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Effective Use of Reproductive Hormones in Breeding Programs

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A. General Aspects of Effective Synchronization Programs

1. Normal Estrous Cycle

Figure 1 summarizes some key aspects of the bovine estrous cycle. Changes in the 2 key structures on the ovary, the preovulatory follicle and the corpus luteum, are emphasized in this figure. The function of 4 important hormones is also summarized in this figure. Estradiol-17ß from the preovulatory follicle causes the cow to manifest estrus behaviour and have an LH surge. The LH surge causes ovulation of the preovulatory follicle about 28 h later. The cells that remain from the preovulatory follicle develop into the corpus luteum. The corpus luteum grows in size during the first part of the estrus cycle and then reaches a plateau phase in which it maintains a large size (20-25 mm diameter). The major hormone coming from the corpus luteum is progesterone and the increase in size of the corpus luteum is reflected in increased concentrations of progesterone in the blood. If the cow becomes pregnant the corpus luteum maintains a large size and progesterone concentrations remain elevated. These high progesterone concentrations prevent the cow from coming into estrus or having a subsequent ovulation. If the cow does not become pregnant then the corpus luteum will decrease at about day 17-20 of the estrous cycle (day of estrus = 0). The reason the corpus luteum regresses is because of secretion of prostaglandin F?? (PGF??) from the non-pregnant uterus. After exposure to PGF?? there is a decrease in circulating progesterone concentrations as well as a subsequent decrease in size of the corpus luteum. Thus, a major feature of the estrous cycle is the development and regression of the corpus luteum.

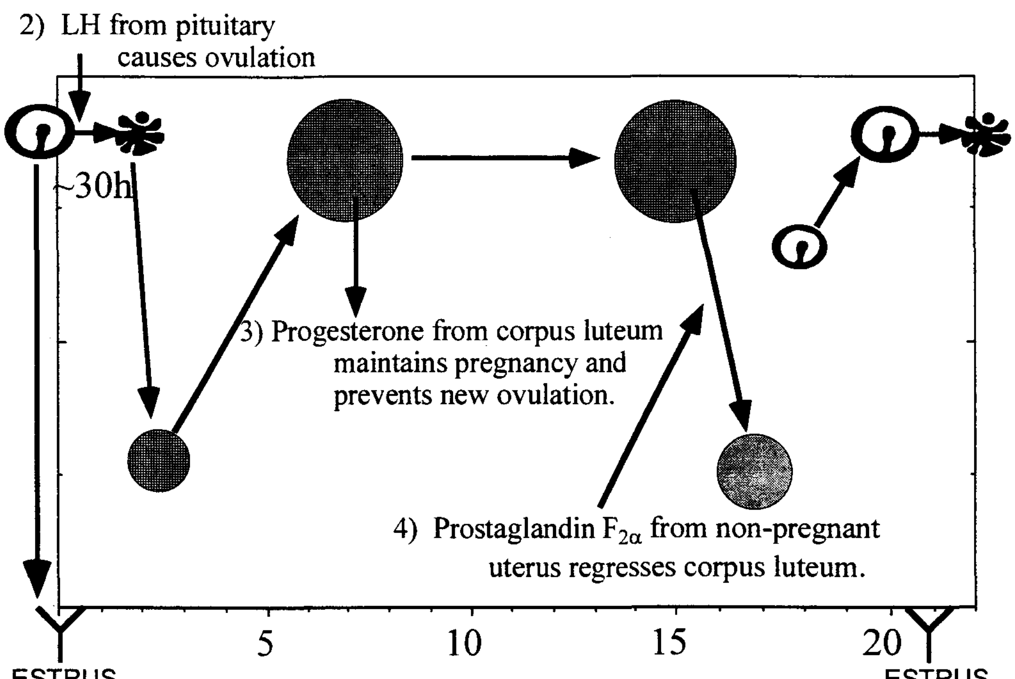

Figure 1. General features of the bovine estrous cycle emphasizing 2 key ovarian structures (preovulatory follicle and corpus luteum) and 4 key hormones.

The information in Figure 1 has been well known for some time. However in the last few years some important data has become available regarding growth of follicles and the relationship to Follicle-Stimulating Hormone (FSH) concentrations. An understanding of this new information is important for understanding recent synchronization protocols and therefore a brief summary of this information is provided below.

A number of recent studies have used transrectal ultrasound to analyze the final stages of follicular growth in cattle. In Figure 2 is shown a typical pattern for cows that have 2 follicular waves during an estrous cycle. Near the time of ovulation a group of small follicles begins to grow on the ovaries and this growth has been termed a follicular wave. From this group of follicles a single dominant follicle is selected to continue growth; whereas, other follicles of the follicular wave undergo regression. Due to the presence of a functional corpus luteum and high progesterone concentrations, this first dominant follicle does not cause estrus behavior and does not continue to ovulation. The first dominant follicle will become non-functional and a second follicular wave begins at about mid-cycle. Again a dominant follicle is selected from this second follicular wave and this follicle continues to ovulation because it's growth corresponds to the time of regression of the corpus luteum. Some cows also show 3 waves of follicular growth such that the second dominant follicle regresses, a third follicular wave is initiated, and the third dominant follicle becomes the ovulatory follicle. Also shown in Figure 2 is the pattern of circulating FSH concentrations in cows with 2 follicular waves during an estrous cycle. Both the first and second follicular waves are preceded by an increase in FSH concentrations (Adams et al., 1992). This increase in FSH is essential for the initiation of a follicular wave. The subsequent decrease in FSH is essential for selection a single dominant follicle.

