Proceedings:

Applied Reproductive Strategies
in Beef Cattle Workshop

September 5 – 6, 2002
Kansas State University
Manhattan, Kansas

Edited by S. K. Johnson

Co-Sponsored by:
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College of Veterinary Medicine, Kansas State University

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Conference Objectives

This workshop is designed to:

1) Improve the understanding of the physiological processes of the bovine estrous cycle, the procedures available to synchronize estrus and ovulation and the proper application of these systems in beef cattle operations.

2) Improve the understanding of methods to assess male bovine fertility and how it affects the success of AI programs.

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REVIEW OF FOLLICULAR GROWTH AND THE BOVINE ESTROUS CYCLE

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Introduction

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$_{2\alpha}$ (PGF$_{2\alpha}$) from the non-pregnant uterus. After exposure to PGF$_{2\alpha}$ 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. 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 behaviour 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. Both the first and second follicular waves are preceded by an increase in FSH concentrations (Adams et al., 1992). These increases in FSH are essential for the initiation of a follicular wave. The subsequent decrease in FSH is essential for selection a single dominant follicle. There is also a FSH surge in association with the LH surge that causes ovulation. This FSH surge occurs near the onset of estrus and is shorter of duration than the other FSH surge. The physiological function of this LH surge-associated FSH surge is not defined at this time.

This manuscript will be divided into 3 sections. The first will discuss the hormonal changes near the time of ovulation. The second section will deal with some functional aspects of the corpus luteum and factors that determine the circulating progesterone concentration. The third section will focus on follicular waves and mechanisms for selection of a single dominant follicle.

I. Key events around estrus:

Artificial insemination programs are mainly built around detection of estrus and breeding the cow in relation to the time of that detected estrus. The pregnancy that results from this breeding is obviously not due to the estrous behavior of the cow but is due to ovulation of an oocyte near the time of this estrous behavior. The timing of the hormonal and ovarian events around the time of estrus have been very well characterized using hormonal assays and observation of the ovaries with transrectal ultrasound. Figure 2 shows a simplification of key events around estrus: the increase in estradiol, standing estrus, the luteinizing hormone (LH) surge, and ovulation. All of the events are initiated by high circulating estradiol concentrations. The elevated estradiol is due to growth of a large preovulatory follicle on the ovary. After regression of the corpus luteum the dominant follicle grows and produces increasing amounts of estradiol. The cow becomes sexually active prior to the onset of standing estrus due to the increasing amounts of estradiol in the absence of circulating progesterone. Progesterone is low due to regression of the corpus luteum. If the corpus luteum does not regress and progesterone
remains elevated some or all of the subsequent events (LH surge, estrus, ovulation) do not occur even when estradiol is elevated.

After estradiol concentrations have reached sufficient concentrations for a certain time period there is a change in the brain that causes the cow to begin to stand solidly during mounting (onset of estrus). There is also secretion of the hormone gonadotropin releasing hormone (GnRH) in large amounts from a part of the brain called the hypothalamus. The secretion of GnRH is what causes the LH surge. These 2 events (estrous and GnRH/LH surge) happen very close to the same time but are actually due to 2 distinct events in the brain that are generally synchronized. It is possible to have estrus occur in some cows without a corresponding LH surge. For example, some cows that are cystic will show standing estrous behavior without any LH surge or ovulation. Using daily ultrasound on lactating dairy cows (n = 175) we have observed estrus without an ovulation in about 11% of lactating cows (Sartori and Wiltbank, unpublished results). In general, these cows did not have follicles that were sufficiently large to qualify as cystic cows. The lack of ovulation is probably due to lack of an estradiol-induced LH surge. Alternatively, there are also times in lactating cows that no standing estrous behavior is demonstrated but the cows have ovulation due to an LH surge (~9%). Thus, although estrus and the LH surge normally happen almost simultaneously, there are instances when either of these 2 events can occur independently.

Figure 2. Diagram of the key events near estrus. High circulating estradiol causes the LH surge and standing estrous behavior. Estrus lasts longer, on average, in heifers than in lactating cows. Cows and heifers ovulate about 28 h from the onset of estrus and the LH surge.

The onset of estrus is due to the high circulating estradiol concentrations. Estrous behavior ends prior to ovulation in cattle. The end of estrus may be due to decreased circulating estradiol because estradiol production in the follicle is dramatically reduced following the LH surge. As shown in Figure 1 the duration of estrus is greater in heifers
than in lactating cows (Nebel et al., 1997). We have also found that circulating peak estradiol concentrations near the time of estrus is lower in lactating cows than heifers (Sartori et al., 2000). Thus, the reduced intensity and duration of estrus in lactating cows may be due to lower circulating estradiol concentrations.

The time from the onset of estrus until ovulation is between 25 and 34 h (Walker et al., 1996). The time of the LH surge is the event that sets the time of ovulation. Induction of an LH surge and ovulation with a GnRH injection prior to the normal time of ovulation (Pursley et al., 1995) will also cause an ovulation in about 28 h after the GnRH treatment (range of 24-32 h).

It is critical to note that AI to estrous behavior is based on the idea that estrus is a good sign of the time of ovulation. If there is no ovulation, there will be no fertilization or pregnancy even if the cow showed very clear signs of estrus. A commonly used procedure in commercial dairy operation, the Ovsynch protocol, was developed to allow breeding of cows to a synchronized ovulation without the need for detection of estrus (Pursley et al., 1995; Pursley et al., 1997). This protocol relies on an injection with GnRH to induce an LH surge and subsequent ovulation. This protocol has also allowed extensive studies on the optimal time of AI in relation to an induced ovulation. It is possible to compare the optimal time of AI between studies using estrus and studies using GnRH because the onset of estrus should correspond to the time of the GnRH/LH surge. Thus, the time of injection of GnRH will approximate the time of onset of estrus and both will be about 28 h from the time of ovulation.

II. The corpus luteum and circulating progesterone.

A. What determines size of the corpus luteum?

It seems obvious that the size of the ovulatory follicle will have an effect on size of the corpus luteum. This relationship was clearly demonstrated in a study that we recently published (Vasconcelos et al., 2001). We used the Ovsynch protocol (GnRH-7 days- PGF2a-2 days-GnRH) to synchronize an ovulation but we reduced the size of the ovulatory follicle in some cows by aspirating the dominant follicle at day 3 or 4 after the first GnRH. At the time of the second GnRH injection the follicle size was much less in the aspirated group (11.5±0.2mm) than the non-aspirated group (14.5±0.4mm). The volume of the CL was dramatically reduced by ovulation of the smaller follicle when measured either at day 7 (2,862±228 vs. 5,363±342 mm³) or at day 14 (4652±283 vs. 6526±373 mm³) after the second GnRH injection. Thus, size of the ovulatory follicle can dramatically alter size of the corpus luteum, probably due to a greater number of granulosa and/or thecal cells contributing to the corpus luteum.

The amount of LH support can also regulate the growth of the corpus luteum. In Figure 3 is shown data from Peters et al., 1994. These investigators treated heifers with a GnRH antagonist on different days of the estrous cycle to evaluate the effect on subsequent luteal function and serum progesterone concentrations. Treatment with the GnRH antagonist eliminated LH pulses but it did not cause regression of the corpus luteum. It appears that the corpus luteum did not grow as well when the cows were treated with GnRH antagonist during the early luteal phase. This is reflected in the serum progesterone concentrations shown in Figure 3. Thus, in addition to an effect of size of the ovulatory follicle, there also is a stimulatory effect of LH on growth of the corpus luteum and these 2 factors appear to be important in determination of size of the corpus luteum.
B. What determines circulating progesterone concentrations?

The amount of luteal tissue volume is probably a key determinant of progesterone production from the corpus luteum. In the experiment discussed above (Vasconcelos et al., 2001) in which aspiration was used to reduce the size of the ovulatory follicle, a reduction in size of the corpus luteum also was reflected in reduced circulating progesterone concentrations. However, in most data sets with cows that naturally ovulated there appears to weak or no relationship between size of the corpus luteum and circulating progesterone concentrations. Although, at first glance, it seems likely that these 2 parameters should be closely correlated, it must be remembered that the circulating concentration of a hormone is determined not only by the rates of production but also by the rates of degradation of the hormone.

Our recent studies have shown that lactating cows have much greater rates of progesterone metabolism than heifers or non-lactating cows. This appears to be due to the very high rates of liver blood flow that are present in lactating dairy cows. The physiological scenario appears to be that high milk production requires very high levels of feed intake. This high level of feed intake increases blood flow to the gut and subsequently increases blood flow to the liver. Steroids, such as progesterone and estradiol are primarily metabolized in the liver and therefore elevated liver blood flow leads to increased rates of steroid metabolism. In recent studies we have found that lactating dairy cows have much larger corpora lutea but lower circulating progesterone concentrations than found in heifers. This is probably due to the high rates of progesterone metabolism in lactating dairy cows. The reason for the larger corpora lutea appears to be due to ovulation of larger follicles in lactating dairy cows. Ovulation of larger follicles may be due to the high estradiol metabolism in lactating dairy cows not allowing circulating estradiol to reach critical levels that will induce an LH surge until

![Graph of serum progesterone concentrations in heifers treated with GnRH antagonist from days 2-7, 7-12, or 12-17 of the estrous cycle or left untreated (Control). The serum progesterone concentrations were reduced in heifers treated on days 2-7 or days 7-12 compared to the other 2 groups (data from Peters et al., 1994).]
Follicular estradiol production is greatly elevated. Thus, lactating dairy cows may grow larger follicles but circulating estradiol may not be correspondingly increased due to high estradiol metabolism. Similarly, larger corpora lutea formed due to ovulation of these larger follicles may not produce elevated circulating progesterone concentrations because progesterone is metabolized at a greater elevated rate in lactating dairy cows.

**Beef cattle**

\~ 40 days postpartum

![Diagram showing serum progesterone concentrations](image)

**Figure 4.** Summary of serum progesterone concentrations observed following the first ovulation of beef cattle that was preceded or not preceded by progestin treatment.

### C. Why does the corpus luteum regress too early or too late?

Regression of the corpus luteum is due to a physiological sequence of events that involves progesterone, estradiol, oxytocin, and prostaglandin (PG) F2α (Silvia et al., 1991; Salfen et al., 1999). It has been known for many years that regression of the corpus luteum is primarily due to uterine secretion of PGF2α. Short estrous cycles appear to be primarily due to premature secretion of PGF2α from the uterus. Figure 4 shows a summary of data from various studies on short estrous cycles. In the absence of prior progestin treatment, beef cows will have a short luteal phase following first ovulation. In contrast, prior treatment with progestin results in normal serum progesterone concentrations following first ovulation. The reason for the short luteal phase is premature secretion of PGF2α (Hunter, 1991). Probably most instances of either early or late regression of the corpus luteum ultimately relate to an alteration in uterine PGF2α secretion.
III. Follicular Waves and Selection of a Single Dominant Follicle

A. General Aspects of Follicular Waves.

A number of recent studies have used transrectal ultrasound to analyze the final stages of follicular growth in cattle. In Figure 5 is shown a schematic of follicle growth and FSH for a cow that has 2 follicular waves during a 21 d 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 an LH surge, behavioral estrus, 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 this dominant follicle is functional at the time of regression of the corpus luteum. Some cows also have 3 waves of follicular growth such that the second dominant follicle regresses, a third follicular wave is initiated, and the third dominant follicle is functional at the time of luteolysis and therefore is the ovulatory follicle. The pattern of circulating FSH concentrations has an important functional relationship to the pattern of follicular growth. Both the first and second follicular waves are preceded by an increase in FSH concentrations (Adams et al., 1983). Near the time of estrus there are 2 surges in FSH that are difficult to discriminate because they are temporally adjacent. The first surge corresponds to the GnRH/LH surge that induces ovulation and a second occurs near the time of ovulation and is associated with emergence of the first follicular wave. This increase in FSH is essential for initiation of a follicular wave. Emergence of the follicular wave has generally been retrospectively determined as the time when the first follicles of the follicular wave reached ≥ 4 mm. The time of emergence is generally at the peak of the FSH surge. Following emergence, follicles continue growth and circulating FSH begins to decline up until the time of follicular deviation.

Figure 5. Schematic of follicle growth and FSH for a cow that has 2 follicular waves during a 21 d estrous cycle.
Follicular deviation has been identified in many studies in which follicular growth was followed on a regular basis using transrectal ultrasound. Follicular deviation has been defined as the beginning of the greatest difference in growth rates (diameter changes between successive ultrasound examinations) between the largest follicle (i.e., dominant follicle) and the second largest follicle (i.e., largest subordinate follicle) at or before the examination when the second largest follicle reached its maximum diameter (Ginther et al., 1996). A representation of the time of follicular deviation is shown in Figure 5. At the time of follicular deviation the diameter of the future dominant follicle averages 8.5 mm and the future largest subordinate follicle averages 7.2 mm (Ginther et al., 1996). The circulating FSH concentrations reach a nadir near the time of follicular deviation and this decrease is probably essential for selection of a single dominant follicle. Selection of the dominant follicle either occurs at the time of follicular diameter deviation or is closely associated with this process. Later sections will discuss the potential mechanisms involved in follicular selection and growth of the follicle after follicular selection.

Follicular waves do not occur only in cycling cattle (Table 1). Follicular waves are present in prepubertal calves by 2 months of age (Evans et al., 1994) and are also present in dairy or beef cattle prior to onset of cyclicity. Follicular waves also occur during most of pregnancy (Ginther et al., 1996a). The maximal diameter of the largest follicle decreases from 15.7 mm in wave 1 to 13.1 mm in wave 2 of pregnancy (Ginther et al., 1994). There is a subsequent decrease in the maximal size of the largest follicle from 12.7 mm at Day 90 of pregnancy to 10.3 mm at the third wave after day 90 and further decreases down to a maximal size of 8.5 mm by the ninth month (Ginther et al., 1996a). As in other physiological states there is an FSH surge that, on average, corresponds to emergence of each follicular wave during pregnancy. The time of follicular selection appears to be similar in most of these states; although, selection may occur at slightly smaller follicles during the first 4 weeks after birth (Evans et al., 1994) and in the last months of pregnancy (Ginther et al., 1996a). The regulation of follicular waves in some of these anovulatory states will be discussed in the next section of this manuscript.

Table 1. Physiologic states in which follicular waves have been found.

