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# Current Estrus Synchronization and Artificial Insemination Programs for Cattle

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**ABSTRACT:** Traditional methods of estrus synchronization developed over the past 60 yr have involved controlling estrous cycle length by administering progestin and(or) regressing the corpus luteum with luteolytic drugs. Treatment with progestins alone to extend the estrous cycle length was effective in synchronization of estrus, but fertility and embryonic development following long-term progestin administration were compromised. Shorter periods of progestin treatment combined with luteolytic drugs or the use of luteolytic drugs alone to synchronize estrus result in pregnancy rates similar to those following a spontaneous estrus when cows or heifers are bred by artificial insemination after detection. The synchrony of estrus following most traditional methods is not precise enough to allow a single, timed insemination, and effectiveness of those treatments in synchronizing estrus varies depending on the stage of the estrous cycle during which animals are treated. The most recently developed synchronization treatments combine traditional methods of controlling cycle length with the manipulation of follicular development in order to "program" or "select" the ovulatory follicle. The new methods synchronize estrus more precisely and control the time of ovulation more exactly in order to allow a single, timed insemination without the need for detection of behavioral estrus. Investigations of other means of altering the development of the ovulatory follicle prior to ovulation are under way in an effort to improve the fertility of lactating cows and heifers over that of untreated animals exhibiting a spontaneous estrus.

Key Words: Cattle, Estrus Synchronization, Artificial Insemination, Corpus Luteum, Follicle, Ovulation

## Introduction

The history of estrous cycle synchronization and the use of AI in cattle is a testament to how discoveries in basic science can be applied to advance the techniques used for livestock breeding and management. Several authors described the experiments that have been conducted since the discovery of ovarian steroids and that have led to the effective control of the length of the bovine estrous cycle and the timing of estrus and ovulation (Hansel and Schechter, 1972; Hansel and Beal, 1979; Patterson et al., 1989; Odde, 1990; Larson and Ball, 1992). The purpose of this review is to describe the current status of estrus synchronization and ovulation control programs and to relate AI practices to those programs. A brief recap of historical events will be useful to better understand current techniques, but the focus will be on methods of estrus synchronization and AI that are emerging today with an eye toward the potential for future developments.

The past 50 yr of research and development of estrus synchronization products has left us with the "tools" to control the timing of the onset of estrus by controlling the length of the estrous cycle. The choice of approaches for controlling cycle length are 1) to regress the corpus luteum ( **CL** ) of the animal before the time of natural luteolysis, and thereby shorten the cycle, or 2) to administer exogenous progestins to delay the time of estrus following natural or induced luteolysis that may extend the length of the estrous cycle. In either case, the emphasis is placed on controlling or mimicking luteal function to control the time of estrus. Variations on one of the two approaches to cycle control are the basis for commercially available products that successfully synchronize estrus in the majority of cows or heifers within a 5- to 7-d period and that yield conception rates following estrus detection and AI breeding that are similar to those following AI after a spontaneous estrus.

Concurrent with the last 25 yr of research on controlling estrous cycle length has been the development of a better understanding of follicular development. Methods of interrupting or manipulating the wave-like pattern of follicular growth and controlling ovulation have been developed. The current and future

Table 1. Estrus response and fertility of heifers treated with exogenous progesterone for various periods to synchronize estrus[a]

| Treatment period | No. of heifers | Estrus response, %[b] | Conception rate, %[c] | Pregnancy rate, %[d] |
|---|---|---|---|---|
| 0 days (control) | 22 | 96 | 68 | 65 |
| 9 days | 54 | 70 | 53 | 37 |
| 12 days | 38 | 81 | 65 | 52 |
| 18 days | 24 | 100 | 38 | 38 |
| 21 days | 12 | 92 | 36 | 33 |

[a]Adapted from Roche (1974).

[b]Detected in estrus within 144 h after treatment or 21 d (control).

[c]Conception rate = (no. pregnant/no. inseminated 12 h after estrus) × 100.

[d]Pregnancy rate = (no. pregnant/no. in group) × 100.

direction of estrus synchronization is to focus on combining traditional methods of controlling cycle length with the manipulation of follicular development in order to "program" or "select" the ovulatory follicle. The immediate goal of controlling both CL function and follicular development is to devise a treatment that will synchronize estrus more precisely and to control the time of ovulation more exactly to allow a single, timed insemination without the need for detection of behavioral estrus. The ultimate goal may be to improve the fertility of lactating cows and heifers over that of untreated animals exhibiting a spontaneous estrus.