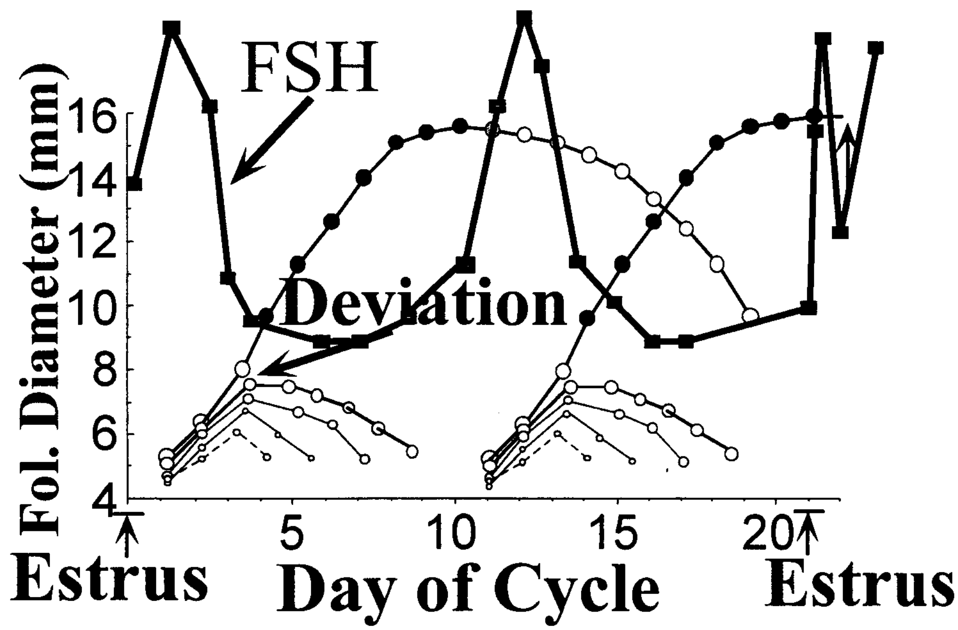

Figure 2. Representation of the follicle growth patterns and Follicle-Stimulating Hormone (FSH) concentrations in cows with 2 follicular waves during the estrous cycle.

An understanding of this basic information on reproductive physiology can provide a good framework for a discussion of different synchronization strategies that are currently available.

2. General synchronization strategies

Classically, synchronization strategies have tried to regulate corpus luteum function in order to regulate the estrous cycle. One method has been to extend the period of elevated progesterone concentration until the natural corpus luteum has undergone regression. This requires a time period longer than the normal lifespan of the corpus luteum (14-16 d). The problems and effective methodologies related to these progestin based strategies are discussed in section II below. A second method that has been used to control the corpus luteum is to induce early regression of the corpus luteum in order to synchronize the estrous cycle. Early investigators used estrogen-based strategies to induce luteal regression but this strategy was not consistently effective. A few years after the discovery that prostaglandin (PG) F?? was the natural hormone that caused regression of the corpus luteum, commercial products containing PGF?? or it's analogs became available for use in bovine reproductive management. Section III below summarizes some relevant information on PGF?? synchronization strategies.

Along with regulating the corpus luteum, recent synchronization strategies have focused on synchronizing follicular waves in order to improve precision and fertility following synchronization protocols. Some strategies use estrogens to reduce FSH concentrations in order to regress the current follicular wave and initiate a new follicular wave. Other strategies use gonadotropin releasing hormone (GnRH) to ovulate any dominant follicles on the ovary and thereby initiate a new follicular wave. These strategies still require synchronization of the luteal function in order to produce effective synchronization strategies as discussed in Sections III and IV below.

Recent emphasis has been placed on producing protocol that synchronize the time of ovulation with sufficient precision to allow a single timed insemination in all synchronized cows. These strategies use either estrogens (in the absence of progesterone) to induce a GnRH surge, a subsequent LH surge and ovulation. These estrogen-based programs also induce a synchronized behavioral estrus due to elevated estrogen concentrations. Other programs use a treatment with GnRH to induce an LH surge and ovulation. These strategies also can induce synchronous ovulation but may not be associated with behavioral estrus due to a decreasing serum estradiol concentrations following exposure of the dominant follicle to the LH surge.

Thus, the optimal programs for synchronization would: 1. **Synchronize luteal function** so that progesterone concentrations would be low at the appropriate times, 2. **Synchronize follicular waves** so that a dominant follicle would be at an optimal stage of development at a synchronous time, and 3. **Synchronize ovulation** precisely so that insemination can occur at one time in all cows at the optimal time for maximal fertility. An additional expectation is that problem cows, such as cows with nutritional anovulation or follicular cysts, would be effectively treated and have ovulation synchronized with the optimal synchronization system. Obviously, these optimal synchronization systems have to be weighed against the ease of use for the producer or veterinarian and the cost vs. financial reward per each synchronized cow. Although there is not currently an optimal strategy for all situations, the information can be used in designing acceptable strategies for specific situations.