<table>
<thead>
<tr>
<th>Physiologic state</th>
<th>Follicular waves</th>
<th>Length of follicular wave</th>
<th>FSH surge before wave</th>
</tr>
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<tr>
<td>Estrous Cycle</td>
<td>Yes</td>
<td>9-14 days</td>
<td>Yes</td>
</tr>
<tr>
<td>Postpartum Anestrus</td>
<td>Yes</td>
<td>7-12 days</td>
<td>Not evaluated</td>
</tr>
<tr>
<td>Prepubertal</td>
<td>Yes</td>
<td>7 days</td>
<td>Yes</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Yes</td>
<td>6-12 days (vary by stage)</td>
<td>Yes</td>
</tr>
<tr>
<td>Follicular Cysts</td>
<td>Yes</td>
<td>7-25 days</td>
<td>Yes</td>
</tr>
</tbody>
</table>

B. Follicle Growth from Emergence to Selection.

One critical interaction occurring during almost all phases of reproduction in cattle is an interaction between secretion of pituitary FSH and secretion of ovarian inhibitors of FSH. This has been termed two-way functional coupling between FSH and follicles (Ginther et al., 2000). In the absence of any follicles following ovariectomy of cattle there is a rapid increase in circulating FSH that reaches maximum by 24 h with a subsequent continuous maximal secretion (Gibbons et al., 1997). Aspiration of all
follicles greater than 3 mm results in a similar elevation in FSH indicating that the follicular FSH inhibiting activity is coming from follicles greater than 3 mm in diameter (Gibbons et al., 1997). The 2 primary inhibitors of FSH that are secreted by the follicle are inhibin and estradiol. Inhibin appears to be secreted by follicles of all sizes; however, circulating estradiol only appears to increase after a dominant follicle has been selected following deviation. Maximal FSH concentrations are observed at the time of emergence of a new follicular wave probably due to low circulating concentrations of inhibin and estradiol. As the follicular wave progresses, larger follicles appear to produce greater FSH inhibitory activity, probably inhibin, reducing circulating FSH. On average, the nadir in circulating FSH is reached at the time of follicular selection with the dominant follicle of approximately 8.5 mm in diameter. Diminished FSH continues until a few days before emergence of a subsequent follicular wave. Circulating estradiol increases from ~0.2 to ~1 pg/ml near the time of follicle selection (Kulick et al., 1999) and this is probably responsible for the final depression in circulating FSH at this time. Estradiol alone is a very weak inhibitor of FSH secretion but synergizes with inhibin to strongly inhibit FSH secretion.

Figure 1 shows a simplified diagram of the interaction that exists between FSH and the follicle. Circulating FSH is elevated at the time of follicular emergence due to lack of the circulating inhibitors of FSH, inhibin and estradiol. As the follicles grow, circulating FSH declines due to increasing circulating inhibin. Some smaller follicles do not continue growth in the lower FSH environment (Ginther et al., 1996). Near the time of follicular selection there is a further depression in FSH probably due to the combined actions of circulating inhibin and low amounts of estradiol. One critical aspect of this postulated model is that follicle growth up to the time of deviation only involves an interaction between the pituitary and follicle with no involvement of the hypothalamus. Thus, this pituitary-follicle interaction can continue to occur in the presence of widely divergent ovulatory or anovulatory physiological states. Obviously, specific events can set the timing of follicle wave emergence such as removal of dominant follicle inhibitory activities at the time of ovulation or inhibition of post-deviation follicular growth in prepubertal calves or early postpartum cows. The critical concept is that an underlying physiological motif that is present during almost all physiological states is a dynamic interaction between pituitary FSH and follicular growth from emergence to follicular selection.

C. Changes in LH Receptor during Selection.

There are probably multiple cellular mechanisms involved in the deviation process including increased free IGF-1, decreased IGF binding proteins, and increased follicular estradiol production. This section will focus on the data related to increased LH receptor near the time of diameter deviation because of the wealth of information on this subject and because of the well documented importance of LH action in follicular growth after deviation (see section E). Beg et al., 2001 compared the diameter of the largest and second largest follicles from the first follicular wave using slaughterhouse ovaries to estimate diameter deviation and determined granulosa cell LH receptor mRNA by quantitative RT-PCR. The increased difference in LH receptor mRNA expression between the 2 largest follicles occurred, on average, an equivalent of 8 h before any increased difference in either follicle diameter (follicular selection) or follicular-fluid estradiol concentration (Beg et al., 2001). In other studies follicular diameter deviation in individual cows was not determined but similar changes in LH receptor mRNA or
binding have been noted near the time of follicular selection. For example, Table 2 shows the results of Xu et al., 1995. There was an increase in LH receptor mRNA on granulosa cells from non-detectable on Day 2 (average of 6.7 mm) to highly expressed levels on Day 4 (average of 10.8 mm; Day 0 = day of wave emergence). Near the expected time of follicular selection, there was also a 4-fold increase in LH receptor mRNA in thecal cells.

Table 2. Expression of mRNAs for LH receptor (LHr) and FSH receptor (FSHr) in theca and granulosa cells of bovine follicles collected on different days of the first follicular wave. No FSHr was detected in theca cells (data not shown) (data from Xu et al., 1995).

<table>
<thead>
<tr>
<th>Day of wave</th>
<th>No. heifers (No. of follicles)</th>
<th>Size of follicle</th>
<th>Theca LHr</th>
<th>Granulosa FSHr</th>
<th>Granulosa LHr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3 (8)</td>
<td>5.3 ± 0.4</td>
<td>15 ± 7a</td>
<td>15 ± 5</td>
<td>0a</td>
</tr>
<tr>
<td>2</td>
<td>4 (12)</td>
<td>6.7 ± 0.5</td>
<td>13 ± 6a</td>
<td>25 ± 4</td>
<td>0a</td>
</tr>
<tr>
<td>4</td>
<td>4 (4)</td>
<td>10.8 ± 0.8</td>
<td>59 ± 10b</td>
<td>31 ± 11</td>
<td>26 ± 10b</td>
</tr>
<tr>
<td>6</td>
<td>5 (5)</td>
<td>12.9 ± 0.6</td>
<td>21 ± 10a</td>
<td>14 ± 4</td>
<td>24 ± 8b</td>
</tr>
<tr>
<td>8</td>
<td>4 (4)</td>
<td>11.1 ± 1.0</td>
<td>28 ± 10a</td>
<td>13 ± 6</td>
<td>9 ± 7a</td>
</tr>
<tr>
<td>10</td>
<td>3 (3)</td>
<td>11.7 ± 0.3</td>
<td>10 ± 7a</td>
<td>6 ± 3</td>
<td>0a</td>
</tr>
</tbody>
</table>

a,bValues with different superscripts differ within columns (P<0.05)

Another study (Bao et al., 1997) also found an increase in detectable (by in situ hybridization) LH receptor mRNA in granulosa cells at 10.8 mm, and LH receptor mRNA increased nearly 2-fold as the follicle grew to 13.2 mm and another 2-fold as the follicle grew to 15.0 mm. Although not statistically significant, Bodensteiner et al., 1996 reported approximately 3-fold greater granulosa cell LH receptor numbers (measured by [125I]-hCG binding) from the largest follicle on Day 2 after ovulation (average of 8.5 mm) compared to the largest follicle on Day 4 after ovulation (average of 13.0 mm). In contrast, Evans and Fortune, 1997 reported no increase in detectable LH receptor mRNA (by in situ hybridization) in granulosa cells from follicles on Day 2 (~9 mm) compared to Day 3 (~11 mm; Day 0 = day of wave emergence); but reported that differences in estradiol concentrations between the dominant and largest subordinate follicle were already detectable on both Day 2 and Day 3. However, Jolly et al., 1994 measured the in vitro cAMP response to LH in granulosa cells from follicles of different sizes and found a clear increase in LH responsiveness as follicles grew to greater than 9-10 mm. We have recently completed a study in which we evaluated when the follicle acquired the ability to ovulate after an injection of LH. We found that follicles just after deviation (10 mm) had the ability to ovulate to a large dose of LH (40 mg). Follicles before deviation (7.0 or 8.5 mm) could not ovulate to this high dose of LH. Thus, at the time of follicular selection (~8.5 mm) there are a variety of measures that indicate that after follicular selection there is acquisition of LH responsiveness in the follicle (LH-induced ovulation) and LH receptors in the granulosa cells (cAMP production, LH binding, LH receptor mRNA).
D. Follicle Growth from Selection to Ovulatory Size.
Follicle growth past the time of deviation as well as post-deviation follicular estradiol production appear to be regulated by LH pulses. Gong et al., 1995 found that follicles failed to grow beyond ~9 mm in diameter (size of largest follicle at deviation) in cows in which LH pulses had been suppressed by chronic treatment with a GnRH agonist. Fike et al.,1997 used a more straightforward animal model by treating cows with a GnRH antagonist to reduce LH pulses. Cows treated on days 2-7 after estrus, i.e. during the first follicular wave, had the largest follicle grow to an average diameter of only 8.0 mm and persisted for only 6.3 d as compared to maximal diameter of 11.8 mm and persistence of 10.4 d in control heifers. There was also a reduction in circulating estradiol following inhibition of LH pulses (Fike et al., 1997). In a different study, inhibition of LH concentrations by treatment with progesterone did not alter the time or diameter characteristics at the time of follicular selection (Ginther et al., 2001). However, the growth rate of the developing dominant follicle was reduced when the follicle reached ~10 mm in diameter. Thus, follicle growth past deviation appears to require LH pulses and maximal size of the follicle may be decreased by reducing LH pulses.

There is additional evidence for the role of LH pulses in post-selection growth from examination of results from studies that increased LH pulses. A reduction in circulating progesterone concentrations was found to increase numbers of LH pulses and to prolong growth and increase maximal diameter of the dominant follicle. This persistent dominant follicle can also be induced by treatment with small exogenous LH pulses (Taft et al., 1996). Of possible physiological importance is the finding that a transient increase in mean circulating LH concentrations encompasses the expected time of follicular deviation, although this transient increase apparently is not required for diameter deviation (Ginther et al., 2001). Thus, maximal growth of the dominant follicle may be regulated by numbers of LH pulses.

E. Model of Follicular Growth.
We propose a follicular growth model based on a plethora of data that shows the importance of FSH and LH in follicular growth. A particularly interesting recent study (Crowe et al., 2001) used heifers that were immunized against GnRH and given either FSH (single bolus of 1.5 mg pFSH), LH pulses (150 µg pLH i.v. every 4 h), or both FSH + LH treatment. In heifers treated with LH alone, there was no growth of follicles to a diameter greater than 5 mm. Treatment with FSH alone caused follicle growth from 5 mm to 9.5 mm. Follicular growth past the expected time of follicular selection (to 10 mm or greater) occurred only in heifers treated with both FSH and LH. The small (< 5mm) and medium (5-9.5 mm) were not estrogen active (only 3 of 131 follicles with Estradiol:Progesterone > 1.0); whereas, almost all larger follicles (> 10 mm) were estrogen active (18 of 21 follicles all in FSH + LH group). Thus, follicle growth before follicular selection requires FSH, but after follicular selection, LH pulses are required. Figure 2 shows a simplified model for the growth of follicles from emergence until ovulation. The emergence of the follicular wave and growth until the time of follicular selection is primarily regulated by circulating FSH. The FSH concentrations are progressively inhibited until they reach a nadir at follicular selection. At this time, continued growth of the follicle and follicular estradiol production requires LH pulses. The dominant follicle continues growth until sufficient circulating estradiol is achieved to induce an LH surge and ovulation of the dominant follicle.
Figure 6. Simplified model for the relationship between the action of hormones and the growth of follicles from emergence until ovulation.

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HEIFER DEVELOPMENT AND REPRODUCTIVE TRACT SCORING FOR A SUCCESSFUL HEIFER PROGRAM: THE SHOW-ME-SELECT REPLACEMENT HEIFER PROGRAM, A COORDINATED MANAGEMENT CONCEPT

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Introduction and Program Description

The Missouri Show-Me-Select Replacement Heifer Program has developed a comprehensive set of guidelines for a beef replacement heifer development and marketing program. The program coordinates established management practices known to be beneficial to appropriate heifer development into a total quality management approach. The specific objectives of the program are:

- Improve existing developing programs through a Total Quality Management approach.
- Provide a reliable source of quality replacements through genetics and management.
- Increase marketing opportunities for and add value to Missouri-raised heifers.

The following description of this program can serve as a guide for others trying to develop or improve systems of heifer development.

Participants enrolled in the Show-Me-Select Replacement Heifer Program begin with a comprehensive health and vaccination program on their heifers starting at or before weaning. The health program is administered under the advice and guidance of the producer’s veterinarian to insure proper use of health products according to label directions. The health program is focused on maintaining good health and providing adequate protection against the major diseases that cause reproductive losses and reduced reproductive performance in cattle.

Pre-breeding examinations served as a monitor point to evaluate the post-weaning to pre-breeding phase of heifer development. These examinations were scheduled to take place when the average age of the heifers was 12 to 13 months (range 10-14 months). These examinations included reproductive tract scores (RTS), pelvic measurements, weights, and visual observations for structural soundness.

Pre-breeding examinations were scheduled between 30 and 60 days prior to the planned breeding season. University of Missouri State Extension specialists met with the producer’s veterinarian and regional extension livestock specialist to perform these examinations. Working together as a team insured all involved parties had the same appreciation and understanding of the technical procedures performed and that interpretation of the results would be uniform.
Each participating producer received individual and summary data on their herds from the pre-breeding examinations. These data were used to identify problems associated with heifer development to this point, and provide recommendations on the breeding program of the heifers.

Pregnancy examinations were scheduled on heifers from enrolled herds such that they were performed prior to 120 days of gestation in order to determine fetal age. Herds that used artificial insemination allowed a minimum of 2 weeks between the AI period and natural service cleanup. This permitted the examiner to distinguish AI bred heifers from natural serviced bred heifers.

Each producer received individual and summary data from the pregnancy examinations. These data included stage of gestation (in days) for each heifer and a projected calving date based on the observation. Producers utilizing synchronization and AI were provided with synchronization response and AI conception rates. The summary data included total pregnancy rates and pregnancy rates by 21-day intervals.

**Program Summary**

The program was initiated as a pilot project in two regions of Missouri in 1997. A programmatic effort to establish the program statewide was supported by a grant from the University of Missouri Outreach and Extension Outreach Development Fund in 1998. Four additional regions participated in the program in 1998 and another four regions participated in 1999. Since 1999, several regions of the state have coordinated a program for fall born heifers. Table 1 shows the number of participants in the Show-Me-Select program since 1997.