## Estrous Cycle Control

*Exogenous Progestins.* Isolation and synthesis of estrogen (Allen and Doisy, 1923) and progesterone (Corner and Allen, 1929) were followed by studies that revealed estrus could be delayed and thereby synchronized by exogenous administration of progesterone to cattle or sheep (Christian and Casida, 1948; Dutt and Casida, 1948). This led to a flurry of activity in which progesterone or synthetic progestins were injected, released intravaginally, or fed for a period up to and exceeding the length of the estrous cycle to synchronize estrus following the cessation of progestin administration (see Hansel and Beal, 1979). In general, the longer the progestin was administered to cattle, the higher the rate of estrus synchronization, but the lower the fertility of the synchronized animals (Table 1).

Twenty-five years after long-term progestin feeding to control estrus was abandoned due to low fertility, several laboratories have been able to use ultrasonography to demonstrate that progestin administration at "sub-luteal" levels inhibits estrus and ovulation and synchronizes estrus in cattle, but that a persistent, estrogen-secreting follicle develops when progestin treatment extends the estrous cycle (Lucy et al., 1990; Sirous and Fortune, 1990; Cupp et al., 1992).

Development of the persistent follicle is caused by increased pulsatile secretion of gonadotropins during the period when the exogenous progestin is inhibiting estrus, but the CL has regressed (Kojima et al., 1992; Savio et al., 1993; Stock and Fortune, 1993; Custer et al., 1994). The low fertility of cows bred at the synchronized estrus following long-term administration of progestin is due to premature resumption of meiosis of ova or abnormal development of embryos derived from ova of persistent follicles (Wishart and Young, 1974; Mihm et al., 1994; Ahmad et al., 1995; Revah and Butler, 1996).

Increasing the level of exogenous progestin administered to synchronize estrus to a level that mimics the amount of progesterone secreted by the CL reduces the likelihood of developing a persistent follicle in progestin-treated cattle (Roberson et al., 1989; Savio et al., 1993). It is clear today that increasing the level of progestin administered to delay estrus or the application of a consistent method of "turning over" a persistent follicle and initiating a new wave of follicular development could improve the fertility of cattle treated with long-term exogenous progestin to synchronize estrus (Anderson and Day, 1994; Schmitt et al., 1996).

*Exogenous Progestins and Estradiol.* Although at the time they lacked the knowledge of why progestin-treated cattle exhibited lower fertility, Wiltbank et al. (1961) recognized that reducing the period of progestin administration improved conception rates at the synchronized estrus. Simultaneously, Kaltenbach et al. (1964) and others (Loy et al., 1960; Wiltbank 1966) demonstrated that estradiol was luteolytic when administered early in the bovine estrous cycle. Hence, combining progestin treatment with the administration of estradiol at the initiation of treatment enabled the period of progestin treatment to be shortened (9 to 14 d) without reducing the percentage of animals exhibiting a synchronized estrus. This treatment regimen is the basis for the commercial product SYNCRO-MATE B® (**SMB**) marketed in the United States, as well as the PRID® (progesterone-releasing intravaginal device) and EAZI-BREED™ CIDR® (controlled intravaginal drug release device) marketed in Europe, Australia, and New Zealand.

The treatment of cyclic cows or heifers with exogenous progestin preceded by an injection of estradiol is usually followed by a high incidence (> 90%) of estrus during the 5 d following progestin removal. Odde (1990) reviewed 15 trials conducted with 1,032 puberal heifers that were observed for signs of estrus following SMB treatment. Of those heifers, 92.5% were observed in estrus within 5 d after treatment. The failure to achieve synchronization rates of 100% in cyclic heifers or cows treated with SMB was related to the response of animals treated at different stages of the estrous cycle. Miksch et al. (1978) indicated that the SMB treatment was effective in only 80 to 86% of the heifers that began

treatment on d 1 through 8 of the cycle. Pratt et al. (1991) went on to report that estrus was synchronized in only 48% of the cows treated on d 3, but that synchronization was 100% when treatment began on d 9 of the estrous cycle.

The distribution of estrus following SMB treatment is highly synchronized. In 15 separate trials in which the standard SMB treatment was used to synchronize estrus in 736 cows or heifers, a majority (65%) of the animals were observed in estrus between 24 and 48 h after implant removal (Miksch et al., 1978; Spitzer et al., 1978). The "tight" synchrony of estrus that occurs following either SMB treatment of heifers or SMB treatment and 48-h calf removal in postpartum beef cows makes these treatments logical for use with timed insemination. Mares et al. (1977) reported that pregnancy rates following timed breeding were actually higher (51%) than when SMB-treated heifers were inseminated 12 h after estrus detection (39%) in herds in which the majority of heifers were cycling prior to SMB treatment. Hence, one of the principal advantages of SMB treatment is the tight synchrony of estrus that makes this treatment compatible with timed insemination, especially when careful estrus detection is difficult.