II. Use of Progestins

The first synchronization methods for cows used progesterone. Soon after discovery of progesterone it was found that progesterone prevented an animal from coming into estrus. Thus, if progesterone were delivered for more than the normal length of an estrus cycle cows would have a synchronized estrus after terminating progesterone delivery. This method could fairly reliably synchronize estrus in cows but unfortunately there was a substantial decrease in fertility in the synchronized cows. The decreased fertility is due to abberant follicular growth patterns.

As shown in Figure 2 if dominant follicles are produced at a time when progesterone concentration is high (mid-cycle) they will become non-dominant and the subsequent increase in FSH will cause formation of a new follicular wave. In all currently available methods for synchronizing cattle with progestins there is less progestin activity than is normally produced by the corpus luteum. For example, the Controlled Internal Drug Releasing Device (CIDR) from New Zealand secretes enough progesterone to produce a blood concentration of about 2 ng/ml of progesterone. The normal progesterone concentration is about 4 ng/ml. Thus, insufficient progesterone activity is produced by this device to cause the dominant follicle to die and allow formation of a new follicular wave. This scenario produces a very large dominant follicle that has been termed a persistent follicle.

Figure 3 illustrates what occurs when a dominant follicle grows in either high or low progesterone concentrations. In the lower part of the figure is shown that a dominant follicle exposed to high progestin will become non-dominant and a new follicular wave will emerge. In the upper part of the figure is shown that in a low progestin enviroment the dominant follicle will continue to grow and become a persistent follicle. No new follicular waves will emerge in the presence of a dominant persistent follicle. On the right hand side is shown the conception rates in heifers or cows that are allowed to ovulate a persistent (upper) or a normal dominant (lower) follicle. It is clear that ovulation of a persistent follicle produces much lower conception rates. The low fertility produced by persistent follicles is the main reason that longer treatments with progestins such as melengesterol acetate (MGA), norgestomet implants (implant in Syncro-Mate-B), or intravaginal progesterone releasing devices (CIDR, PRID) will produce reduced conception rates.

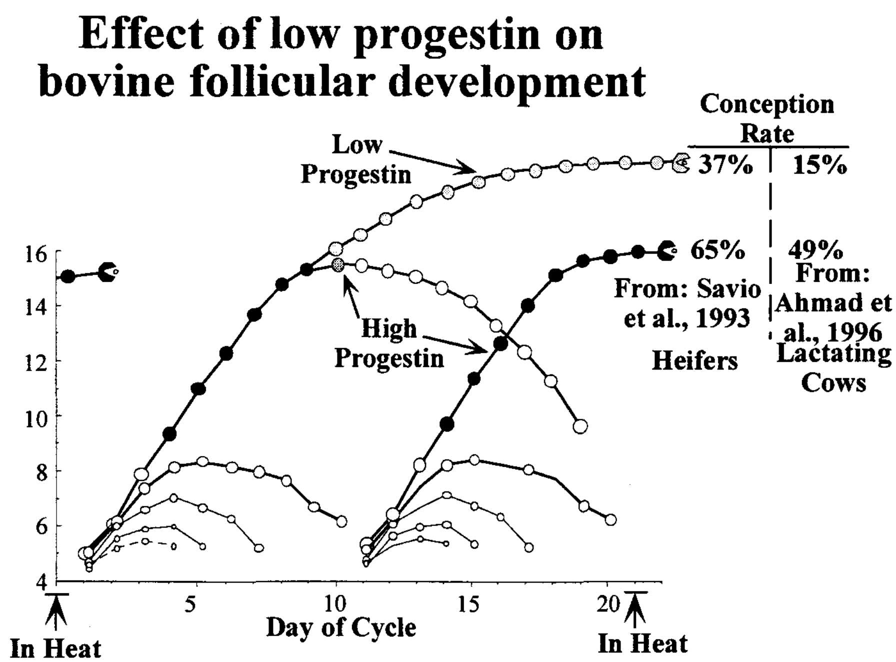

Figure 3. Schematic diagram of the growth patterns and conception rates in cows that ovulate a persistent follicle (upper) due to exposure to low progestin or ovulate a normal follicle due to exposure to the normal high progesterone concentrations.

III. Combination of Estrogens with Progestins

Empirical research in the 1960s and 1970s demonstrated that the time of progestin treatment that was needed to produced synchronization could be reduced to about 9 days if estradiol was given near the beginning of progestin treatment (Wiltbank and Kasson, 1968; Wiltbank et al., 1971; Woody and Abenes, 1975). An injection of 5 mg or more of estradiol regresses the bovine corpus luteum (Wiltbank et al., 1961) and this was the main reason that the estradiol injection was used in this treatment. It was found that combining estradiol with progestin treatment in a 9 day schedule produced fertility at the synchronized estrus that was similar to fertility at a spontaneous estrus (Wiltbank and Kasson, 1968; Wiltbank et al., 1971; Woody and Abenes, 1975). This led to development and FDA approval of syncro-mate-B, a product that contains a 6 mg norgestomet ear implant that is inserted for 9 days combined with an injection of 5 mg estradiol valerate and 3 mg norgestomet given at the time of implant insertion. Cows and heifers are fairly well synchronized at the end of this treatment with good fertility; although, some variability is reported (see Kesler and Faver, 1995 and 1996 for review).