<table>
<thead>
<tr>
<th>Year of program</th>
<th>Number of herds</th>
<th>Number of heifers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>33</td>
<td>1,873</td>
</tr>
<tr>
<td>1998</td>
<td>123</td>
<td>5,189</td>
</tr>
<tr>
<td>1999</td>
<td>232</td>
<td>8,799</td>
</tr>
<tr>
<td>2000</td>
<td>186</td>
<td>8,038</td>
</tr>
<tr>
<td>2001</td>
<td>176</td>
<td>7,367</td>
</tr>
<tr>
<td>1999 Fall-Born Heifers</td>
<td>21</td>
<td>1,436</td>
</tr>
<tr>
<td>2000 Fall-Born Heifers</td>
<td>14</td>
<td>1,353</td>
</tr>
<tr>
<td>2001 Fall-Born Heifers</td>
<td>34</td>
<td>1,970</td>
</tr>
<tr>
<td>1997-2001</td>
<td>393*</td>
<td>36,025</td>
</tr>
</tbody>
</table>

*Number of producers participating in one or more years.

Table 2 shows the adoption rate of various management practices by herds enrolled in the Show-Me-Select Replacement Heifer program from 1997 to 2001 compared to the percent of operations utilizing these practices reported in the 1994 NAHMS survey.
Table 2. Adoption of management practices in beef replacement heifer development

<table>
<thead>
<tr>
<th>Management practice</th>
<th>Percent of herds enrolled</th>
<th>Percent of Operations (NAHMS, 1994)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed Program*</td>
<td>69%</td>
<td>N/A</td>
</tr>
<tr>
<td>Completed Records*</td>
<td>90%</td>
<td>N/A</td>
</tr>
<tr>
<td>Reproductive Tract Scores</td>
<td>100%</td>
<td>1.2%</td>
</tr>
<tr>
<td>Pelvic Measurements</td>
<td>100%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Pregnancy Examination</td>
<td>100%</td>
<td>15.9%</td>
</tr>
<tr>
<td>Weighed</td>
<td>87%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Artificial Insemination</td>
<td>71%</td>
<td>3.3%</td>
</tr>
<tr>
<td>Synchronized Estrus</td>
<td>79%</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

*Completed Program - Enrolled in program and provided data from pre-breeding, breeding season, and pregnancy examination.

*Completed Records – Submitted records completed such that data could be used in calculations and analyses.

Reproductive Summary

Table 3 summarizes the results of pregnancy examinations statewide and the relationship between reproductive tract scores and reproductive performance. The minimum criteria for heifers included in this analysis were a complete pre-breeding examination performed within 30 to 70 days prior to scheduled breeding, and complete pregnancy examination records.

Table 3. Reproductive performance by RTS

<table>
<thead>
<tr>
<th>RTS</th>
<th>Exposed</th>
<th>Pregnant</th>
<th>Open</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>46</td>
<td>29</td>
<td>61.3</td>
</tr>
<tr>
<td>2</td>
<td>1055</td>
<td>854</td>
<td>201</td>
<td>81.0</td>
</tr>
<tr>
<td>3</td>
<td>4504</td>
<td>3911</td>
<td>593</td>
<td>86.8</td>
</tr>
<tr>
<td>4</td>
<td>4912</td>
<td>4322</td>
<td>590</td>
<td>87.9</td>
</tr>
<tr>
<td>5</td>
<td>3675</td>
<td>3261</td>
<td>414</td>
<td>88.7</td>
</tr>
<tr>
<td>TOTALS</td>
<td>14221</td>
<td>12394</td>
<td>1827</td>
<td>87.2</td>
</tr>
</tbody>
</table>

Reproductive performance by cycle of breeding season

<table>
<thead>
<tr>
<th>RTS</th>
<th>Exposed</th>
<th>1st 21 days</th>
<th>2nd 21 days</th>
<th>3rd + 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>26</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>1055</td>
<td>505</td>
<td>48</td>
<td>185</td>
</tr>
<tr>
<td>3</td>
<td>4504</td>
<td>2443</td>
<td>54</td>
<td>842</td>
</tr>
<tr>
<td>4</td>
<td>4912</td>
<td>2875</td>
<td>59</td>
<td>855</td>
</tr>
<tr>
<td>5</td>
<td>3675</td>
<td>2269</td>
<td>62</td>
<td>586</td>
</tr>
<tr>
<td>TOTALS</td>
<td>14221</td>
<td>8118</td>
<td>57</td>
<td>2481</td>
</tr>
</tbody>
</table>

The reproductive performance of heifers with an RTS of 1 or 2 is less than that of heifers with an RTS of 3 or greater. These data indicate that heifers tract scoring 1 or 2 are less likely to be cycling at the beginning of the breeding season and therefore are less
likely to become pregnant or if they do become pregnant, they do so later in the breeding season. Current management recommendations advocate that heifers bred to calve as 2-yr-olds should be exposed for breeding before mature herd mates, and that early calving periods should be used as a means of increasing production efficiency. This practice often results in heifers being bred on their pubertal estrus (Wiltbank, 1970). Fertility of heifers that are bred at the pubertal estrus was 21% lower than those bred on their third estrus (Byerley et al., 1987). This means that heifers should reach puberty 1 to 3 months before the average age at which they are to be bred. Earlier age at puberty in relation to breeding is to ensure that a high percentage of heifers are cycling and that the effects of lowered potential fertility at the pubertal estrus are minimized (Short et al., 1990).

The reproductive summary from herds that utilized MGA/prostaglandin protocol for estrous synchronization and artificial insemination in their breeding program is presented in Table 4. The minimum criteria for heifers included in this analysis were a complete pre-breeding examination performed within 30 to 70 days prior to scheduled breeding, and complete synchronization and pregnancy examination records. These data are similar to those reported by Patterson and Bullock (1995).

<table>
<thead>
<tr>
<th>RTS</th>
<th>Exposed</th>
<th>Synchronization Response</th>
<th>Synchronized Pregnancy</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hd</td>
<td>%</td>
<td>Hd</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>14</td>
<td>37%</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>509</td>
<td>341</td>
<td>67%</td>
<td>194%</td>
</tr>
<tr>
<td>3</td>
<td>2475</td>
<td>1806</td>
<td>73%</td>
<td>1085%</td>
</tr>
<tr>
<td>4</td>
<td>3163</td>
<td>2530</td>
<td>80%</td>
<td>1544%</td>
</tr>
<tr>
<td>5</td>
<td>2417</td>
<td>2004</td>
<td>83%</td>
<td>1240%</td>
</tr>
<tr>
<td>TOTALS</td>
<td>8602</td>
<td>6695</td>
<td>78%</td>
<td>4073%</td>
</tr>
</tbody>
</table>

**Synchronization Response** – Total number of heifers with recorded heat within 7 days of the start of the breeding season. The percent is equal to synchronized/exposed.

**Synchronized Pregnancy** – Total number of heifers conceiving within synchronized period based on staged pregnancy diagnosis. The percent is equal to synchronized pregnancy/exposed.

The following tables summarize the herd reproductive performance from 1997 to 2001. The criteria for herds included in these analyses were a complete pre-breeding examination performed between 30 and 70 days prior to scheduled breeding with complete records.

Table 5 summarizes the reproductive performance in all herds irrespective of the type of breeding program utilized. These herds include natural service breeding as well as herds incorporating estrus synchronization and artificial insemination. There is a trend of improvement in weaning to pre-breeding development as evidenced by the increase in percent cycling and average pre-breeding weight by year. Pregnancy rates are not different between years and are lower than an anticipated pregnancy rate of 90 percent or better. The percent of animals becoming pregnant in the first cycle of the
breeding season are comparable to expected values, however the percent of animals becoming pregnant in the second and subsequent cycles are much lower than expected.

Table 5. Herd reproductive summary

<table>
<thead>
<tr>
<th>Year</th>
<th>Cycling %</th>
<th>Avg Wt kgs</th>
<th>PR %</th>
<th>PR 1st Cycle</th>
<th>PR 2nd Cycle</th>
<th>PR 3rd + Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>49</td>
<td>342</td>
<td>86</td>
<td>65</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td>1998</td>
<td>55</td>
<td>325</td>
<td>85</td>
<td>55</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>1999</td>
<td>59</td>
<td>326</td>
<td>86</td>
<td>54</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>2000</td>
<td>61</td>
<td>339</td>
<td>86</td>
<td>58</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>2001</td>
<td>65</td>
<td>339</td>
<td>87</td>
<td>57</td>
<td>42</td>
<td>43</td>
</tr>
</tbody>
</table>

Cycling – A recorded 4 or 5 RTS at pre-breeding examination
PR – Pregnancy rate

Table 6 summarizes the reproductive performance in herds incorporating MGA/prostaglandin protocol for estrus synchronization and artificial insemination. The same trend of improvement in weaning to pre-breeding development is seen as above evidenced by the increase in percent cycling and average pre-breeding weight by year. Estrus response to synchronization (ER) is expected to approach 80% given properly developed heifers capable of responding to synchronization and adequate heat detection. ER is defined in these herds as a recorded heat within the first 7 days of the breeding season. AI pregnancy rate (AI PR) in herds using synchronization and one insemination based on observed estrus is expected to approach 50%. The AI PR in these herds is defined as the number of heifers diagnosed as AI pregnant divided by the number of heifers exposed at the start of the breeding season. The AI conception rate (AI CR) is a measure of accurate heat detection and the inseminator’s success at AI. The AI CR is defined at the number of heifers conceiving to AI divided by the number of heifers inseminated. The number reported in these herds represents the first AI service only. The anticipated AI CR is 60%. Taken together, the ER, AI CR, and AI PR reported in these herds indicate successful synchronization and artificial insemination, however pregnancy rates (PR) are lower than an anticipated. This is due primarily to the fact that the percent of animals becoming pregnant in the second and subsequent cycles are much lower than expected.

Table 6. Herd reproductive summary from synchronized and AI’ed herds

<table>
<thead>
<tr>
<th>Year</th>
<th>Cycling %</th>
<th>Avg Wt kgs</th>
<th>ER %</th>
<th>AI PR %</th>
<th>AI CR %</th>
<th>PR %</th>
<th>% Preg 1st Cycle</th>
<th>% Preg 2nd Cycle</th>
<th>% Preg 3rd + Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>54</td>
<td>340</td>
<td>76</td>
<td>71</td>
<td>78</td>
<td>87</td>
<td>75</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>1998</td>
<td>56</td>
<td>327</td>
<td>74</td>
<td>54</td>
<td>62</td>
<td>84</td>
<td>58</td>
<td>37</td>
<td>41</td>
</tr>
<tr>
<td>1999</td>
<td>61</td>
<td>328</td>
<td>80</td>
<td>55</td>
<td>58</td>
<td>86</td>
<td>58</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>2000</td>
<td>64</td>
<td>340</td>
<td>78</td>
<td>60</td>
<td>62</td>
<td>87</td>
<td>63</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>2001</td>
<td>69</td>
<td>340</td>
<td>77</td>
<td>54</td>
<td>59</td>
<td>87</td>
<td>57</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

Cycling – Recorded 4 or 5 RTS at pre-breeding examination
ER – Estrus Response: No. of animals with recorded heat within first 7 days of breeding season
AI PR – AI Pregnancy Rate: No. of animals AI pregnant/No. of animals exposed for breeding
AI CR – AI Conception Rate: No. of animals AI pregnant/No. of animals inseminated
PR – Pregnancy Rate: No. of animals pregnant/No. of animals exposed for breeding
Table 7 stratifies herds using synchronization and artificial insemination into quartiles (bottom 25% to top 25%) based on pregnancy rates. The top 25% of the herds meet or exceed all expectations in terms of developing heifers to breeding and successfully incorporating synchronization and artificial insemination into their breeding programs. Only the bottom 25% fell short of anticipated outcomes. Final pregnancy rates differ primarily due to the differences in the number of heifers becoming pregnant in the second and subsequent cycles of the breeding season.

Table 7. Herd Reproductive Summary from Synchronized and AI’ed Herds by Quartile based on Preg Rate

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Cycling %</th>
<th>Avg Wt kgs</th>
<th>ER %</th>
<th>AI PR %</th>
<th>AI CR %</th>
<th>PR %</th>
<th>PR 1st cycle %</th>
<th>PR 2nd cycle %</th>
<th>PR 3rd+ cycle %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom 25%</td>
<td>61</td>
<td>325</td>
<td>73</td>
<td>49</td>
<td>54</td>
<td>72</td>
<td>51</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Mean</td>
<td>63</td>
<td>334</td>
<td>78</td>
<td>56</td>
<td>61</td>
<td>86</td>
<td>60</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Top 25%</td>
<td>65</td>
<td>341</td>
<td>79</td>
<td>63</td>
<td>68</td>
<td>97</td>
<td>68</td>
<td>56</td>
<td>68</td>
</tr>
</tbody>
</table>

Cycling – Recorded 4 or 5 RTS at pre-breeding examination
ER – Estrus Response: No. of animals with recorded heat within first 7 days of breeding season
AI PR – AI Pregnancy Rate: No. of animals AI pregnant/No. of animals exposed for breeding
AI CR – AI Conception Rate: No. of animals AI pregnant/No. of animals inseminated
PR – Pregnancy Rate: No. of animals pregnant/No. of animals exposed for breeding

Summary

Results from the Show-Me-Select Replacement heifer program indicate that the coordination of management practices known to be beneficial for beef replacement heifer development into a total quality management approach have been positive. Data collected can be effectively used to institute management changes necessary to improve development of beef replacement heifers and reproductive performance. The program provides a valuable source of information and education for beef producers and also indicates areas of needed research to refine certain parameters and procedures related to replacement female production.

Literature Cited


REVIEW OF ESTROUS SYNCHRONIZATION SYSTEMS: GnRH

G. Cliff Lamb
North Central Research and Outreach Center, University of Minnesota, Grand Rapids

Introduction

Synchronization of estrus contributes to optimizing the use of time, labor, and financial resources by shortening the calving season, in addition to increasing the uniformity of the calf crop. The major limitation of estrus-synchronization programs is their inability to induce a potentially fertile estrus and ovulation in noncycling cattle (i.e., prepubertal heifers and anestrous suckling cattle). Because initial estrus-synchronization programs were not designed for successful treatment of noncycling cattle, their use in cow-calf operations generally has not produced results that would encourage greater A.I. use in beef cattle. Currently, less than 7% of beef cows and an estimated 8 to 10% of beef heifers are A.I.-bred in the U.S. The potential for increasing A.I. in beef cattle is great if a system can resolve successfully the problem of the noncycling female at the beginning of the breeding season.

The premise behind synchronizing cows and heifers is to first control the timing of onset of estrus by controlling the length of the estrous cycle. The choice of approaches for controlling cycle length are: 1) to regress or “kill” the corpus luteum (CL) of the animal before the time of natural luteolysis, and thereby shorten the cycle (by administration of a prostaglandin F\textsubscript{2\alpha} [PGF\textsubscript{2\alpha}]), 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. A further approach is to “select” the ovulatory follicle by an injection of GnRH, which should cause premature ovulation of that follicle. Using these concepts, researchers have made tremendous strides in developing numerous systems to synchronize the estrous cycle for an A.I. after a detected estrous or a timed-A.I. Table 1 summarizes common products available for use in cattle estrus synchronization systems.