Conception rates of cattle treated with SMB and bred 12 h after estrus detection have been reported to be not significantly different from those of untreated controls in the same trials (see Odde, 1990). However, upon closer inspection of the fertility of cattle treated with SMB, it became apparent that although the reduction in conception rates of all the animals treated may not have been statistically significant, the conception rates of those cattle that began SMB treatment late in the estrous cycle (> d 14) were significantly lower. Improving fertility at the estrus immediately following SMB administration depends on beginning treatment before the animals are late (> d 14) in the estrous cycle. Given the need to avoid treatment early in the estrous cycle in order to maximize estrus response, this suggests that the optimum time for SMB treatment to begin is between d 8 and d 12 of the estrous cycle.

*Luteolytic Prostaglandins.* In 1972 several groups reported that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) caused luteolysis and synchronized estrus (Lauderdale, 1972; Louis et al., 1972; Rowson et al., 1972). A single intramuscular injection of $PGF_{2\alpha}$, two injections separated by 24 h, or a single intrauterine infusion of $PGF_{2\alpha}$ were each luteolytic when administered between d 5 and 21 of the estrous cycle (Kaltenbach and Graves, 1975). Animals treated with similar doses of $PGF_{2\alpha}$ prior to d 5 of the cycle, however, did not consistently experience luteolysis (Lauderdale et al., 1974); hence, the original recommendation to synchronize estrus in a herd of cattle was to administer two injections of $PGF_{2\alpha}$ 11 to 14 d apart. Today, $PGF_{2\alpha}$ or analogues of $PGF_{2\alpha}$ are commercially available under a variety of tradenames in the United States and other countries.

Prior to 1982 it was believed that after d 4 of the estrous cycle all cows were equally responsive to a luteolytic dose of $PGF_{2\alpha}$. King et al. (1982) and others (Tanabe and Hahn, 1984; Stevenson et al., 1984; Watts and Fuquay, 1985), however, demonstrated that cattle injected with $PGF_{2\alpha}$ between d 5 and 9 of the cycle were less responsive than those injected later in the cycle. Chenault (unpublished data) summarized the effect of day of the estrous cycle on percentage of cattle synchronized by a single injection of $PGF_{2\alpha}$. He noted that whereas the majority of cattle responded when treated between d 5 and 17, synchronized estrus response was lowest (67%) among heifers treated on d 5 through 9, moderate (77%) when heifers were treated on d 9 through 12, and highest (> 91%) among those injected after d 12 of the cycle.

The average interval from injection of prostaglandin to estrus is usually 60 to 72 h. Variation in the timing of estrus is created in part by differences among cows in the rate of regression of the CL following treatment. The interval from $PGF_{2\alpha}$ treatment to estrus has also been related to the time required for an ovulatory follicle to develop (Kastelic et al., 1990). Estrus occurred an average of 48 to 59 h after treatment in four studies in which a prostaglandin product was administered on d 5 to 8 of the estrous cycle (King et al., 1982; Stevenson et al., 1984; Tanabe and Hahn, 1984; Watts and Fuquay, 1985). In contrast, the average time of estrus was 53 to 72 h if heifers in the same studies were treated between d 12 and 15 of the estrous cycle. Hence, although estrus is synchronized within a 5-d period following $PGF_{2\alpha}$ treatment, regardless of the stage of the cycle at the time of treatment, the precision of the synchrony of estrus is reduced by variation due to differences in the stage of follicular development at the time of treatment.

Fertility is high following prostaglandin synchronization. Results of most studies indicate that conception rates are similar for beef cows or heifers synchronized with $PGF_{2\alpha}$ and those bred after a naturally occurring estrus. In one of the largest experiments (3,443 animals) Moody and Lauderdale (1977) reported that lactating cows or heifers bred 12 h after detection of a $PGF_{2\alpha}$-synchronized estrus had a conception rate of 59%. Untreated cows and heifers in the same herds achieved a 62% conception rate when bred 12 h after a natural estrus. Although some studies have demonstrated a tendency for animals treated with $PGF_{2\alpha}$ late in the estrous cycle to have higher fertility, that trend has been inconsistent.