The reason for the fairly normal fertility when estradiol is combined with progestin treatments is now clear. Estradiol not only regresses the corpus luteum but also regresses follicles that are present on the ovary. Estradiol is inhibitory to FSH secretion and this effect is potentiated by inhibin. An injection of 5 mg estradiol valerate during emergence of a follicular wave will stop growth of the dominant follicle (Bo et al., 1993; Bo et al., 1994). Growing follicles stop growing on about the same day as the estradiol valerate injection probably due to the decreased FSH concentrations (Bo et al., 1993; Bo et al., 1994).

We performed a study to characterize the effects of Syncro-Mate-B on follicular growth, corpus luteum function, circulating FSH concentrations, and time of ovulation (Vasconcelos et al., 1994). In addition, a previous study found that combining Syncro-Mate-B with an injection of GnRH at 30 hr after implant removal caused increased pregnancy rates per AI (Troxel et al., 1993). Thus, a second objective was to evaluate the effect of GnRH injection at 30 hr after Syncro-Mate-B treatment on time to ovulation.

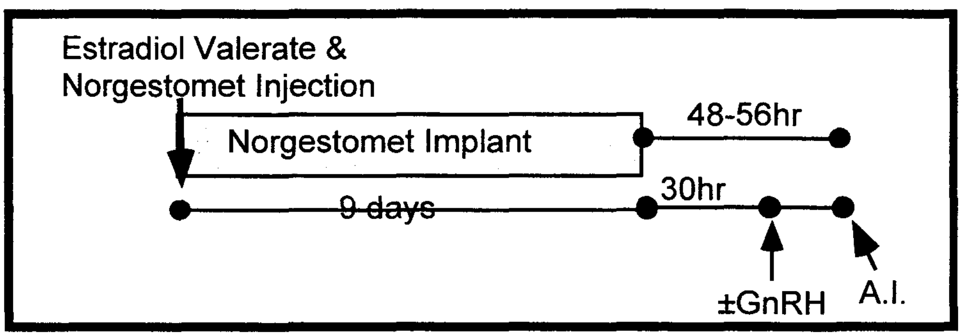

Figure 4. Syncro-Mate-B treatments and timing of GnRH injection in experiment done to evaluate ovarian dynamics during syncro-mate-B treatment and to assess the effect of GnRH on the synchrony of ovulation after syncro-mate-B. Timed-AI was performed on all heifers in this experiment at about 48 h after implant removal and the label directions indicate that this timed AI can occur from 48-56 hr after removal of Norgestomet implant.

Twenty Holstein heifers were treated with the full Syncro-Mate-B system (Figure 4) and ovarian activity was monitored by daily transrectal ultrasound. Day 0 represents the day of injection of norgestomet and estradiol valerate and the day of treatment with the norgestomet implant. Following these treatments the largest growing follicle began to regress probably due to decreased FSH concentrations (Figure 5). At 4-6 days after treatment a new wave of follicles emerged, and the dominant follicle from this wave became the ovulatory follicle (Figure 5). Emergence of this new follicular wave is most likely due to increased FSH concentrations (Figure 7). Thus, all heifers treated with Syncro-Mate-B had regression of the largest follicle present at the time of the initial injection and there was subsequent emergence of a new follicular wave.