Table 1. Products, commercial names, and doses for synchronization products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Commercial name</th>
<th>Administration</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandins</td>
<td>Lutalyse®</td>
<td>i.m. injection</td>
<td>5 mL</td>
</tr>
<tr>
<td></td>
<td>Estrumate®</td>
<td>i.m. injection</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>In-Synch®</td>
<td>i.m. injection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prostamate®</td>
<td>i.m. injection</td>
<td></td>
</tr>
<tr>
<td>Progestins</td>
<td>Melengestrol Acetate</td>
<td>Feed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CIDR</td>
<td>Vaginal implant</td>
<td>0.5 mg/hd/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 implant</td>
</tr>
<tr>
<td>Gonadotropin Releasing Hormone</td>
<td>Cystorelin®</td>
<td>i.m. injection</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>Factrel®</td>
<td>i.m. injection</td>
<td>2 mL</td>
</tr>
<tr>
<td></td>
<td>Fertagyl®</td>
<td>i.m. injection</td>
<td>2 mL</td>
</tr>
</tbody>
</table>
Initial estrous synchronization systems focused on altering the estrous cycle by regressing the CL with an injection of PGF$_{2\alpha}$ followed by a detected estrus between 18 and 80 hours after the injection. After systems involving a single injection of PGF$_{2\alpha}$ became successful, researchers focused on multiple injections of PGF$_{2\alpha}$ to further reduce the days required for heat detection and AI (Lauderdale et al., 1974; Seguin et al., 1978). The next generation of estrous synchronization systems involved progestins, which (while administered) prevent estrus from occurring. Progestins were used to delay the time of estrus following a natural or induced luteolysis and extend the length of the estrous cycle. The use of progestins will be covered extensively by Dr. Dave Patterson (MGA) and Darrel Kesler (CIDR) and their written reports.

Until recently researchers primarily have focused on the timing of estrus; however, the availability of GnRH has given researchers an opportunity to aim their efforts at timing ovulation rather than the event of estrus alone. The obvious advantage is the development of time-A.I. protocols allowing cattlemen to inseminate cows that have no visible signs of estrus. These efforts should optimize the use of time, labor, and financial resources and allow more cattle to become pregnant to A.I. This paper will specifically cover ovulation synchronization protocols emphasizing the use of GnRH.

**GnRH Ovulation Synchronization Protocols**

![Diagram of GnRH protocols](image)

Figure 1. GnRH protocols frequently used for synchronizing beef cattle.
Refer to Figure 1 for GnRH protocol descriptions.

- **Select Synch.** Select Synch effectively initiates estrous cycles in postpartum cows that are not cycling. The duration of the system only requires one week from the start of synchronization until cows begin showing signs of estrus. As many as 5-20% of cows may exhibit estrus at least three days before the PGF$_{2\alpha}$ injection so insemination of these cows will improve overall response. For cows that are inseminated before the PGF$_{2\alpha}$ injection, there is no need to inject those cows with PGF$_{2\alpha}$ on day 0.

- **Ovsynch.** A protocol developed for lactating dairy cows that can be used with success in beef cattle operations. A drawback of Ovsynch is that cows are required to be processed through the working facility a minimum of four times; however, in smaller, more intensive cow/calf operations this protocol can be effective in obtaining excellent overall pregnancy rates.

- **CO-Synch.** In beef cattle operations that are fairly extensive, or would like to incorporate an AI program into their operation, and feel that labor and time associated with heat detection are limiting opportunities for AI use, then using the CO-Synch protocol is a good option. CO-Synch was modified from Ovsynch to reduce the total number of times the cows were to be processed. Even so, pregnancy rates around 50% can be consistently achieved in well-managed herds that use this system.

- **Hybrid Synch.** By using the benefits of Select Synch and CO-Synch, Hybrid Synch maximizes the opportunity for obtaining the greatest overall pregnancy rates. Detection of estrus for two to three days followed by a fixed-time A.I. should increase overall pregnancy rates. As with Select Synch, estrus detection at least three days before the PGF$_{2\alpha}$ injection and inseminating cows then will increase the heat detection rate by between 5 and 20%.

**Advantages and Disadvantages of GnRH Ovulation Synchronization Protocols**

**Cows**

Table 2 summarizes the conception and pregnancy rates of numerous reports evaluating GnRH based synchronization systems. Thompson et al. (1999) scanned the ovaries of 40 early postpartum, suckled beef cows before, during, and after treatments of GnRH and (or) norgestomet and reported that luteal structures were induced from dominant follicles in 75% of the noncycling cows treated, resulting in elevated progesterone after 7 d. In contrast, Stevenson et al. (2000) reported that the rates of induced ovulation for noncycling cows treated with Select Synch were 38% and 49% in two experiments.
<table>
<thead>
<tr>
<th>Reference and treatment description</th>
<th>No. of cows</th>
<th>Conception rate(^a), %</th>
<th>Pregnancy rate(^b), %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Geary et al., 1998</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovsynch + calf removal</td>
<td>220</td>
<td>-</td>
<td>119/220 (54)</td>
</tr>
<tr>
<td>Syncromate-B</td>
<td>216</td>
<td>-</td>
<td>91/216 (42)</td>
</tr>
<tr>
<td><strong>Stevenson et al., 2000</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Synch</td>
<td>289</td>
<td>115/175 (66)</td>
<td>115/289 (38)</td>
</tr>
<tr>
<td>Select Synch + Norgestomet</td>
<td>289</td>
<td>123/208 (59)</td>
<td>123/289 (42)</td>
</tr>
<tr>
<td>(2 \times \text{PGF}_2\alpha)</td>
<td>294</td>
<td>86/142 (61)</td>
<td>86/294 (28)</td>
</tr>
<tr>
<td>Exp. 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Synch</td>
<td>184</td>
<td>80/115 (70)</td>
<td>80/184 (44)</td>
</tr>
<tr>
<td>CO-Synch</td>
<td>175</td>
<td>-</td>
<td>58/175 (33)</td>
</tr>
<tr>
<td>Hybrid Synch</td>
<td>177</td>
<td>60/177 (34)</td>
<td>60/184 (34)</td>
</tr>
<tr>
<td><strong>Dejarnette et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Synch (6 day interval)</td>
<td>24</td>
<td>17/22 (77)</td>
<td>17/24 (71)</td>
</tr>
<tr>
<td>Select Synch (7 day interval)</td>
<td>27</td>
<td>19/25 (76)</td>
<td>19/27 (70)</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Synch</td>
<td>77</td>
<td>40/60 (67)</td>
<td>40/77 (52)</td>
</tr>
<tr>
<td>Select Synch + MGA from d -7 to -1</td>
<td>73</td>
<td>43/61 (72)</td>
<td>43/73 (60)</td>
</tr>
<tr>
<td><strong>Dejarnette et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid Synch (72h)</td>
<td>45</td>
<td>15/20 (75)</td>
<td>20/45 (71)</td>
</tr>
<tr>
<td>Hybrid Synch (72h)+ GnRH on d-16</td>
<td>42</td>
<td>19/24 (79)</td>
<td>23/42 (55)</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid Synch (72h)</td>
<td>638</td>
<td>278/467 (60)</td>
<td>299/632 (47)</td>
</tr>
<tr>
<td>Hybrid Synch (72h)+ GnRH on d-16</td>
<td>638</td>
<td>287/447 (64)</td>
<td>333/634 (53)</td>
</tr>
<tr>
<td><strong>Greiger et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Synch + horn bred</td>
<td>118</td>
<td>47/64 (73)</td>
<td>47/118 (40)</td>
</tr>
<tr>
<td>Select Synch + body bred</td>
<td>119</td>
<td>46/66 (70)</td>
<td>46/119 (39)</td>
</tr>
<tr>
<td>CO-Synch + horn bred</td>
<td>108</td>
<td>-</td>
<td>45/108 (42)</td>
</tr>
<tr>
<td>CO-Synch + body bred</td>
<td>115</td>
<td>-</td>
<td>61/115 (53)</td>
</tr>
<tr>
<td><strong>Geary et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovsynch</td>
<td>123</td>
<td>-</td>
<td>64/123 (52)</td>
</tr>
<tr>
<td>Ovsynch + calf removal</td>
<td>114</td>
<td>-</td>
<td>70/114 (61)</td>
</tr>
<tr>
<td>CO-Synch</td>
<td>117</td>
<td>-</td>
<td>63/117 (54)</td>
</tr>
<tr>
<td>CO-Synch + calf removal</td>
<td>119</td>
<td>-</td>
<td>75/119 (63)</td>
</tr>
<tr>
<td><strong>Geary et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO-Synch (GnRH)</td>
<td>117</td>
<td>-</td>
<td>57/117 (49)</td>
</tr>
<tr>
<td>CO-Synch (GnRH) + calf removal</td>
<td>121</td>
<td>-</td>
<td>56/121 (46)</td>
</tr>
<tr>
<td>CO-Synch (hCG)</td>
<td>114</td>
<td>-</td>
<td>39/114 (34)</td>
</tr>
<tr>
<td>CO-Synch (hCG) + calf removal</td>
<td>115</td>
<td>-</td>
<td>40/115 (35)</td>
</tr>
<tr>
<td><strong>Lamb et al., 2001</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO-Synch</td>
<td>287</td>
<td>-</td>
<td>138/287 (48)</td>
</tr>
<tr>
<td>CO-Synch + CIDR from d –7 to 0</td>
<td>273</td>
<td>-</td>
<td>160/273 (59)</td>
</tr>
</tbody>
</table>

\(^a\) Percentage of cows pregnant exposed to AI.

\(^b\) Percentage of cows pregnant of all cows treated.
Response of ovaries to GnRH is dependent on the stage of follicular growth that GnRH is administered (Geary et al., 2000). A high percentage of cows during the later stages of their estrous cycle (d 15 to 17) failed to ovulate a follicle after administration of GnRH and exhibited estrus prior to an injection of PGF$_{2\alpha}$ (Geary et al., 2000). In addition, Moreira et al. (2000) observed that day of the estrous cycle in which the Ovsynch protocol was initiated in dairy heifers affected dynamics of follicular development, plasma progesterone profiles, and occurrence of premature ovulation. We (Lamb et al., 2001) determined that when treatments were initiated (d -7), 99 of 333 cows (29.7%) considered to be cycling subsequently had low concentrations of progesterone on d 0. In this class of cows treated by Cosynch, 43.3% (26/60) were pregnant after AI.

Of the estrus or ovulation synchronization protocols currently used for suckled beef cows, CO-Synch tends to be more cost effective and less labor intensive than other timed-AI synchronization protocols (Twagiramungu et al., 1995; Geary et al., 2001; Kojima et al., 2000). A disadvantage of this protocol is that approximately 10 to 20% of suckled beef cows exhibit estrus prior to and immediately after the PGF$_{2\alpha}$ injection. Unless these cows are detected in estrus and inseminated, they will fail to become pregnant after the CO-Synch protocol.

**Figure 2.** Conception rates in suckled beef cows treated with CO-Synch or Ovsynch (Geary et al., 2001).
Comparison of the CO-Synch and CO-Synch with a CIDR insert from day –7 to day 0 treatments indicated that addition of a CIDR for progesterone supplementation improved pregnancy rates after a fixed time AI (Lamb et al., 2001). But progesterone did not seem to improve pregnancy rates in suckled beef cows cycling at the initiation of treatments. Progesterone seemed to be more effective in at enhancing pregnancy rates in cows that were cycling but in the later stages of the estrous cycle at first injection of GnRH and subsequently no luteal structure at the PGF$_{2\alpha}$ injection or in noncycling cows. Along with parity, days postpartum, calf removal, and cow condition (Figure 2, Table 3) our previous report (Lamb et al., 2001) also indicated that location variables, which could include differences in pasture and diet, breed composition, body condition, postpartum interval, and geographic location, may affect the success of fixed-time AI protocols. Therefore, a sound strategy for utilizing a GnRH protocol in the absence of progesterone may be to select cows that calved earlier in the calving season that tend to be in good body condition. A high percentage of these cows should be cycling, resulting in acceptable fertility rates.

Reports by Dr. Dave Patterson and Darrel Kesler will address additional GnRH protocols that also utilize a progestin for enhancing fertility rates.

Table 3. Pregnancy rates in suckled beef cows after treatment with CO-Synch, Select Synch, and 2 × PGF$_{2\alpha}$.

<table>
<thead>
<tr>
<th></th>
<th>Lamb et al., 2001</th>
<th>Stevenson et al., 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cosynch</td>
<td>Select Synch</td>
</tr>
<tr>
<td><strong>Body condition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 4.5</td>
<td>12/40 (30)</td>
<td>14/50 (28)</td>
</tr>
<tr>
<td>4.5 to 5.5</td>
<td>30/74 (41)</td>
<td>19/49 (39)</td>
</tr>
<tr>
<td>≥ 5.5</td>
<td>19/32 (59)</td>
<td>38/76 (50)</td>
</tr>
<tr>
<td><strong>Days postpartum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 50</td>
<td>23/60 (38)</td>
<td>12/52 (23)</td>
</tr>
<tr>
<td>51-60</td>
<td>25/62 (47)</td>
<td>31/67 (46)</td>
</tr>
<tr>
<td>61-70</td>
<td>28/49 (62)</td>
<td>42/106 (40)</td>
</tr>
<tr>
<td>71-80</td>
<td>18/41 (44)</td>
<td>31/70 (44)</td>
</tr>
<tr>
<td>&gt; 80</td>
<td>44/75 (59)</td>
<td></td>
</tr>
</tbody>
</table>

Advantages and Disadvantages of GnRH Ovulation Synchronization Protocols

**Heifers**

Heifers are an easier group of females to synchronize within a beef herd. Because heifers are not nursing calves and can be maintained in areas where they can be fed they have responded extremely well to the MGA/PGF$_{2\alpha}$ system (Wood et al., 2001; Brown et al., 1988; Lamb, et al., 2000). In addition, MGA delivered in feed has the ability to induce puberty in some peripubertal heifers (Patterson et al., 1992). However, the length of time to apply this system (31 to 33 d) is a drawback. During a late spring/early
summer breeding season, MGA must be delivered in a grain carrier when cattle tend to be grazing forage pastures. Thus, the challenge is to ensure that each heifer receives the required MGA dose. Therefore, producers could benefit from an alternative estrous synchronization system that eliminates the use of MGA.

**Table 4.** Occurrence of Estrus Before, During, and After the Target Breeding Week (days 0 to 7; day 0 = PGF$_{2\alpha}$; Stevenson et al., 1999).