When a single, timed insemination has been employed following $PGF_{2\alpha}$, some timed-bred groups of cows have had acceptable conception rates; however, there has been significant variation and a greater incidence of very low conception rates have occurred

Table 2. Estrus response[a], conception rate[b], and pregnancy rate[c] of beef heifers following treatment with melengestrol acetate and $PGF_{2\alpha}$[d] to synchronize estrus

| Reference | No. heifers | Estrus response, %[a] | Conception rate, %[b] | Pregnancy rate, %[c] |
|---|---|---|---|---|
| Brown et al. (1988) | 157 | 83 | 69 | 57 |
| Patterson (1990) | 323 | 83 | 74 | 61 |
| Jaeger et al. (1992) | 40 | 71 | 77 | 54 |

[a]A synchronized estrus was defined as estrus within 120 h after treatment.
[b]Conception rate = (no. preg./no. inseminated 12 h after estrus) × 100.
[c]Pregnancy rate = (no. preg./no. treated) × 100.
[d]Melengestrol acetate (.5 mg/d) fed for 14 d followed 17 d by $PGF_{2\alpha}$ (25 mg).

when timed breeding was used. In a large trial using 45 herds, Fogwell et al. (1986) recorded a 22% lower conception rate when a single, timed breeding at 80 h after $PGF_{2\alpha}$ was compared to breeding 12 h after detecting a synchronized estrus. The lower fertility, and in particular the range in conception rates (6.7 to 85.7%), in those herds of cattle timed-bred after $PGF_{2\alpha}$ is most likely related to the greater variation in the timing of estrus following $PGF_{2\alpha}$ treatment as compared to other synchronization programs.

Combining exogenous progestin administration and $PGF_{2\alpha}$ treatment has been used to synchronize estrus (see Hansel and Beal, 1979; Beal, 1995). The most popular of those programs has been the feeding of melengestrol acetate (**MGA**) for 14 d followed 17 d later by injection of a luteolytic dose of $PGF_{2\alpha}$ ($MGA^{14}$-$PGF_{2\alpha}^{17}$). This system initially synchronizes estrus within the 7 d following the last MGA feeding (Patterson et al., 1989; Odde, 1990; King and Odde, 1993). The administration of $PGF_{2\alpha}$ 17 d after the last MGA feeding causes the timing of $PGF_{2\alpha}$ to occur after d 10 of the estrous cycle in the majority of cattle. As noted above, the estrus response and fertility of cattle treated with $PGF_{2\alpha}$ are expected to be maximized if $PGF_{2\alpha}$ treatment occurs after d 10 of the cycle.

The rate of synchronization of estrus following $MGA^{14}$-$PGF_{2\alpha}^{17}$ is usually greater than that following the use of $PGF_{2\alpha}$ alone (Patterson et al., 1995) but may be less than that of heifers treated with SMB (Brown et al., 1988). King et al. (1994) indicated that the percentage of heifers in estrus during the peak 24-h period following $MGA^{14}$-$PGF_{2\alpha}^{17}$ treatment varied from 56 to 71%. This is similar to the distribution of estrus following $PGF_{2\alpha}$ alone and therefore is probably inadequate to allow satisfactory pregnancy rates following a single, timed insemination.

The most consistent finding among research reports of cycling cows or heifers bred 12 h after a detected estrus synchronized with $MGA^{14}$-$PGF_{2\alpha}^{17}$ is that conception rates were equal to or greater than those of untreated control animals or animals synchronized with $PGF_{2\alpha}$ alone (Table 2; Patterson, 1990; Jaeger et al., 1992; King and Odde, 1993). Patterson et al. (1995) suggested that feeding MGA prior to $PGF_{2\alpha}$ may actually enhance fertility, but they noted a 15%

increase in twinning rate of cows treated with $MGA^{14}$-$PGF_{2\alpha}^{17}$.

Synchronization of estrus achieved by controlling cycle length with $PGF_{2\alpha}$ or the combination of a progestin and estradiol or $PGF_{2\alpha}$ has resulted in pregnancy rates of synchronized cows and heifers bred by AI after estrus detection that were variable, but acceptable in most cases. The variability in results and the interest in eliminating the need for detection of estrus have fueled the effort to develop a method to improve estrus synchrony, to enable single, timed insemination, and to enhance AI conception rate. The greatest achievements in those areas have (will) come through the discovery of methods to control follicular development and the time of ovulation. In essence, estrus synchronization in the past has been achieved by controlling (or mimicking) CL function. Improved estrus synchronization methods of the future will likely involve control of CL function and control of the selection of the ovulatory follicle and the timing of ovulation.