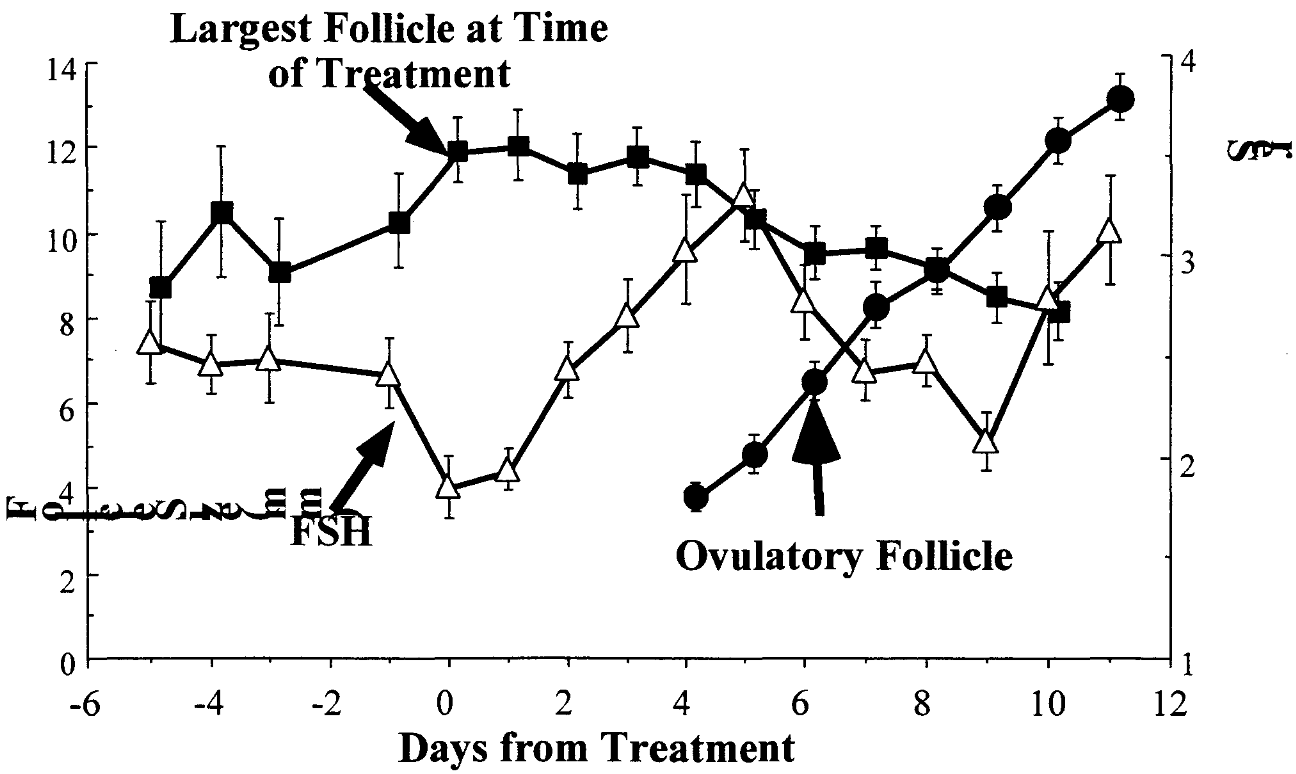

Figure 5. Effect of Syncro-Mate-B treatment on follicle growth and serum FSH concentrations.

The corpus luteum regressed in all cows treated after Day 5 of the estrous cycle. However, half the cows treated on Day 0-4 did not manifest regression of the corpus luteum. This is in agreement with previous results (Pratt et al., 1991) and can be corrected by either increasing the amount of norgestomet in the initial injection to 6 mg (Fanning et al., 1992) or by injecting PGF?? prior to implant removal.

Other synchronization treatments that combine estradiol with progestin have been developed but have not yet been approved in the U.S. A commonly used treatment in some parts of the world is to insert the CIDR into the vagina and to give a treatment with 2 mg of estradiol benzoate. Prostaglandin F2a is given on day 7 to regress the corpus luteum and the CIDR is removed on day 9. This protocol provides fairly synchronous expression of estrus and good fertility.

IV. Use of Prostaglandin F??.

Soon after PGF?? was found to be the hormone that normally regresses the corpus luteum, commercial PGF?? products were developed for synchronizing estrus in order to improve reproductive efficiency. Currently there are 2 commercial products available: Lutalyse (Upjohn-Pharmacia) and Estrumate (Bayer). These programs have been highly successful, particularly in lactating dairy cows, and this hormone is probably the most utilized reproductive hormones at this time. There are 3 problems with PGF?? for synchronization programs. The first is that the early corpus luteum (before day 7) will not regress after treatment with PGF??. This problem is primarily overcome but giving a second injection of PGF?? at an interval after the first injection that will allow all cows to have a functional corpus luteum. For heifers this interval is about 10 or 11 days; however, in lactating cows a 13-14 d interval is required before all cows have a PGF??-responsive corpus luteum. The second problem is that non-cyclic cows are not induced to cycle after treatment with PGF??. The third problem is that cows that respond to PGF?? come into estrus at a highly variable time after treatment.

To illustrate the extent of variability in response to PGF?? we analyzed data from a group of cows that were synchronized using a normal PGF?? protocol (Figure 6). All lactating cows (n = 154) were given PGF?? at a random stage of the estrous cycle and were observed extensively for signs of estrus during the next 7 days. As shown below about half of the cows (49%) were observed in estrus and bred after the first PGF?? treatment and 46% of those bred became pregnant. Most came into estrus on Day 2, 3, or 4 after PGF??. After a second PGF??, another third of the cows were bred with most cows in estrus on Days 2, 3, 4, or 5. After a third PGF??, the remaining cows were timed-bred at 72 hrs with low success (4% pregnant/AI). It is obvious that the lactating cows that do not show estrus after 2 PGF?? injections are likely to have poor fertility to timed-AI after PGF??. It is not possible to choose a proper time of AI because estrus occurs over a 3 to 4 day period. Thus, synchrony of estrus after PGF?? is not sufficiently precise in lactating dairy cattle to permit successful timed AI. In addition, acyclic cows are unlikely to be substantially improved by PGF?? treatment. It should be mentioned that timed-AI at 72 hrs after PGF?? treatment gave much better success (50% pregnancy rate per AI) in heifers (Pursley et al., 1997b).