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>2×PGF$_{2\alpha}$</th>
<th>MGA+PGF$_{2\alpha}$</th>
<th>Select Synch</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td></td>
<td>139</td>
<td>289</td>
<td>160</td>
</tr>
<tr>
<td>Before: days -5 to -1</td>
<td></td>
<td>8.6 ( 12)</td>
<td>5.9 ( 17)</td>
<td>12.5 ( 20)</td>
</tr>
<tr>
<td>During: days 0 to 7</td>
<td></td>
<td>74.8 (104)</td>
<td>82.0 (237)</td>
<td>72.5 (116)</td>
</tr>
<tr>
<td>(Average days to estrus)</td>
<td></td>
<td>(3.0 ± .1)</td>
<td>(3.2 ± .1)</td>
<td>(2.3 ± .1)</td>
</tr>
<tr>
<td>After: &gt;day 7</td>
<td></td>
<td>8.6 ( 12)</td>
<td>8.3 ( 24)</td>
<td>8.7 ( 14)</td>
</tr>
<tr>
<td>No estrus</td>
<td></td>
<td>7.9 ( 11)</td>
<td>3.8 ( 11)</td>
<td>6.2 ( 10)</td>
</tr>
</tbody>
</table>

More recently researchers have incorporated gonadotropin-releasing hormone into (GnRH) estrus synchronization systems, which can induce preovulatory LH surges in prepubertal heifers (Skaggs et al., 1986) and consistently induce ovulation of large follicles ($\geq$ 10 mm) present at the time of injection (Thompson et al., 1999; Wood et al., 2001). The majority of these systems have relied on visual detection of estrus for suitable results (Cassady et al., 1999; Stevenson et al., 1999). In most cases heifers have failed to achieve the fertility rates in a GnRH protocol that equals the fertility of the standard MGA/PGF system. In addition, synchrony of estrus after PGF$_{2\alpha}$ in an MGA system tends to be tighter with more heifers being artificially inseminated during a shorter period of time than when using a GnRH protocol (Table 4; Stevenson et al., 1999; Funston et al., 2002). Nonetheless, fertility in heifers that are estrus detected and inseminated after a detected estrus does not appear to be compromised over a normal 2× PGF$_{2\alpha}$ system (Table 5), whereas heifers inseminated after a fixed time with or without an additional injection of GnRH before the CO-Synch protocol appears to have improved fertility over a 2× PGF$_{2\alpha}$ system, especially in heifers with poorly developed reproductive tracts (Table 6; Dahlen et al., 2001).
Table 5. Rates of Estrus, Conception, and Pregnancy for Heifers Detected during the Target Breeding Week (days 0 to 7; day 0 = PGF$_{2\alpha}$; Stevenson, et al., 1999).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Item</th>
<th>2 × PGF$_{2\alpha}$</th>
<th>MGA+PGF$_{2\alpha}$</th>
<th>Select Synch</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td></td>
<td>139</td>
<td>289</td>
<td>160</td>
</tr>
<tr>
<td>Estrus detection$^2$, %</td>
<td></td>
<td>74.8</td>
<td>82.0</td>
<td>72.5</td>
</tr>
<tr>
<td>Conception rate$^3$, %</td>
<td></td>
<td>69.2</td>
<td>68.2</td>
<td>63.8</td>
</tr>
<tr>
<td>Pregnancy rate$^4$, %</td>
<td></td>
<td>51.8</td>
<td>56.0</td>
<td>46.2</td>
</tr>
</tbody>
</table>

1 Percent of heifers expressing estrus of all heifers synchronized
2 Percent of heifers conceiving of heifers inseminated
3 Percent of heifers pregnant of all heifers synchronized

Table 6. Synchronized Pregnancy rates of heifers synchronized with prostaglandin F$_{2\alpha}$ and GnRH (Dahlen et al., 2001)

<table>
<thead>
<tr>
<th>Reproductive tract score</th>
<th>Treatment</th>
<th>2 × PGF$_{2\alpha}$ (d –12 and 0)</th>
<th>CO-Synch (GnRH on d –6)</th>
<th>CO-Synch (GnRH on d –12 and –6)</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%)</td>
<td>no. (%)</td>
</tr>
<tr>
<td>2</td>
<td>0/53 (0)</td>
<td>6/55 (11)</td>
<td>9/53 (17)</td>
<td>15/161 (9)$^p$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12/72 (17)</td>
<td>12/71 (17)</td>
<td>21/73 (29)</td>
<td>45/216 (21)$^q$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6/38 (16)</td>
<td>17/38 (45)</td>
<td>12/35 (34)</td>
<td>35/111 (32)$^q$</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4/10 (40)</td>
<td>3/8 (38)</td>
<td>1/8 (13)</td>
<td>8/26 (31)$^{pq}$</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22/173 (13)$^x$</td>
<td>38/172 (22)$^y$</td>
<td>43/169 (25)$^y$</td>
<td>103/514 (20)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ RTS = Reproductive tract scores.
$^p,q$ Percentages within a item and column lacking a common superscript differ (P < 0.01).
$^x,y$ Percentages within a row lacking a common superscript differ (P < 0.05).

Implications

Most beef herds in the mid-western United States initiate the breeding season with between 25 and 70% of their cows in an anestrus state. For most AI programs to work, a sound nutrition program is essential to ensure a high percentage of cows are cycling. Synchronization does decrease the labor associated with AI programs; additional benefits include a shorter breeding season and consequently a shorter calving season. In essence, the value of progeny is greater due to a more uniform calf crop and more older calves at weaning.
To achieve optimal pregnancy rates with a GnRH synchronization protocol, cows should be in good body condition (BCS ≥5) and treatments should be initiated only when cows are at least 50 days postpartum. Treatment of suckled cows with GnRH 7 d before an injection of PGF$_{2\alpha}$ partially resolved the problem of anestrus before the beginning of the breeding season that cannot be resolved with PGF$_{2\alpha}$ systems alone.

**Literature Cited**


Introduction

The beef cattle industry has seen rapid gains in economically desirable traits largely due to the selection and expanded use of genetically superior sires made available through artificial insemination (AI). Recent surveys indicate, however, that less than 5 percent of the beef cows in the United States are bred by AI, and only half of the cattle producers who practice AI use any form of estrus synchronization to facilitate their AI programs. The inability to predict time of estrus for individual cows or heifers in a group often makes it impractical to use AI because of the labor required for detection of estrus. Available procedures to control the estrous cycle of the cow can improve reproductive rates and speed up genetic progress. These procedures include synchronization of estrus in cycling females, and induction of estrus accompanied by ovulation in heifers that have not yet reached puberty or among cows that have not returned to estrus after calving.

The following protocols and terms will be referred to throughout this manuscript.

**Protocols:**

- **PG**: Prostaglandin F$_{2\alpha}$ (PG; Lutalyse, Estrumate, ProstaMate, InSynch).
- **MGA-PG**: Melengestrol acetate (MGA; 0.5 mg/hd/day) is fed for a period of 14 days with PG administered 17 to 19 days after MGA withdrawal.
- **GnRH-PG (Select Synch)**: Gonadotropin-releasing hormone injection (Cystorelin, Factrel, Fertagyl) followed in 7 days with an injection of PG.
- **MGA-GnRH-PG (MGA Select)**: MGA is fed for 14 days, GnRH is administered 10 or 12 days after MGA withdrawal, and PG is administered 7 days after GnRH.
- **7-11 Synch**: MGA is fed for 7 days, PG is administered on the last day MGA is fed, GnRH is administered 4 days after the cessation of MGA, and a second injection of PG is administered 11 days after MGA withdrawal.

**Terms:**

- **Estrus response**: The number of females that exhibit estrus during a synchronized period.
- **Synchronized period**: The period of time during which estrus is expressed after treatment.
- **Synchronized conception rate**: The proportion of females that become pregnant of those exhibiting estrus and inseminated during the synchronized period.
- **Synchronized pregnancy rate**: Proportion of females that become pregnant of the total number treated.

There are several advantages to a successful estrus synchronization program. These include: 1) Cows or heifers are in estrus during a predictable interval, which allows for artificial insemination, embryo transfer or other planned reproductive techniques; 2)
The time required to detect estrus is reduced, which in turn decreases labor expense associated with the breeding program; 3) Cattle will conceive earlier during the breeding period; and 4) Calves will be older and weigh more at weaning.

To avoid problems when using estrus synchronization, females should be selected for a program when the following conditions are met: 1) Adequate time has elapsed from calving and the time synchronization treatments are implemented (a minimum of 40 days postpartum at the beginning of treatment is suggested); 2) Cows are in average or above-average body condition (scores of at least 5 on a scale of 1 to 9); 3) Cows experience minimal calving problems; 4) Replacement heifers are developed to prebreeding target weights that represent at least 65 percent of their projected mature weight; and 5) Reproductive tract scores (RTS) are assigned to heifers no more than two weeks before a synchronization treatment begins (scores of 3 or higher on a scale of 1 to 5) and at least 50 percent of the heifers are assigned a RTS of 4 or 5 (Patterson et al., 2000a).

Development of Methods to Synchronize Estrus

The development of methods to control the estrous cycle of the cow has occurred in five distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited preovulatory follicular maturation and ovulation. Regulation of estrous cycles was believed to be associated with control of the corpus luteum, whose life span and secretory activity are regulated by trophic and lytic mechanisms. Phase I included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in Phase II; whereas Phase III involved prostaglandin F2α (PG) and its analogs as luteolytic agents. Treatments that combined progestational agents with PG characterized Phase IV.

Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the change that occurs during a follicular wave. Growth of follicles in cattle occurs in distinct wave-like patterns, with new follicular waves occurring approximately every 10 days (6-15 day range). We now know (Phase V) that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan.

A single injection of gonadotropin-releasing hormone (GnRH) to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronized ovulation or luteinization of most large dominant follicles. Consequently, a new follicular wave is initiated in all cows within 2 to 3 days of GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 days later (Twagiramungu et al., 1995). This method will be referred to as the GnRH-PG protocol throughout this manuscript. The GnRH-PG protocol increased estrus synchronization rate in beef (Twagiramungu et al., 1992a,b) and dairy (Thatcher et al., 1993) cattle. A drawback of this method is that approximately 5 to 15% of the cows are detected in estrus on or before the day of PG injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period (Kojima et al., 2000).
Synchronization of estrus and ovulation with the GnRH-PG-GnRH protocol.

Administration of PG alone is commonly utilized to synchronize an ovulatory estrus in cycling cows. However, this method is ineffective in anestrous females and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of estrus during the synchronized period (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). Consequently, the GnRH-PG-GnRH protocol was developed to synchronize follicular waves and timing of ovulation. The GnRH-PG-GnRH protocol for fixed-time AI results in development of a preovulatory follicle that ovulates in response to a second GnRH-induced LH surge 48 hours after PG injection. Addition of a GnRH injection 48 hours after PG has been given the trademark, Ovsynch (Pursely et al., 1995). Ovsynch was validated recently as a reliable means of synchronizing ovulation for fixed-time AI in lactating dairy cows. Time of ovulation with Ovsynch occurs between 24 to 32 hours after the second GnRH injection and is synchronized in 87 to 100% of lactating dairy cows. Pregnancy rates among cows that were inseminated at a fixed time following Ovsynch ranged from 32 to 45%, rates comparable to controls. The Ovsynch protocol, however, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PG controls).

Recently, variations of the Ovsynch protocol (CO-Synch and Select Synch) were tested in postpartum beef cows (Figure 1). It is important to understand that treatment variations of Ovsynch currently being used in postpartum beef cows have not undergone the same validation process that Ovsynch underwent in lactating dairy cows. At this point we do not know whether response in postpartum beef cows to the protocols outlined in Figure 1 is the same or different from lactating dairy cows due to potential differences in follicular wave patterns. Differences in specific response variables may include: a) the relative length of time to ovulation from the second GnRH injection; b) the anticipated range in timing of ovulation; and c) the degree of ovulation synchrony that occurs.

Two variations from Ovsynch being used most extensively in postpartum beef cows are currently referred to as CO-Synch and Select Synch. CO-Synch (Geary et al., 1998) is similar to Ovsynch in that timing and sequence of injections are the same and all cows are inseminated at a fixed time. CO-Synch differs from Ovsynch, however, in that cows are inseminated when the second GnRH injection is administered, compared to the recommended 16 hours after GnRH for Ovsynch treated cows. Select Synch (Downing
et al., 1998) differs too, in that cows do not receive the second injection of GnRH and are not inseminated at a fixed time. Cows synchronized with this protocol are inseminated 12 hours after detected estrus. It is currently recommended for Select Synch treated cows that detection of estrus begin as early as day 4 after GnRH injection and continue through day 5 after PG (Kojima et al., 2000). Select Synch, similar to Ovsynch, was less effective than the melengestrol acetate (MGA)-PG protocol in synchronizing estrus in beef heifers (Stevenson et al., 1999).

The MGA Program

This manuscript reviews recently developed methods using MGA to control estrous cycles of cows or heifers in breeding programs involving natural service or artificial insemination. Four methods will be outlined for using the melengestrol acetate (MGA®Premix, Pharmacia Animal Health, Kalamazoo, Mich.) program to facilitate estrus synchronization in heifers or cows. The choice of which system to use depends largely on a producer’s goals. Melengestrol acetate is the common denominator in each of the systems presented here. MGA is an orally active progestin. When consumed by cows or heifers on a daily basis, MGA will suppress estrus and prevent ovulation. MGA may be fed with a grain or a protein carrier and either top-dressed onto other feed or batch mixed with larger quantities of feed. MGA is fed at a rate of 0.5 mg/animal/day.

The duration of feeding may vary between protocols, but the level of feeding is consistent and critical to success. Animals that fail to consume the required amount of MGA on a daily basis may prematurely return to estrus during the feeding period. This can be expected to reduce the synchronization response. Therefore, adequate bunk space must be available so that all animals consume feed simultaneously.

Animals should be observed for behavioral signs of estrus each day of the feeding period. This may be done as animals approach the feeding area and before feed distribution. This practice will ensure that all females receive adequate intake. Cows and heifers will exhibit estrus beginning 48 hours after MGA withdrawal, and this will continue for 6 to 7 days. It is generally recommended that females exhibiting estrus during this period not be inseminated or exposed to natural service because of the reduced fertility females experience at the first heat after MGA withdrawal.

Method 1: MGA with Natural Service

The simplest method involves using bulls to breed synchronized groups of females. This practice is especially useful in helping producers make a transition from natural service to artificial insemination. In this process, cows or heifers receive the normal 14-day feeding period of MGA and are then exposed to fertile bulls about 10 days after MGA withdrawal (Figure 2).