## The Ovulatory Follicle and the Time of Ovulation

Development of follicles during the bovine estrous cycle occurs in waves of growth during which several follicles begin growing until a single, dominant follicle becomes significantly larger than the subordinate follicles (Ginther et al., 1989; Kastelic, 1994). The dominant follicle inhibits growth of subordinate follicles, and those subordinate follicles become atretic and regress. In the presence of a CL the dominant follicle proceeds through a "growth phase", a "static phase" and into a "regression phase" in which it loses the ability to inhibit folliculogenesis and a new follicular wave is initiated. Conversely, in the absence of a CL, increased pulsatile secretion of gonadotropins causes the dominant follicle to mature, secrete estradiol, and ovulate. Estrous cycles of most cows consist of two or three waves of follicular development, the last wave giving rise to the ovulatory follicle (Figure 1).

Altering development of the ovulatory follicle can affect the timing of estrus and the fertility of the ovulated ovum. For example, in a group of cattle treated with a luteolytic dose of $PGF_{2\alpha}$ on d 8 of the
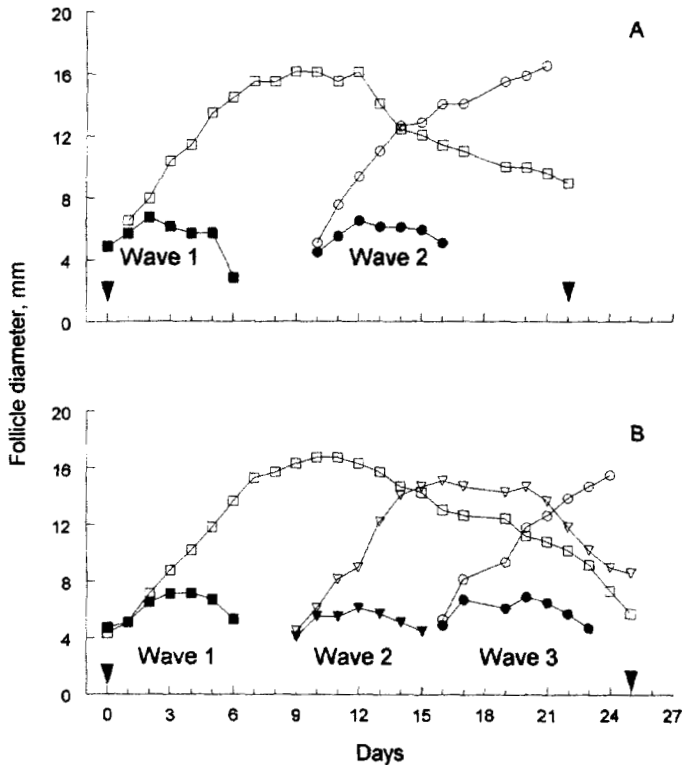
Figure 1. The development of the dominant (open symbols) and largest subordinate follicle (solid symbols) in heifers exhibiting two (A) or three waves (B) of follicular growth during the bovine estrous cycle. Solid arrows indicate the day of ovulation. Adapted from Kastelic (1994).

estrous cycle, a time when the first dominant follicle had lost or was about to lose its ability to inhibit subordinate follicles, part of the group reached estrus and ovulated the first wave dominant follicle quickly (4 d). The remaining cows in the group experienced regression of the first wave dominant follicle and developed a new ovulatory follicle, but this group required an average 6 d after $PGF_{2\alpha}$ to ovulate (Kastelic et al., 1990; Kastelic and Ginther, 1991). In a herd of cows randomly distributed throughout the estrous cycle, a follicular wave would be emerging in some animals while others would have dominant follicles capable of immediate maturation and rapid ovulation. Unless follicular development in such a mixed group is controlled, the timing of a synchronized estrus and ovulation would be variable.

Sequential monitoring of the ovulatory follicle in animals that are treated with progestins in the absence of a CL has revealed that follicular waves were interrupted and a large, dominant follicle developed and persisted on the ovary throughout the treatment period in 80% of the progestin-treated cows (Beal et al., 1990; Custer et al., 1994). The ability to "hold" a large, dominant follicle was characteristic of treatment with MGA, a progesterone-containing CIDR

or a subcutaneous norgestomet implant, and in each case the persistent follicle ovulated after a synchronized estrus (Roberson et al., 1989; Savio et al., 1993; Custer et al., 1994). The pregnancy rate of cows bred following progestin treatment that caused a persistent, dominant follicle to ovulate was reduced by 17 to 35% (Sanchez et al., 1993; Savio et al., 1993; Wehrmann et al., 1993).