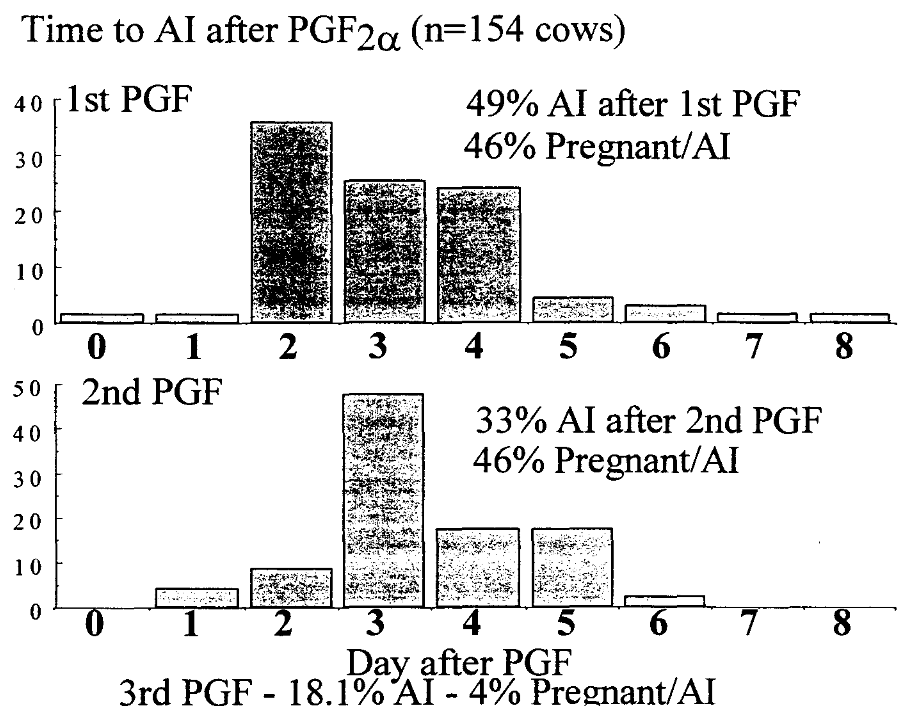

Figure 6. Time of AI after first or second injection of PGF?? in lactating dairy cows.

It is now clear that the variability in time to estrus after PGF?? is due to differences between cows in follicular growth and not due to differences in time to luteal regression. Figure 7 shows that heifers or cows that respond to an injection of PGF?? will show estrus at an earlier time if PGF?? is given on Day 7 than on Day 10 of the estrous cycle (Momont and Seguin, 1983). This is not due to a difference in time from PGF?? until low progesterone concentration. On Day 7 a functional dominant follicle is present on the ovary and only 2 days (heifers) or 2.5 days (lactating cows) are required until estrus. On Day 10 the dominant follicle will have lost functional dominance in many cows and a new follicular wave will be emerging. Unlike the first follicular wave, this second follicular wave does not emerge in a synchronous fashion. Thus, heifers or cows could be at varying stages of follicular growth at the time of the PGF?? on Day 10 resulting in a longer, more variable, time to estrus.

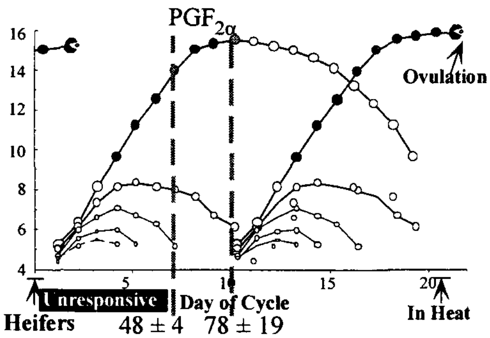

Figure 7. A schematic representation of the number of hrs from PGF?? to behavioral estrus in heifers and lactating cows given PGF?? on different days of the cycle (adapted from Momont & Seguin, 1983).

V. Combinations of Gonadotropin Releasing Hormone (GnRH) and PGF??:

Recent studies have shown that use of GnRH prior to PGF?? can increase synchrony in luteal regression and time to estrus. How does this work? An injection of GnRH will cause an LH surge and subsequent ovulation of a responsive follicle. What determines if a follicle will ovulate in response to a GnRH-induced LH surge? In our studies we have generally found that growing follicles greater than 9-10mm in diameter will ovulate after an injection of GnRH. It does not matter if there is a corpus luteum present or not. In a recent study we have evaluated the response of lactating dairy cows to an injection of GnRH at various times of the cycle (Table 1). Ovaries were evaluated by ultrasound for 25 days prior to an injection of 100µg GnRH. Four of the 33 had not ovulated prior to injection of GnRH and all of these anestrous cows ovulated after GnRH. The two stages of the cycle when ovulation did not occur after GnRH were in the early cycle (Days 0-2) or mid cycle (Days 9-14). A total of 85% of the cows ovulated after GnRH supporting our previous report that a high percentage of lactating cows ovulate after an injection of GnRH. In a previous study we found that 18/20 (90%) lactating cows ovulated after injection of GnRH at a random time during the estrous cycle, whereas only 13/24 (54%) heifers ovulated (Pursley et al., 1995).

Table 1. Effect of time from previous ovulation on response of lactating dairy cows to an injection of GnRH (4 cows with no previous ovulation are included in total).

	Day after Previous Ovulation							
	0-2	3-5	6-8	9-11	12-14	15-17	18-21+	Total
No. Ovulating	0/2	3/3	5/5	2/3	5/7	3/3	6/6	28/33

After an injection of GnRH there is growth of a new follicular wave (Figure 8). As shown in Figure 8, there is a great deal of variability in follicular growth prior to the injection of GnRH (as shown by error bars) and much less variability in follicle size after GnRH. The new follicular wave emerges on mean Day 1.61 after the injection of GnRH.