![Figure 2. MGA and natural service (adapted from Patterson et al., 2000b).](image-url)
This system works effectively, however, careful attention to bull to female ratios should be observed. It is recommended that 15 to 20 synchronized females be exposed per bull. Age and breeding condition of the bull and results of breeding soundness examinations should be considered carefully.

**Method 2: MGA + Prostaglandin**

A more precise means of estrous cycle control involves the combination of MGA with prostaglandin F$_{2\alpha}$ (PG). Prostaglandin F$_{2\alpha}$ (PG) is a luteolytic compound normally secreted by the uterus of the cow. Prostaglandin F$_{2\alpha}$ can induce luteal regression but cannot inhibit ovulation. When PG is administered in the presence of a functional corpus luteum (CL) during days 6 to 16 of the estrous cycle, premature regression of the CL begins and the cow returns to estrus.

In this program, prostaglandin should be administered 19 days after the last day of MGA feeding. This treatment places all animals in the late luteal stage of the estrous cycle at the time of injection, which shortens the synchronized period and maximizes conception rate (Figure 2). Although a 19-day interval is optimal, 17- to 19-day intervals produce acceptable results and provide flexibility for extenuating circumstances (Brown et al., 1988; Lamb et al., 2000). Four available PG products for synchronization of estrus in cattle can be used after the MGA treatment: Lutalyse®, ProstaMate®, InSynch®, or Estrumate®. Label-approved dosages differ with each of these products; carefully read and follow directions for proper administration before their use.

**Figure 2.** The MGA-PG protocol (adapted from Brown et al., 1988; Lamb et al., 2000).

Figure 3 (Patterson et al., 2000b) illustrates the distribution of estrus comparing the MGA-PG system to an MGA-only system. The combined MGA-PG system is best suited for use with AI programs because of the high degree of synchrony that can be achieved, which decreases the amount of time required for detection of estrus. Under natural mating conditions there may be an advantage to distribute estrus over several additional days to prevent overworking of bulls used in these programs.

**Figure 3.** Distribution of estrus comparing the MGA-PG system to an MGA-only system (adapted from Patterson et al., 2000b).
Table 1 provides a summary of field trials involving heifers where MGA was used in conjunction with natural service or MGA-PG was used prior to AI (Patterson et al., 2000b). One of the major advantages in using MGA to control estrous cycles of cattle, as seen from the data presented in Table 1, is the flexibility in matching specific synchronization protocols with the particular management system involved.

<table>
<thead>
<tr>
<th>Breeding program</th>
<th>Number of heifers</th>
<th>Estrus response No.</th>
<th>Estrus response %</th>
<th>Synchronized conception rate No.</th>
<th>Synchronized conception rate %</th>
<th>Synchronized pregnancy rate No.</th>
<th>Synchronized pregnancy rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural service</td>
<td>1749</td>
<td>--------</td>
<td>------</td>
<td>1151/1749</td>
<td>66</td>
<td>1151/1749</td>
<td>66</td>
</tr>
<tr>
<td>AI</td>
<td>4245</td>
<td>3354/4245</td>
<td>79</td>
<td>2414/3354</td>
<td>72</td>
<td>2414/4245</td>
<td>57</td>
</tr>
</tbody>
</table>

**Method 3: MGA® Select**

The MGA® Select treatment (Wood et al., 2001; Figure 4) is useful in maximizing estrus response and reproductive performance in postpartum beef cows. The MGA® Select protocol is a simple program that involves feeding MGA for 14 days followed by an injection of GnRH (Cystorelin®, Factrel®, or Fertagyl®) on day 26 and an injection of PG on day 33. The addition of GnRH to the 14-19 day MGA-PG protocol improves synchrony of estrus, while maintaining high fertility in postpartum beef cows.

![Figure 4. The MGA® Select protocol. MGA is fed for a period of 14 days followed in 12 days (day 26) by an injection of GnRH, and PG 19 days after MGA withdrawal (day 33).](image)

We conducted experiments during the spring 2000 and 2001 breeding season to compare the 14-19 day MGA-PG protocol with or without the addition of GnRH on day 12 after MGA withdrawal and 7 days prior to PG in postpartum suckled beef cows (Patterson et al., 2001; Figure 5). These experiments were conducted at the University of Missouri Thompson Farm at Spickard, MO.

![Figure 5. Cows were fed MGA for 14 days; 19 days after MGA withdrawal PG was administered to all cows. GnRH was administered to ½ of the cows 7 days prior to PG (Patterson et al., 2001).](image)
The following tables provide a summary of the results from the study conducted during the 2001 breeding season. Table 2 provides a summary of the number of cows within age group by treatment, the average number of days postpartum and body condition score on the first day of MGA feeding, and the percentage of cows that were cycling prior to the time treatment with MGA began. Cyclicity status was determined based on two blood samples for progesterone obtained 10 days before and on the first day of MGA.

### Table 2. Number of cows within age group per treatment, days postpartum, body condition and cyclicity status at the time treatment with MGA began (Patterson et al., unpublished data).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age group (yrs)</th>
<th>No. of cows</th>
<th>Days postpartum</th>
<th>Body condition score</th>
<th>Cycling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-PG</td>
<td>2, 3 &amp; 4</td>
<td>52</td>
<td>47</td>
<td>5.2</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>48</td>
<td>39</td>
<td>5.2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>100</td>
<td>44</td>
<td>5.2</td>
<td>40</td>
</tr>
<tr>
<td>MGA-GnRH-PG</td>
<td>2, 3 &amp; 4</td>
<td>53</td>
<td>47</td>
<td>5.3</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>48</td>
<td>40</td>
<td>5.3</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>101</td>
<td>44</td>
<td>5.3</td>
<td>53</td>
</tr>
</tbody>
</table>

1Average number of days postpartum on the day treatment with MGA began. Body condition scores were assigned one day prior to the day treatment with MGA was initiated using a scale 1 = emaciated to 9 = obese. Cyclicity was determined from 2 blood samples for progesterone obtained 10 days and 1 day prior to the day treatment with MGA was initiated.

Table 3 provides a summary of estrus response, synchronized conception and pregnancy, and final pregnancy rates for cows assigned to the two treatments. Estrus response was significantly higher among MGA Select treated cows compared with the MGA-PG treated cows. Synchronized pregnancy rates were higher among the 5-year-old and older cows assigned to the MGA Select treatment.

### Table 3. Estrus response, synchronized conception and pregnancy rate, and final pregnancy rate at the end of the breeding period (Patterson et al., 2001). a,b Percentages within column and category with unlike superscripts are different (P<.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age group (yrs)</th>
<th>Estrus response (no.) (%)</th>
<th>Synchronized conception rate (no.) (%)</th>
<th>Synchronized pregnancy rate (no.) (%)</th>
<th>Final pregnancy (no.) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-PG</td>
<td>2, 3 &amp; 4</td>
<td>44/52 85</td>
<td>36/44 82</td>
<td>36/52 69</td>
<td>49/52 94</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>32/48 67</td>
<td>22/32 69</td>
<td>22/48 46 a</td>
<td>48/48 100</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>76/100 76 b</td>
<td>58/76 76</td>
<td>58/100 58</td>
<td>97/100 97</td>
</tr>
<tr>
<td>MGA-GnRH-PG</td>
<td>2, 3 &amp; 4</td>
<td>46/53 87</td>
<td>33/46 72</td>
<td>33/53 62</td>
<td>51/53 96</td>
</tr>
<tr>
<td></td>
<td>5+</td>
<td>42/48 88</td>
<td>34/42 81</td>
<td>34/48 71 b</td>
<td>47/48 98</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>88/101 87 b</td>
<td>67/88 76</td>
<td>67/101 66</td>
<td>98/101 97</td>
</tr>
</tbody>
</table>

The objective of a second experiment during the spring 2000 breeding season was to determine if MGA pretreatment could improve conception rates following a GnRH-PG-GnRH protocol (Perry et al., 2001). Cows from two University of Missouri herds
[Greenley Farm (n= 90); South Farm (n=137)] were assigned by age and days postpartum to one of two treatments. Control and MGA-treated (Figure 6) cows were fed a supplement carrier with or without MGA for 14 days. GnRH was administered to all cows 12 days after MGA or carrier withdrawal and 7 days prior to PG. All animals were administered GnRH and artificially inseminated 72 hours after PG.

Pregnancy rates to fixed-time AI were determined 50 days after insemination (Table 4). There was no difference between treatments at location 1 [MGA = 58% (26/45); Control = 51% (23/45)]. However, there was a difference (P<.03) in pregnancy rate to fixed-time AI between treatments at location 2 [MGA=63% (44/70); Control = 45% (30/67)]. Furthermore, when the data from both locations was combined the overall difference remained significant [MGA=70/115 (61%); Control=53/112 (47%); P<.05]. These data indicate that pregnancy rates resulting from fixed-time insemination are improved significantly when treatment with MGA precedes the GnRH-PG-GnRH protocol.

### Table 4. Fixed-time AI and final pregnancy rates of MGA-treated and Control cows (Perry et al., 2001.)

<table>
<thead>
<tr>
<th>Item</th>
<th>Location 1 No.</th>
<th>Location 1 %</th>
<th>Location 2 No.</th>
<th>Location 2 %</th>
<th>Total No.</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate to fixed-time AI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGA-treated</td>
<td>26/45</td>
<td>58%</td>
<td>44/70</td>
<td>63%</td>
<td>70/115</td>
<td>61%</td>
</tr>
<tr>
<td>Control</td>
<td>23/45</td>
<td>51%</td>
<td>30/67</td>
<td>45%</td>
<td>53/112</td>
<td>47%</td>
</tr>
<tr>
<td>Final pregnancy rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGA-treated</td>
<td>38/45</td>
<td>84%</td>
<td>64/70</td>
<td>91%</td>
<td>102/115</td>
<td>89%</td>
</tr>
<tr>
<td>Control</td>
<td>38/45</td>
<td>84%</td>
<td>59/67</td>
<td>88%</td>
<td>97/112</td>
<td>87%</td>
</tr>
</tbody>
</table>

^a,bPercentages within column and category with unlike superscripts are different (P<.05).

### Figure 6. Treatment schedules and timing of fixed-time insemination for MGA-treated and Control (modified CO-Synch) cows (Perry et al., 2001).

#### Method 4: 7-11 Synch
Recently we developed an estrus synchronization protocol for beef cattle that was designed to: 1) shorten the feeding period of MGA without compromising fertility; and 2)
improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development (Figure 7A; Kojima et al., 2000). This new treatment, 7-11 Synch, was compared with the GnRH-PG protocol. Synchrony of estrus during the 24-hour peak response period (42 to 66-hour) was significantly higher among 7-11 Synch treated cows. Furthermore, the distribution of estrus was reduced from 144 hours for GnRH-PG treated cows to 60 hours for cows assigned to the 7-11 Synch treatment (Figure 7B; Kojima et al., 2000). The 7-11 Synch protocol resulted in a higher degree of estrus synchrony (91%) and greater AI pregnancy rate (68%) during a 24-hour peak response period compared to the GnRH-PG protocol (69% and 47%, respectively).

**Figure 7A.** Illustration of the treatment schedule and events associated with the 7-11 Synch protocol (Kojima et al., 2000). **Figure B.** Estrus response of cows treated with the 7-11 Synch or GnRH-PG protocols (Kojima et al., 2000).

**Additional Considerations**

An additional consideration for Methods 2, 3 and 4 pertains to cows or heifers that fail to exhibit estrus after the last PG injection. In this case, cows or heifers would be re-injected with PG 11 to 14 days after the last injection of PG was administered. These females would then be observed for signs of behavioral estrus for an additional 6 to 7 days. This procedure would maximize efforts to inseminate as many females within the first 2 weeks of the breeding period as possible. Cows that were inseminated during the first synchronized period should not be re-injected with PG. In addition, the decision to use Methods 3 or 4 in heifers should be based on careful consideration of the heifer’s age, weight, and pubertal status.

**Summary and Conclusions**

Expanded use of AI and/or adoption of emerging reproductive technologies for beef cows and heifers requires precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. Efforts to develop a more effective estrus synchronization protocol have focused recently on synchronizing follicular waves by injecting GnRH followed 7 days later by injection of PG (Ovsynch, CO-Synch, Select Synch). A factor contributing to reduced synchronized pregnancy rates in dairy and beef cows treated with the preceding protocols is that 5 to 15% of cycling cows show estrus on or before PG injection. We developed new protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and beef heifers in which the GnRH-PG protocol is preceded by either short- or long-term progestin treatment.
Although other types of progestin treatments (CIDR, PRID, or norgestomet) can be substituted in these estrus synchronization protocols, we chose to use MGA for the following reasons: a) MGA is economical to use (≈ 2 cents per animal per day to feed); b) MGA was recently cleared for use in reproductive classes of beef and dairy cattle (Federal Register, 1997); c) methodology and understanding of the use of MGA is documented in the literature (Zimbelman, 1963; Zimbelman and Smith, 1966; Patterson et al., 1989), dating back as early as the 1960’s; and d) MGA is easily administered in feed and does not require that animals be handled or restrained during administration. Perhaps more importantly, MGA is currently the only progestin approved for use and available in the U. S., making research of methods to improve and broaden the scope of its use all the more significant.

Table 5 provides a summary of various estrus synchronization protocols for use in postpartum beef cows. The table includes estrus response for the respective treatments and the synchronized pregnancy rate that resulted. These data represent results from our own published work, in addition to unpublished data from DeJarnette and Wallace, Select Sires, Inc. The results shown in Table 5 provide evidence to support the sequential approach to estrus synchronization in postpartum beef cows we describe.

Our preliminary studies identified significant improvements in specific reproductive endpoints among cows that received MGA prior to the administration of PG compared with cows that received PG only, including increased estrus response and improved synchronized conception and pregnancy rates. More recently we observed a significant improvement in synchrony of estrus without compromising fertility in postpartum beef cows and beef heifers that were pretreated, either short- or long-term, with MGA prior to GnRH and PG. We propose the general hypothesis that progestin (MGA) treatment prior to the GnRH-PG estrus synchronization protocol will successfully: 1) induce ovulation in anestrous postpartum beef cows and peripubertal beef heifers; 2) reduce the incidence of a short luteal phase among anestrous cows induced to ovulate; 3) increase estrus response, synchronized conception and pregnancy rate; and 4) increase the likelihood of successful fixed-time insemination. Our data suggest that new methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers in which the GnRH-PG protocol is preceded by a progestin offer significant potential to more effectively synchronize estrus with resulting high fertility.

