. Britt. 1977. Detection of estrus by three methods. *J. Dairy Sci.* 60:1994-1998.
- Stevenson, J. S., Y. Kobayashi, M. P. Shipka, and K. C. Rauchholz. 1996. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin F_{2 α} . *J. Dairy Sci.* 79:402-410.
- Stevenson, J.S., Y. Kobayashi, and K. E. Thompson. 1997. Conception and pregnancy rates in dairy cattle after various programmed breeding systems. *J. Dairy Sci.* 80(Suppl. 1):238 (Abstr.).
- Stevenson, J. S., M. C. Lucy, and E. P. Call. 1987. Failure of timed inseminations and associated luteal function in dairy cattle after two injections of prostaglandin F₂ alpha. *Theriogenology* 28:937-946.
- Stevenson, J. S., and J. R. Pursley. 1994. Use of milk progesterone and prostaglandin F₂ in a scheduled artificial insemination program. *J. Dairy Sci.* 77:1755-1760.
- Thatcher, W. W., R. L. de la Sota, E. J.-P, Schmitt, T. C. Diaz, L. Badinga, F. A. Simmen, C. R. Staples, and M. Drost. 1996. Control and management of ovarian follicles in cattle to optimize fertility. *Reprod. Fertil. Dev.* 8:203-217.
- Thatcher, W. W., K. L. Macmillan, P. J. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31:149-164.
- Tjondronegro, S., P. Williamson, G. J. Sawyer, and S. Atkinson. 1987. Effect of progesterone intravaginal devices on synchronization of estrus in postpartum dairy cows. *J. Dairy Sci.* 70:2162-2167.
- Twagiramungu, H., L. A. Guilbault, and J. J. Dufour. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A review. *J. Anim. Sci.* 73:3141-3151.
- Vasconcelos, J. L. M., R. W. Silcox, J. A. Lacerda, J. R. Pursley, and M. C. Wiltbank. 1997a. Pregnancy rate, pregnancy loss, and response to heat stress after AI at two different times from ovulation in dairy cows. *Biol. Reprod.* 56(Suppl. 1):140 (Abstr.).
- Vasconcelos, J. L. M., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1997b. Synchronization rate, size of the ovulatory follicle, and conception rate after synchronization of ovulation with GnRH on different days of the estrous cycle. *J. Dairy Sci.* 80(Suppl. 1)148 (Abstr.).
- Wolfenson, D., W. W. Thatcher, J. D. Savio, L. Badinga, and M. C. Lucy. 1994. The effect of a GnRH analogue on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. *Theriogenology* 42:633-644.
- Xu, Z. Z., and L. J. Burton. 1998. Synchronization of estrus with PGF_{2 α} administered 18 days after a progesterone treatment in lactating dairy cows. *Theriogenology* 50:905-915.

Notes

1. Correspondence: phone:919-513-1115; fax: 919-515-7780; E-mail: Scott_whisnant@ncsu.edu.

Table 1. Comparison of results from studies that examined conception and pregnancy rates between cows treated with the 14-d PGF treatment (PGF) or the Ovsynch method (OV)

Study	Conception rate %		Pregnancy rate %	
	PGF	OV	PGF	OV
Stevenson et al., 1996	47	35	26	35
Burke et al., 1996	42	27	30	27
Britt and Gaska, 1998	32	47	18	47
Momcilovic et al., 1998	22	33	11	33
Pursley et al., 1997ab	46.3	37.8	38.9	37.8
Stevenson et al., 1997	31.7	37.3	15.9	37.3
Jobst et al., 1999	42.2	30.1	— ^a	30.1
Unpublished results by authors	38.5	31.7	25	31.7
Unweighted means ± SEM from above	37.7 ± 3.1	34.9 ± 2.2	19.8 ± 2.7	34.9 ± 2.2

^aData not reported.**Table 2.** Summary of pregnancy rate results from variations of the Ovsynch protocol

Study	0 h	24 h	30–36 h	48 h
Pursley et al., 1995 ^a	11% ^x	46% ^y	—	55% ^z
Schmitt et al., 1996 ^b	—	25.8%	—	45.5%
Stevenson et al., 1996	—	—	35.3%	—
Peterson and Pursley, 1999 ^c	14.7%	—	—	31.3%

^aPGF was given at 9^x, 8^y, or 7^z d after the first GnRH injection.^bUsed buserelin.^cPGF was given at 9 d after the first GnRH injection.

Table 3. An example schedule for use of the Ov-Synch protocol allowing for timed rebreeding of cows that fail to become pregnant to the first insemination

Date	Ovsynch+
April 15	Give GnRH
April 22	Give PGF
April 24	Give GnRH
April 25	Breed 16 to 24 h after GnRH
May 13 ^a	Give GnRH (18 d after breeding) ^a
May 20	Ultrasonography for pregnancy detection and give PGF to nonpregnant cows
May 22	Give GnRH
May 23	Breed 16 to 24 h after GnRH
June 10	Give GnRH
June 17	Ultrasound and give PGF to nonpregnant cows
June 19	Give GnRH
June 20	Breed 16 to 24 h after GnRH

^aIf a second replicate is used, the second sets of respective treatments would begin here at 28 d after the first replicate.