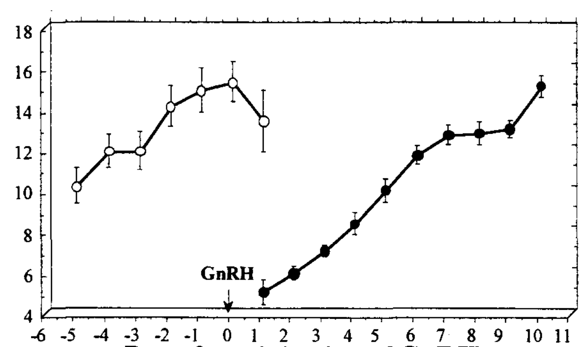

Figure 8. Growth of dominant follicles before or after an injection of GnRH in lactating dairy cows (n = 20).

In 1992 Twagiramungu et al. reported that injection of GnRH 6 days prior to PGF?? increased synchronization rate in beef cattle. Use of GnRH 7 days prior to PGF?? has also been proposed to improve synchronization rate in lactating dairy cattle (Thatcher et al., 1993). We reported that injection of GnRH prior to PGF?? synchronizes growth of ovarian follicles (Pursley et al., 1995). We found that this growing follicle can be ovulated in a very precise manner by using a second injection of GnRH at 1.5 to 2 days after the PGF?? injection (Pursley et al., 1995). This information was consolidated into a practical reproductive management program for lactating dairy cattle that has become known as the Ovsynch protocol. This program requires 3 injections as shown in Figure 9. After the second injection of GnRH the cows are bred without regard to estrous behavior. The pregnancy rate per AI is similar with cows that have a timed AI after this protocol as those that are bred to a normal estrus and this is about 40% in lactating cows. This program only synchronizes ovulation in about 60-70% of heifers as compared to about 85% synchronization of lactating dairy cows. Thus, this new protocol allows more effective management of reproduction in lactating dairy cows because cows can be bred at the correct time without continuous detection of estrus.

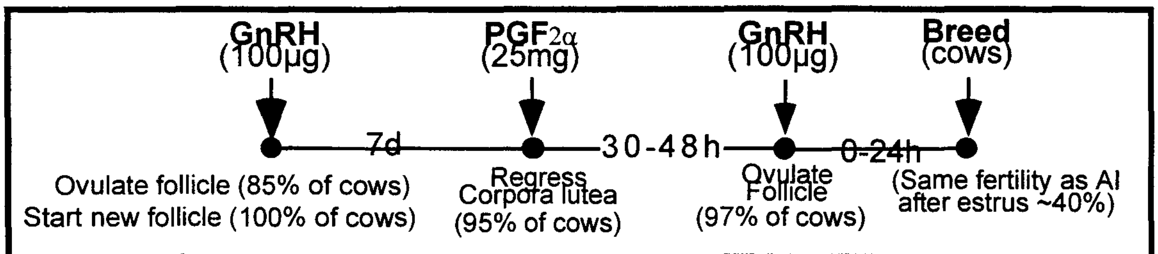

Figure 9. Timing and purpose of hormonal injections during the synchronization of ovulation procedure in lactating dairy cows (Ovsynch).

The AI submission rate is dramatically improved after using Ovsynch. This is because all cows can be routinely bred on a given day of lactation. We performed a study in 3 Wisconsin dairy herds to evaluate the efficiency of the Ovsynch program for reproductive management (see Pursley et al., 1997a). All cows were randomly assigned (n = 333 cows) to either a control group with the herds typical reproductive management (heat detection with occasional PGF?? use) or to the Ovsynch group with all cows bred only to timed AI on one day of the week. Cows remained on the reproductive management programs throughout lactation so that days open could be compared. Cows in the Ovsynch group that were detected in estrus could not be bred until they were detected not pregnant at a pregnancy diagnosis and resynchronized with Ovsynch. Thus, no estrus detection was used in the Ovsynch group. Not surprisingly the Ovsynch cows had fewer days to first AI (54 vs. 81 days) because they were all serviced between 50 and 58 days in lactation. However, first service pregnancy rate/AI was not different between Ovsynch vs Control (37% vs. 39% respectively). Interestingly, days open was less (99 vs. 118) in the Ovsynch group even though no estrus detection was utilized. Thus, it is possible to have a good reproductive management program without estrus detection.

What are the disadvantages of Ovsynch? The most obvious disadvantage is the cost of the hormones. It is a good possibility that the improvements in reproductive efficiency more than pay for the increased hormone costs; however, this needs to be carefully evaluated on each farm. Second, the Ovsynch program only allows a normal pregnancy rate/AI and therefore there needs to be an effective method for detecting non-pregnant cows after Ovsynch. The most practical method is probably an intensive estrus detection program at 18-25 days after the Ovsynch program. Some veterinarians will also use ultrasound to detect early pregnancies. Third, the safeguard of cows not showing estrus during times of low fertility is removed with the Ovsynch program. For example, during a hot summer many cows will not show estrus and therefore not be bred during this time of low fertility. With the Ovsynch program many cows will continue to have AI during the summer but pregnancy rate per AI may be reduced by heat stress (Hansen, 1994).