**Table 5.** Comparison of estrus response and fertility in postpartum beef cows after treatment with various estrus synchronization protocols.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estrus response</th>
<th>Synchronized pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 shot PG</td>
<td>241/422</td>
<td>147/422 57% 35%</td>
</tr>
<tr>
<td>Select Synch</td>
<td>353/528</td>
<td>237/528 67% 45%</td>
</tr>
<tr>
<td>MGA-PG 14-17 d</td>
<td>305/408</td>
<td>220/408 75% 54%</td>
</tr>
<tr>
<td>MGA-2 shot PG</td>
<td>327/348</td>
<td>243/348 93% 70%</td>
</tr>
<tr>
<td>MGA-PG 14-19 d</td>
<td>161/206</td>
<td>130/206 83% 63%</td>
</tr>
<tr>
<td>MGA Select</td>
<td>174/204</td>
<td>134/204 85% 66%</td>
</tr>
<tr>
<td>MGA Select + GnRH at AI</td>
<td>Fixed-time AI</td>
<td>70/115 91% 61%</td>
</tr>
<tr>
<td>7-11 Synch</td>
<td>40/44</td>
<td>30/44 91% 68%</td>
</tr>
</tbody>
</table>
References


REVIEW OF ESTROUS SYNCRHONIZATION SYSTEMS: CIDR INSERTS

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Introduction

Although estrous synchronization has been cited over the past several decades to have significant impact on reproductive performance of beef cattle (Schafer et al., 1990), only a limited percent of beef cattle operations in the U.S. synchronize estrus. In a summary by the National Animal Health Monitoring Systems (NAHMS) only 3.3% of beef cattle in the U.S. were being synchronized (NAHMS, 1994) even though estrous synchronization and AI have been documented to increase production and profitability in beef operations (Lesmeister et al., 1973; Bellows and Short, 1990; Wiltbank, 1990).

Estrous synchronization is a valuable reproductive tool for beef producers. A survey was conducted in Illinois in 2000 in attempt to understand why producers were not using estrous synchronization (Kesler, 2000). In completing the survey, producers were asked to indicate the reasons (listed in Table 1) they did not synchronize estrus in their heifers and cows. Although there was support for all provided reasons, one factor emerged as the primary reason: lack of time/labor. The second ranked reason was poor results. These are the two most important factors that must be considered in new product development and education. Products that were made available up until the 1990's were limited in efficacy and consistency and may have caused producers to lose interest in or question new developments.

Table 1. Reasons why producers don’t synchronize estrus.

<table>
<thead>
<tr>
<th>Reason</th>
<th>Frequency³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too difficult to use</td>
<td>11%</td>
</tr>
<tr>
<td>Don’t see the value</td>
<td>11%</td>
</tr>
<tr>
<td>Lack of time/labor</td>
<td>43%</td>
</tr>
<tr>
<td>High cost</td>
<td>11%</td>
</tr>
<tr>
<td>Poor results</td>
<td>17%</td>
</tr>
<tr>
<td>Requires heat detection</td>
<td>8%</td>
</tr>
</tbody>
</table>

³Producers were asked “If applicable, check which of the following explain why you do not routinely use estrous synchronization.” The number of checks in each category were divided by the total number of checks to determine frequency.

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A new reproductive tool—the CIDR—was approved by FDA in 2002 for the synchronization of estrus (Food and Drug Administration, 2002). The CIDR, an intravaginal progesterone insert, used in conjunction with PGF$_2$α advances the first pubertal estrus in beef heifers, advances the first postpartum estrus in suckled beef cows, and synchronizes estrus in replacement beef and dairy heifers and suckled beef cows. The CIDR was developed in New Zealand and has been used there for several years.

The CIDR is a “T” shaped device—referred to as an insert by FDA. The wings of the device collapse to form a rod that can be inserted into the vagina with an applicator. The CIDR is left in the vagina for seven days. An injection of PGF$_2$α is administered on day 6 and the insert is removed on day 7; however, many academic researchers are suggesting that the PGF$_2$α be administered at the same time the insert is removed. On the end opposite the wings of the insert a tail is attached that facilitates removal of the insert seven days after administration. The backbone of the CIDR is a nylon spine covered by a progesterone (1.38 g) impregnated silicone skin. Upon insertion blood progesterone concentrations rise rapidly. Maximal concentrations are reached within an hour (Figure 1). Progesterone concentrations are maintained at a relatively constant level during the seven days the insert is in the vagina (Figure 2). Upon removal of the insert, progesterone concentrations are quickly eliminated (Figures 3).
Retention rate of the insert during the seven-day period is exceptionally high: generally 97-98%. In recent research on dairy cows, vaginal irritation was monitored. This study with 863 lactating dairy cows demonstrated that most cows had a clear, cloudy, or yellow mucus associated with the insert upon removal; however, only 2% of the cows had a score suggestive of potential vaginitis. Caution is advised when working with the CIDRs, individuals handling the CIDRs should wear latex or nitrile gloves to prevent exposure to progesterone on the surface of the insert and to prevent the introduction of contaminants from the hands into the vagina of the treated females. The inserts are developed for a one-time use and multiple use—which is not approved by FDA—may cause vaginal infections.

Results from the studies that support the claims were published (Lucy et al., 2001). That study included 724 beef heifers, 851 beef cows, and 260 dairy heifers from seven sites across the United States demonstrating that the protocol is efficacious (Table 2).

Table 2. Synchrony, conception rates, and pregnancy rates of beef cows and heifers administered the CIDR + PGF$_2$α procedure.a

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>PGF$_2$α</th>
<th>CIDR + PGF$_2$α</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beef Cows:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrus 1st 3 Days</td>
<td>15%</td>
<td>33%</td>
<td>59%</td>
</tr>
<tr>
<td>Synchronization Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anestrous Cows</td>
<td>11%</td>
<td>19%</td>
<td>45%**</td>
</tr>
<tr>
<td>Cyclic Cows</td>
<td>19%</td>
<td>49%</td>
<td>72%**</td>
</tr>
<tr>
<td>Conception Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anestrous Cows</td>
<td>38%</td>
<td>57%</td>
<td>57%</td>
</tr>
<tr>
<td>Cyclic Cows</td>
<td>58%</td>
<td>70%</td>
<td>63%</td>
</tr>
<tr>
<td>Pregnancy Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anestrous Cows</td>
<td>4%</td>
<td>11%</td>
<td>26%**</td>
</tr>
<tr>
<td>Cyclic Cows</td>
<td>11%</td>
<td>34%</td>
<td>46%**</td>
</tr>
<tr>
<td><strong>Beef Heifers:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrus 1st 3 Days</td>
<td>13%</td>
<td>27%</td>
<td>65%</td>
</tr>
<tr>
<td>Synchronization Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal Heifers</td>
<td>7%</td>
<td>11%</td>
<td>48%**</td>
</tr>
<tr>
<td>Cyclic Heifers</td>
<td>17%</td>
<td>37%</td>
<td>80%**</td>
</tr>
<tr>
<td>Conception Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal Heifers</td>
<td>75%</td>
<td>55%</td>
<td>58%</td>
</tr>
<tr>
<td>Cyclic Heifers</td>
<td>52%</td>
<td>52%</td>
<td>61%</td>
</tr>
<tr>
<td>Pregnancy Rate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal Heifers</td>
<td>6%</td>
<td>6%</td>
<td>28%**</td>
</tr>
<tr>
<td>Cyclic Heifers</td>
<td>9%</td>
<td>19%</td>
<td>49%**</td>
</tr>
</tbody>
</table>

*aModified from Lucy et al. (2001).

**Denotes a significant (P<.01) treatment effect.
CIDR/PGF$_{2\alpha}$ Protocol: Mechanism of Action

During the seven days of CIDR insertion, progesterone diffusion from the CIDR does not affect spontaneous luteolysis. Assuming all beef heifers and cows have 21 day estrous cycles, there will be two populations of females after six days of CIDR treatment: females without corpora lutea and females with corpora lutea more than six days after ovulation. All females, therefore, have corpora lutea that are potentially responsive to an injection of PGF$_{2\alpha}$. Although most research data suggests that only about 90% of corpora lutea in heifers and cows more than six days after ovulation regress promptly to an injection PGF$_{2\alpha}$, only about 60% of the females will have corpora lutea at the time of PGF$_{2\alpha}$ treatment (assuming that spontaneous corpora lutea regression begins about 18 days after ovulation). Therefore, about 95% of the females treated with the FDA approved CIDR/PGF$_{2\alpha}$ protocol are synchronized to exhibit estrus within a few days of CIDR insert removal. However, more than 95% of the treated females will be synchronized to exhibit estrus if estrous behavior is monitored for five days after removal of the CIDR insert.

An advantage of a progestin-based estrous synchronization protocol is that administration of progestins to prepubertal heifers and postpartum anestrous cows have been demonstrated to hasten puberty and cyclicity. Table 3 reports that efficacy of the CIDR in the study conducted by Lucy et al. (2001). In fact, in addition to synchronizing estrus in replacement beef and dairy heifers and suckled beef cows, FDA approved the claim that the CIDR advances the first pubertal estrus in beef heifers and advances the first postpartum estrus in suckled beef cows.

Table 3. Efficacy of the CIDR + PGF$_{2\alpha}$ procedure in hastening puberty and cyclicity.\(^a\)

<table>
<thead>
<tr>
<th>Target Animal</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepubertal Beef Heifers</td>
<td>7%</td>
<td>48%**</td>
</tr>
<tr>
<td>Anestrous Beef Cows</td>
<td>11%</td>
<td>45%**</td>
</tr>
</tbody>
</table>

\(^a\)Adapted from Lucy et al. (2001).
**Differs from the control group (P<.01).

Synchronization of Estrus with Alternative Protocols Using the CIDR

Several alterations of the basic protocol are being evaluated, however, much work is yet to be done since field trials with CIDRs were limited during the FDA approval process. Some of the published data will be reviewed, although it should be noted that these results are preliminary and it is unlikely that the best way to utilize CIDRs has yet to be established. The following are alternative protocols; however, caution is advised on the implementation of these protocols until thorough multi-site studies are conducted.

- Inclusion of the CIDR in the CO-Synch procedure.
- Inclusion of an estradiol at the time of CIDR insertion.
- Inclusion of an estradiol about 24 hours after CIDR removal.
- Administration of the CIDR 14±1 days after insemination and removal 21±1 days later for resynchronization; however, this application will be discussed elsewhere in this proceedings.
Inclusion of the CIDR in the CO-Synch Procedure: Lamb et al. (2001) published data in which the CIDR was included in the CO-Synch estrous synchronization procedure. The CIDR was inserted at the time of the first injection of GnRH and removed at the time of the injection of PGF₂α. Overall, there was a positive effect of including the CIDR in the CO-Synch protocol; however, this positive effect was not consistent across all locations. Second, the positive effect of including the CIDR was absent in the cows that were cycling and had high progesterone concentrations at the time of PGF₂α treatment, which may explain why there was not a positive effect at each location (Table 4).

Table 4. Effect of including a CIDR in the CO-Synch protocol for beef cows. a

<table>
<thead>
<tr>
<th>Item</th>
<th>CO-Synch</th>
<th>CO-Synch + CIDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number</td>
<td>287</td>
<td>273</td>
</tr>
<tr>
<td>Station:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>52%</td>
<td>43%</td>
</tr>
<tr>
<td>Kansas</td>
<td>54%</td>
<td>66%</td>
</tr>
<tr>
<td>Minnesota</td>
<td>38%</td>
<td>51%</td>
</tr>
<tr>
<td>Missouri</td>
<td>53%</td>
<td>71%</td>
</tr>
<tr>
<td>Combined</td>
<td>48%</td>
<td>59%</td>
</tr>
<tr>
<td>Reproductive Status:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycling + high P4 at PGF₂α</td>
<td>58%</td>
<td>58%</td>
</tr>
<tr>
<td>Noncycling + high P4 at PGF₂α</td>
<td>40%</td>
<td>53%</td>
</tr>
<tr>
<td>Cycling + low P4 at PGF₂α</td>
<td>43%</td>
<td>79%</td>
</tr>
<tr>
<td>Noncycling</td>
<td>38%</td>
<td>66%</td>
</tr>
</tbody>
</table>

aAdapted from Lamb et al. (2001).

Martinez et al. (2002a) included the CIDR in the CO-Synch protocol for heifers and the data are summarized in the following table (Table 5). Ovsynch and CO-Synch have not been reported to be as effective in heifers as cows. However, Martinez’s data would suggest that the CO-Synch protocol is effective in heifers if the CIDR is included as was done for cows—administered at the time of the first injection of GnRH and removed at the time of the PGF₂α.

Table 5. Effect of CO-Synch alone or along with CIDR treatment in beef heifers. a

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO-Synch</td>
<td>23</td>
<td>39%</td>
</tr>
<tr>
<td>CO-Synch + CIDR</td>
<td>25</td>
<td>68%*</td>
</tr>
</tbody>
</table>

aAdapted from Martinez et al. (Theriogenology 57:1049; 2002a).

Estrada et al. (2002) administered the CO-Synch + CIDR protocol to both cows and heifers. Pregnancy rates in their study were 61% for cows and 60% for heifers. Although Estrada et al. (2002) did not have a similar treatment group without the CIDR; these positive results would support the studies by Lamb et al. (2001) and Martinez et al. (2002a).
Inclusion of an Estradiol at the Time of CIDR Insertion: The injection of estradiol at the time of CIDR insertion is to synchronize the follicular wave. Alternatively, GnRH may be used to synchronize the follicular wave as used in the previously described CO-Synch procedure. Lane et al. (2001a) demonstrated that 0.75 mg of estradiol benzoate given at the time of PRID—an alternative progesterone-releasing intravaginal device—insertion, with PGF$_2$ given one day before PRID removal, was more effective at synchronizing estrus during the first 72 hours after insert removal than was GnRH at PRID insertion. In Table 6 are results from two studies in which various doses of estradiol benzoate were administered at the time of CIDR insertion to both heifers and cows (Lammoglia et al., 1998 and Steckler et al., 2001). These results suggest that inclusion of an estradiol injection at the time of CIDR insertion may improve synchronized pregnancy rates in cows, but not in heifers. As these studies have not been repeated at multiple sites, optimal dosage for cows is uncertain.