Controlling the time of emergence of a new follicular wave and synchronizing the follicular wave status of animals within a group to be synchronized could improve the synchrony of estrus and ovulation and ensure that the ovulatory follicle has the optimum potential for fertilization and embryo development. Several methods of initiating a new follicular wave and controlling follicle "turnover" have been revealed that can be combined with traditional methods of controlling estrous cycle length.

Manipulating Follicular Development. Ablation of ovarian follicles ≥ 5 mm, altering the endogenous release of LH and FSH, or administration of exogenous steroids or gonadotropins can cause regression of a dominant follicle and emergence of a new follicular wave (Roche et al., 1998). The most common methods for altering follicular turnover in conjunction with estrus synchronization have been the administration of gonadotropin-releasing hormone (GnRH) or estradiol administration in conjunction with an exogenous progestin (see Bo et al., 1995).

Estrogen induces follicular atresia (Dierschke et al., 1994), and injection of estradiol benzoate (2 mg) or estradiol 17$\beta$ (5 mg) in cows fitted with a norgestomet implant or a progesterone-containing CIDR causes suppression of a dominant follicle and emergence of a new follicular wave. Bo et al. (1995) summarized data that indicated that development of the dominant follicle was interrupted and the emergence of the next follicular wave was synchronized to occur an average of 4.3 d after cows or heifers were treated with estradiol 17$\beta$. The treatment was effective regardless of the stage of dominant follicle development (growing, static, or regressing) at the time of treatment. They also demonstrated that the effect of estradiol was dependent on concurrent treatment with a progestin and that the effect of estradiol 17$\beta$ on follicular dynamics was direct (i.e., not mediated by changes in endogenous gonadotropins).

Administration of GnRH during the bovine estrous cycle causes regression or ovulation of the dominant follicle and initiates the emergence of a new wave of follicular growth an average of 2.5 d following treatment (Pursley et al., 1995a). Atresia or ovulation of the dominant follicle depends on the status (growing, static, or regressing) of the follicle at the time of GnRH injection (Silcox et al., 1993; Twagiramungu et al., 1994). Ovulation of a growing dominant follicle occurred 100% of the time following GnRH administration; however, ovulation of dominant

Table 3. Estrus response[a], conception rate[b], and pregnancy rate[c] of postpartum beef cows following treatment with $PGF_{2\alpha}$[d] or GnRH and $PGF_{2\alpha}$[e] to synchronize estrus

| Reference and treatment | No. of cows | Estrus response, % | Conception rate, % | Pregnancy rate, % |
|---|---|---|---|---|
| Forbes et al. (1997) | | | | |
| 2×-$PGF_{2\alpha}$ | 314 | 47[x] | 61 | 28[x] |
| GnRH[-7] $PGF_{2\alpha}$ | 306 | 60[y] | 66 | 39[y] |
| Geary and Whittier (1997) | | | | |
| 2×-$PGF_{2\alpha}$ | 82 | 87 | 58 | 51 |
| GnRH[-7] $PGF_{2\alpha}$ | 82 | 74 | 57 | 42 |

[a]A synchronized estrus was defined as estrus within 144 h after treatment.
[b]Conception rate = (no. preg./no. inseminated 12 h after estrus) × 100.
[c]Pregnancy rate = (no. preg./no. tested) × 100.
[d]$PGF_{2\alpha}$ (25 mg) administered twice at a 14-d interval.
[e]GnRH (100 mg) administered 7 d prior to $PGF_{2\alpha}$ (25 mg).
[x,y]Percentages in same column with different superscripts differ ($P < .05$).

follicles in the static or regressing phases occurred 33% and 0% of the time, respectively.

Administration of GnRH 6 or 7 d prior to injection of cows or heifers with a luteolytic dose of $PGF_{2\alpha}$ (GnRH[-7]-$PGF_{2\alpha}$) has been used by several groups in an effort to synchronize follicular development prior to regression of the corpus luteum to synchronize estrus. The synchrony of estrus following GnRH[-7]-$PGF_{2\alpha}$ is more precise than when $PGF_{2\alpha}$ is administered twice at a 14-d interval (2×-$PGF_{2\alpha}$; Figure 2). Forbes et al. (1997) and Geary and Whittier (1997) have reported that the conception rates of beef cows inseminated 12 h after estrus detection were similar among groups treated with GnRH[-7]-$PGF_{2\alpha}$ or 2×-$PGF_{2\alpha}$ (Table 3).