The Ovsynch program has been successfully used on many dairy farms around the country in the last 2 years. It can dramatically improve service rate and overall reproductive efficiency on a farm. Interestingly, it does appear to be effective in some non-cycling cows. It can cause many of these cows to have a synchronized ovulation with some resulting pregnancies. It also has been found to be effective in treating cows with follicular cysts. It is important to remember that it was not designed for heifers and does not effectively synchronize ovulation in heifers. A recent abstract indicates that a GnRH-PGF-GnRH program is effective for timed-AI in suckled beef cows and beef heifers (Roy and Twagiramungu, 1996). These researchers used only 6 days between the first GnRH injection and the PGF?? injection. The pregnancy rate per AI was 62.2% in the fixed-time AI group and 70.0% in the cows bred to a normal estrus (no difference P>0.1). Thus, changing the timing to 6 days rather than 7 days between the GnRH and PGF?? injection could allow this protocol to be effective in heifers.

VI. Time of Insemination after Synchronization Programs

Time of AI in relation to onset of estrus and time of ovulation is one factor that can influence conception rates that are achieved with any AI program. The early studies suggested that there may be a fairly narrow time for optimal success with AI (Table 2). This information led to the AM/PM rule for AI. However, it should be noted that this study suffered from low numbers of cows per treatment group and a lack of statistical comparisons. A number of studies from AI organizations have shown similar non return rates from once-a-day vs twice-a-day AI. Data from Mississippi also shows that breeding once-a-day in the AM is similar to breeding at both AM and PM (Table 3; Gonzalez et al., 1985) and that AI at the onset of estrus only is similar to AI at the onset of estrus and again 12 h later (Table 4; Wahome et al., 1985). In addition, Jersey cows bred once per day had similar pregnancy rates as AI by the AM/PM rule (Table 5; Graves et al., 1997). However, there was some reduction in conception rates in those cows that were first in heat and bred in the same AM (Table 6; Graves et al., 1997).

TABLE 2. Effect of time of insemination on fertility in beef cows (Trimberger and Davis, 1943).

Time of Breeding	Cows Inseminated	Cows conceiving
Start of estrus	25	44%
Middle of estrus	40	82%
End of estrus	40	75%
After estrus - 6 hours	40	63%
12 hours	25	32%
18 hours	25	28%
24 hours	25	12%
36 hours	25	8%
48 hours	25	0%

Table 3. Conception rate after AI in AM only or at 12 h after estrus (AM/PM).

Treatment	# of heifers	Conception rate
AM/PM	132	62.9%
AM	129	62.0%

Table 4. Conception rate after one or two AI.

Treatment	# of heifers	Conception rate
One AI	84	70.2%
Two AI	86	68.6%

Table 5. Effect of once versus twice daily AI

	No. Bred	% Pregnant
a.m.-p.m. rule	172	60.5%
once per day	165	57.6%

Table 6. Effect of time from estrus to AI

	No. Bred	% Pregnant
a.m.-a.m.	112	51.8%[b]
a.m.-p.m.	109	59.6%[a]
p.m.-a.m.	116	65.5%[a]

Some recent data further suggests that there is about a 24 h time period during which acceptable conception rates can be obtained. Diskin, 1996 in Ireland reported the results of 1200 inseminations from bulls of average to above-average fertility (Table 7). AI any time from right after onset of estrus until 24 hr after onset of estrus gave acceptable conception rates. We have recently completed a study (Pursley et al., 1998) in which the optimal time of AI was evaluated after synchronization of ovulation (Ovsynch). The percentage of cows pregnant at 28 days after AI (pregnancy rate per AI) was similar for all the time periods from 0-24 h after the second GnRH injection (Table 8). The only time that was markedly lower was at 32 h after the second injection of GnRH which would be after the expected time of ovulation. The calving rate also showed a similar pattern with only the 32 h time period having lowered fertility. Thus, there is substantial flexibility in the optimal time of AI and it appears to be better to breed too early than too late in relation to ovulation. Interestingly there was also an indication that time of AI may affect the gender of the calf. A subsequent study using 1601 cows also suggested that cows bred at the same time as the second GnRH injection (0 time) had a higher percentage of female calves (54% vs. 46% at 24 h).

Table 7. Effect of time of breeding, relative to first observation of heat, on calving rate.

Interval (hrs)	0-12	12-18	18-24	24-36
Calving rate (%)	51%	58%	54%	35%

Table 8. Hours from GnRH to AI in lactating cows (* - significantly different; P<0.05).

	0	8	16	24	32
Number of cattle	149	148	149	143	143
Pregnancy rate per AI	37%	41%	45%	41%	32%*
Calving Rate	32%	34%	36%	32%	23%*
% Female calves	61%*	45%	54%	54%	65%*

VII. Future Synchronization Programs

There are numerous recent studies analyzing novel synchronization methods using various combinations of progestins, estradiol, GnRH, or PGF??. It is not yet clear the optimal protocol that will emerge from these studies. As mentioned above the goal is to produce future protocols that will synchronize follicular waves as well as corpus luteum function in order to allow an effective timed AI program. As in the Ovsynch program it is likely that the time of ovulation will be precisely synchronized with either treatment with GnRH or estradiol. Researchers are also hopeful that some future protocols will provide increased fertility and effective treatment of non-cyclic (anestrous or cystic) cows.

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