The precise synchrony of estrus following GnRH[-7]-$PGF_{2\alpha}$ treatment makes this treatment a candidate to be combined with a single, timed insemination. However, Pursley et al. (1995a) reported that the range in timing of ovulation in lactating dairy cows following GnRH[-7]-$PGF_{2\alpha}$ treatment extended from 84 to 120 h after $PGF_{2\alpha}$. They demonstrated that the range in ovulation time in cows could be reduced to 8 h (72 to 80 h after $PGF_{2\alpha}$) if a second GnRH injection was administered 48 h after $PGF_{2\alpha}$ in the GnRH[-7]-$PGF_{2\alpha}$ treatment. The resulting GnRH[-7]-$PGF_{2\alpha}$-GnRH[2] treatment has been termed "Ovsynch" because the combination of follicular synchronization, estrus synchronization, and ovulation control are effective in more precisely timing ovulation of a fertile ovum.

Pregnancy rate following timed insemination after the Ovsynch treatment in lactating dairy cows was similar to that of cows bred following 2×-$PGF_{2\alpha}$ and insemination 12 h after detection of estrus (38.9 vs 37.8%; Pursley et al., 1997). Pursley et al. (1995b) indicated that pregnancy rates varied when cows were timed-inseminated at 0, 8, 16, 24, or 32 h after the second injection of GnRH in the Ovsynch program (Table 4). The highest pregnancy rate (44%) was achieved when insemination was 16 h after the second GnRH injection, but the modest reduction in pregnancy rate and the convenience of inseminating cows

at the same time that the second GnRH injection was administered made that combination attractive, especially for use with large numbers of beef cows. Geary and Whittier (1997) compared the Ovsynch program
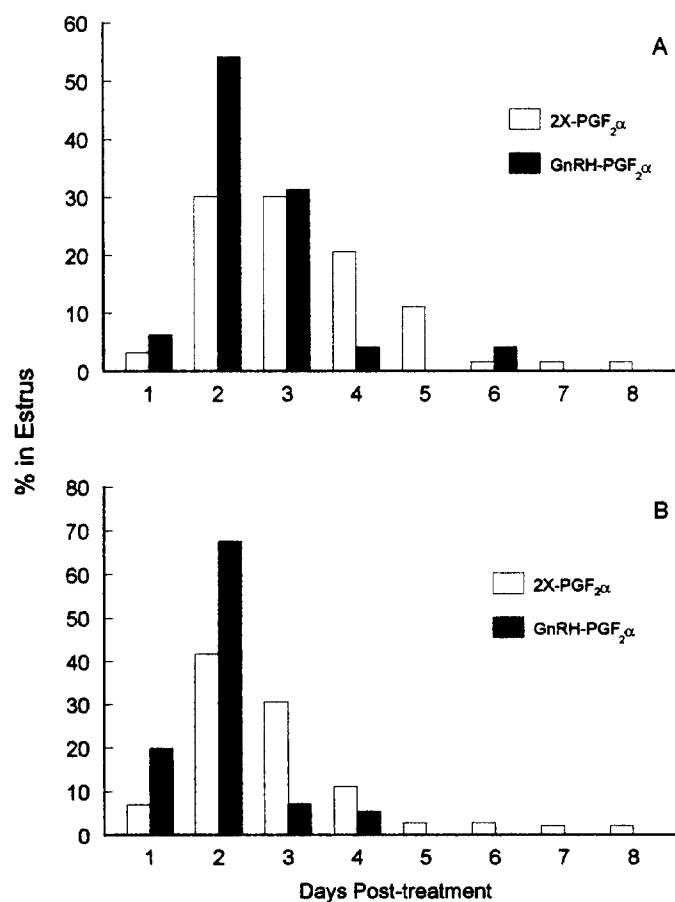


Figure 2. Distribution of behavioral estrus detected in cows (A) or heifers (B) treated with two injections of $PGF_{2\alpha}$ (25 mg each) at a 14-d interval (2×- $PGF_{2\alpha}$; n = 63 cows and 144 heifers) or GnRH (100 μg) followed 7 d later by 25 mg $PGF_{2\alpha}$ (GnRH-$PGF_{2\alpha}$; n = 48 cows and 111 heifers).

Table 4. Conception rate of lactating dairy cows bred at differing
intervals following Ovsynch treatment[ab]

| Item | Interval from last GnRH to insemination | | | | |
| --- | --- | --- | --- | --- | --- |
|  | 0 h | 8 h | 16 h | 24 h | 32 h |
| Conception rate, %[c] | 37[x] | 40[x] | 44[x] | 40[x] | 32[y] |

[a]Adapted from Pursley et al., 1995b.
[b]Ovsynch protocol is GnRH (100 μg) 7 d prior to $PGF_{2\alpha}$ (25 mg) with GnRH (100 μg) 48 h after $PGF_{2\alpha}$.
[c]Conception rate = (no. preg./no. insem) × 100.
[x,y]Percentages in the same row with different superscripts differ ($P < .10$).

in which postpartum beef cows were timed-bred either 0 or 24 h after the second GnRH injection with timed breeding after SMB and detected no differences in pregnancy rates (Table 5).

An alternative method of synchronizing follicular development prior to synchronizing estrus has been reported in which estradiol benzoate or estradiol 17β is administered to regress the dominant follicle and cause the emergence of a new follicular wave. Day et al. (1997) described a treatment in which estradiol benzoate (2 mg) was administered coincident with the insertion of a progesterone-releasing CIDR for 7 d. Each animal received a second injection of estradiol benzoate (1 mg) 48 h after CIDR removal. The purpose of the initial estradiol injection was to initiate a new wave of follicular growth. The purpose of the second estradiol injection was to induce a synchronous behavioral estrus and an endogenous LH surge, thereby controlling the time of ovulation. Animals were bred after detection of estrus, and the conception rate (62%) of treated animals was similar to that of untreated controls (57%). Although timed insemination was not employed in this experiment, the high degree of estrus synchrony (> 72% in estrus in 24 h) indicates that this treatment, like Ovsynch, is likely to result in high pregnancy rates following timed breeding.

Preceding traditional methods of estrus synchronization with a treatment that eliminates a growing or static dominant follicle has had two very exciting effects. First, it synchronizes follicular development among cows prior to the estrus synchronization treatment. In turn, variation in the timing of estrus is reduced because development of the ovulatory follicle is more uniform. Second, and perhaps even more exciting, is the finding that when an LH surge is induced by GnRH or estradiol in a group of animals that have had both follicular development and luteal regression synchronized, the range of the timing of ovulation is remarkably precise and the possibility of achieving high pregnancy rates following a single, timed insemination is enhanced.

## Conclusions and Implications

The current state of estrous cycle control has been elevated by the discovery of methods to regress an existing dominant follicle and to initiate a new wave of follicular development. Administration of GnRH or estradiol synchronizes follicular development prior to the use of $PGF_{2\alpha}$ or progestins to control cycle length. The synchronous emergence of a follicular wave in a herd of cows or heifers treated to synchronize estrus causes a more precisely timed estrus and enables a timed ovulation to be induced that results in the release of an ovum with optimal fertility.

Combining the control of follicular development and ovulation with traditional methods of estrus synchronization seems to be the best method of achieving the immediate goal of achieving acceptable pregnancy rates following a single, timed artificial insemination in conjunction with estrus synchronization. Conversely, the more lofty goal of increasing the conception rate of cows or heifers treated for estrous cycle and ovulation control above that of animals exhibiting a spontaneous estrus has not been achieved. Further research into the effects of altering follicular development prior to a synchronized estrus and ovulation is currently the most promising approach to improving fertility. In particular, altering the steroid hormone milieu or manipulating the time course of development of the ovulatory follicle from the pre-antral stage to ovulation are likely to receive immediate attention by researchers. Perhaps those investigations during the next decade can lead to an enhancement in fertility similar to the improvements that have been

Table 5. Pregnancy rates of postpartum beef cows bred by timed insemination following Ovsynch treatment[ab]

| Item | Ovsynch + 0 h[b] | Ovsynch + 24 h[b] |
| --- | --- | --- |
| No. of cows | 360 | 392 |
| Pregnancy rate, %[c] | 48 | 48 |

[a]Adapted from Geary and Whittier (1997).
[b]GnRH (100 μg) administered 7 d prior to $PGF_{2\alpha}$ (25 mg) with 100 μg GnRH 48 h after $PGF_{2\alpha}$; insemination at time of second GnRH (0 h) or 24 h after second GnRH.
[c]Pregnancy rate = (no. preg./no. treated) × 100.

realized in the synchrony of estrus and timing of ovulation through research conducted over the past decade.

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