Table 6. Effect of administration of estradiol benzoate at the time of CIDR insertion. a

<table>
<thead>
<tr>
<th>Estradiol Benzoate</th>
<th>Synchronized Pregnancy Rates</th>
<th>Synchronized Pregnancy Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lammoglia et al. (1998)</td>
<td>Steckler et al. (2001)</td>
</tr>
<tr>
<td><strong>Heifers:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 mg</td>
<td>35%</td>
<td>40%</td>
</tr>
<tr>
<td>0.20 mg</td>
<td>33%</td>
<td>---</td>
</tr>
<tr>
<td>0.38 mg</td>
<td>35%</td>
<td>---</td>
</tr>
<tr>
<td>0.75 mg</td>
<td>29%</td>
<td>---</td>
</tr>
<tr>
<td>1.00 mg</td>
<td>---</td>
<td>30%</td>
</tr>
<tr>
<td>2.00 mg</td>
<td>---</td>
<td>37%</td>
</tr>
<tr>
<td>4.00 mg</td>
<td>---</td>
<td>27%</td>
</tr>
<tr>
<td><strong>Cows:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00 mg</td>
<td>8%</td>
<td>46%</td>
</tr>
<tr>
<td>0.25 mg</td>
<td>23%</td>
<td>---</td>
</tr>
<tr>
<td>0.50 mg</td>
<td>21%</td>
<td>---</td>
</tr>
<tr>
<td>1.00 mg</td>
<td>58%</td>
<td>53%</td>
</tr>
<tr>
<td>2.00 mg</td>
<td>---</td>
<td>55%</td>
</tr>
<tr>
<td>4.00 mg</td>
<td>---</td>
<td>63%</td>
</tr>
</tbody>
</table>

*aAdapted from Lammoglia et al. (1998) and Steckler et al. (2001).

Inclusion of an Estradiol at the Time of CIDR Removal: The idea behind giving estradiol about 24 hours after the CIDR removal is to hasten the onset of estrus and tighten synchrony. Fike et al. (1997) demonstrated that the administration of 1.0 mg of estradiol benzoate about 24 hours after the removal of the CIDR increased estrus response from 40% for control heifers to 60% for estradiol benzoate treated cows. Results of a study reported by Rasby et al. (1998) supported the Fike et al. study. They administered 1.0 mg of estradiol benzoate 24 to 30 hours after CIDR removal and observed more heifers in estrus within three days of CIDR removal than in the non-estradiol benzoate treated heifers. Lane et al. (2001b) demonstrated that 0.5 mg estradiol benzoate given 24 hours after PRID removal decreased the interval to the onset of estrus and decreased the
variation in onset of estrus; however, it decreased pregnancy rates when given at emergence of a follicle wave. Martinez et al. (2002b) administered estradiol benzoate about 24 hours after CIDR removal in heifers synchronized with the CIDR along with injections of estradiol benzoate and progesterone at the time of CIDR insertion and results are summarized in Table 7. Note that a higher percentage of heifers exhibited estrus even though the heifers were inseminated at a predetermined time. Pregnancy rates were not different from heifers administered the CO-Synch protocol so the additional animal handling would not seem warranted. Furthermore, this protocol included an injection of progesterone at CIDR insertion, which may have circumvented the decrease in pregnancy rates observed by Lane et al. (2001b) by removing heifers with emerging follicular waves at estradiol benzoate treatment after CIDR removal. Meyer et al. (2002), however, reported that an injection of progesterone at the time of CIDR insertion did not influence pregnancy rates to timed AI (TAI).

Table 7. Effect of using GnRH or estradiol benzoate with CIDRs for estrus synchronization in beef heifers.a

<table>
<thead>
<tr>
<th>Item</th>
<th>GnRH b</th>
<th>Estradiol Benzoate c</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td>103</td>
<td>52</td>
</tr>
<tr>
<td>Estrus (%)</td>
<td>65%</td>
<td>92%*</td>
</tr>
<tr>
<td>PG to estrus:</td>
<td>47 h</td>
<td>47 h</td>
</tr>
<tr>
<td>Hours</td>
<td>47 h</td>
<td>47 h</td>
</tr>
<tr>
<td>SD</td>
<td>8.2</td>
<td>3.8*</td>
</tr>
<tr>
<td>Pregnancy Rate to AI</td>
<td>65%</td>
<td>62%</td>
</tr>
</tbody>
</table>

aAdapted from Martinez et al. (J. Anim. Sci. 80:1746; 2002b).
bThe GnRH treated heifers received GnRH at the time of CIDR insertion. CIDRs were left in for 7 days and PGF2α was administered at CIDR removal. Forty-eight hours after PGF2α/CIDR removal they were administered a second injection of GnRH. Heifers were fixed-time inseminated (about 48 hours after CIDR removal).
cThe estradiol benzoate treated heifers received 2 mg of estradiol benzoate and 50 mg of progesterone at CIDR insertion. CIDRs were left in for 7 days and PGF2α was administered at CIDR removal. Twenty-four hours after CIDR removal heifers were administered a 1 mg injection of estradiol benzoate. Heifers were fixed-time inseminated (about 48 hours after CIDR removal).

Protocol and Product Variations: The first variation that should be considered is when to administer the PGF2α in the basic CIDR/PGF2α protocol: day 6 or day 7. The rational for administering the PGF2α on day 6 is to give luteolysis greater time for improved synchrony. Although this strategy seems reasonable, with a five-day estrus observation period for breeding the administration of PGF2α on day 7 will be as effective as on day 6. Day of PGF2α injection may be an issue when TAI protocols are developed; however, these procedures will likely use GnRH or an estradiol and these products may again make this issue irrelevant. Another variation is the difference that exists between CIDRs approved for use in the U.S. and those approved for use outside the U.S. In the U.S. the CIDR contains 1.38 mg of progesterone vs. 1.9 mg progesterone impregnated in the silicone in the CIDRs used outside the U.S. The following figures (Figures 4, 5, and 6) illustrate the different progesterone profiles, which in fact do not differ significantly and certainly will not alter efficacy.
Estradiol benzoate is not commercially available in the U.S. Therefore, there has been interest in estradiol cypionate (ECP), which is commercially available. Colazo et al. (2002) administered ECP (0.5 mg) and 50 mg of progesterone at the time of CIDR insertion. ECP was also administered about 24 hours after insert removal. These researchers reported that inclusion of the ECP and progesterone improved pregnancy rates as compared to CIDR/PGF$_2^\alpha$ treatment. It is difficult to make too many far-reaching conclusions from these studies since estradiol benzoate is not legal in the U.S. and very little data are available on substituting ECP. Furthermore, Rhinehart et al. (2002) reported that estradiol 17$\beta$, estradiol benzoate, and estradiol cypionate are equally effective in stimulating follicular atresia but that recruitment of a new follicle wave after regression may be delayed in beef heifers administered estradiol cypionate.

**A New Tool for the Reproductive Toolbox**

A new reproductive tool is now available in the U.S. for synchronization of estrus. Substantial data exists only for the approved protocol—a seven-day treatment along with an injection of PGF$_2^\alpha$ six days after insertion or one day before removal; however, this may not be the best way to utilize the CIDR for the synchronization of estrus. Protocols that may be used after favorable results emerge from studies conducted at multiple sites follow.

1. Inclusion of the CIDR in the CO-Synch protocol (primary synchronization in heifers and cows).
2. Administration of ECP about 24 hours after removal of the CIDR in the CIDR/PGF$_2^\alpha$ protocol or in place of the second injection of GnRH in the CO-Synch protocol (primary synchronization in heifers and cows).
3. Administration of ECP at the time of CIDR insertion in the CIDR/PGF$_2^\alpha$ protocol (primary synchronization in cows).
4. A combination of 2, 3, and 4 (primary synchronization in cows).

These are all primary synchronization protocols: synchronization of the first estrus of the breeding season. It should be noted that dosage of ECP has not been established and will differ from estradiol benzoate as ECP is a longer acting ester of estradiol than estradiol benzoate. These protocols may involve breeding upon the detection of estrus or TAI. The basic CIDR/PGF$_2^*$ was developed for breeding based upon the detection of estrus. When breeding upon the detection of estrus after any estrus synchronization protocol, a percentage—far greater than desired—of heifers or cows will not exhibit estrus (Table 8) and the timing of estrus will differ from the MGA/PGF$_2^*$ protocol. Although they may be inseminated at a return estrus or after resynchronization, the number of days to breeding females not exhibiting estrus can be shortened by administering a synchronization protocol the day after the estrous detection period. For example, administering the CO-Synch protocol to heifers or cows on day 6 after a five day estrous detection period will allow them to be inseminated 15 days after the first day of the breeding season. This is a gain of about 8 to 9 days per cow not observed in estrus during the primary synchronization period.

Table 8. Estrus response and pregnancy rates in beef heifers synchronized with the MGA/PGF$_2^*$ and CIDR/PGF$_2^*$ protocols.$^a$

<table>
<thead>
<tr>
<th>Time of Estrus</th>
<th>MGA/PGF$_2^*$</th>
<th>CIDR/PGF$_2^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>25-48</td>
<td>6%</td>
<td>34%</td>
</tr>
<tr>
<td>49-72</td>
<td>31%</td>
<td>31%</td>
</tr>
<tr>
<td>73-96</td>
<td>26%</td>
<td>9%</td>
</tr>
<tr>
<td>97-120</td>
<td>8%</td>
<td>2%</td>
</tr>
<tr>
<td>No Estrus</td>
<td>27%</td>
<td>22%</td>
</tr>
<tr>
<td>Conception Rate</td>
<td>69%</td>
<td>65%</td>
</tr>
<tr>
<td>Pregnancy Rate</td>
<td>50%</td>
<td>51%</td>
</tr>
</tbody>
</table>

$^a$These data were collected from studies conducted by the author over a five-year period. A total of 320 heifers were treated with the MGA/PGF$_2^*$ protocol and 113 heifers were treated with the CIDR/PGF$_2^*$ protocol.

The method of breeding heifers and cows after the synchronization with the CIDR is being examined substantially as well. The CO-Synch + CIDR protocol is a TAI protocol. Heifers and cows synchronized with the protocols using estradiol have been bred upon the detection of estrus or at a predetermined time (TAI); however, currently the CO-Synch or CO-Synch + CIDR protocols are the only TAI protocols. Results in Table 7 illustrate why the CIDR/PGF$_2^*$ protocol does not use TAI; however, a modified TAI procedure has been developed for the MGA/PGF$_2^*$ protocol, which has similar variability in the interval from PGF$_2^*$ to estrus (Table 8). If heifers or cows are going to be bred based on the detection of estrus, recommendations are provided in Table 9.
Table 9. Breeding recommendations for synchronized heifers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Breeding Recommendation</th>
</tr>
</thead>
</table>
| 1    | Heifers should be observed for estrus twice a day (early morning and evening) at times when no other activities are occurring. If there is any question don’t hesitate to breed.  
   —A heifer has no chance of conceiving if she is not inseminated |
| 2    | Heifers may be bred using the A.M./P.M. rule that recommends breeding 8 to 12 hours after the first observation of estrus.  
   —Do not extend this interval beyond 12 hours as intervals greater than 12 hours compromise pregnancy rates (Nebel et al., 1994). |
| 3    | If a heifer exhibits estrus at a time that removes the likelihood of using the A.M./P.M. rule correctly (i.e., estrus at 1:00 P.M. and should be bred at 9:00 P.M to 1:00 A.M.) it is better to breed early than late.  
   —Once ovulation has occurred the oocyte has a limited time of viability, whereas the sperm has a far longer time of survival. |
| 4    | Although heifers may be bred by the A.M./P.M. rule, there are significant data that suggest that once a day breeding is as efficacious. Continue to check for estrus twice daily and breed only in the A.M. (i.e., heifers in estrus on Monday P.M. and Tuesday A.M. should be bred Tuesday A.M.).  
   —Once daily breeding reduces the number of animal handlings for breeding by one-half (Nebel et al., 1994). |
| 5    | If a heifer continues to exhibits estrus 12 hours after breeding, don’t hesitate to re-inseminate.  
   —The loss of days to conception is far greater than the cost of the semen. |
| 6    | When breeding previously inseminated heifers, be sure that the heifer is in estrus. This can be confirmed by gently examining the uterus for tone per rectum.  
   —Inseminating a pregnant heifer may terminate that pregnancy. |

**Synchronization Satisfaction Assessment**

Producer satisfaction is a most troubling issue as society is consistently reminded of product satisfaction. What is a satisfactory synchronization pregnancy rate? Maximal pregnancy rates—assuming 100% synchrony—is on the average 68% (61% to 73%; mean ± one standard deviation; synchronized conception rates of 14 published studies). Based on this maximal pregnancy rate the following Synchronization Satisfaction Assessment was created (Table 10).

Table 10. Synchronization satisfaction assessment.
Are the synchronization protocols achieving their goal of maximal pregnancy rates? Using this satisfaction assessment the studies using the alternative protocols discussed herein—the most recent data available—are summarized in Table 11. Clearly, near maximal pregnancy rates are being achieved with the alternative protocols. However, multiple site studies are needed to verify the consistency of these protocols. Further, some protocols are more convenient to use than others. Administration of an estradiol requires an additional animal handling which may not be acceptable in some situations.

Table 11. Satisfaction assessment of alternative CIDR protocols.

<table>
<thead>
<tr>
<th>Study</th>
<th>Protocol(^a)</th>
<th>Females</th>
<th>Satisfaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb et al. (2002)</td>
<td>CO-Synch + CIDR</td>
<td>Beef Cows</td>
<td>High</td>
</tr>
<tr>
<td>Martinez et al. (2002a)</td>
<td>CO-Synch + CIDR</td>
<td>Beef Heifers</td>
<td>Exceptional (68%)</td>
</tr>
<tr>
<td>Estrada et al. (2002)</td>
<td>CO-Synch + CIDR</td>
<td>Beef Cows</td>
<td>Exceptional (61%)</td>
</tr>
<tr>
<td>Estrada et al. (2002)</td>
<td>CO-Synch + CIDR</td>
<td>Beef Heifers</td>
<td>Exceptional (60%)</td>
</tr>
<tr>
<td>Steckler et al. (2001)</td>
<td>CIDR/PGF(_{2\alpha}) + EB(^b)</td>
<td>Beef Cows</td>
<td>Exceptional (63%)</td>
</tr>
<tr>
<td>Martinez et al. (2002b)</td>
<td>CIDR/PGF(_{2\alpha}) + EB(^c)</td>
<td>Beef Heifers</td>
<td>Exceptional (62%)</td>
</tr>
<tr>
<td>Martinez et al. (2002b)</td>
<td>CO-Synch + CIDR</td>
<td>Beef Heifers</td>
<td>Exceptional (65%)</td>
</tr>
<tr>
<td>Colazo et al. (2002)</td>
<td>CIDR/PGF(_{2\alpha}) + ECP</td>
<td>Beef Heifers</td>
<td>Exceptional (65%)</td>
</tr>
</tbody>
</table>

\(^a\)See the full description of the protocol in the manuscript.
\(^b\) Estradiol benzoate was administered at the time of CIDR insertion.
\(^c\) Estradiol benzoate was administered approximately 24 hours after CIDR removal.

Summary

A new product is now available for the synchronization of estrus. During the next several years expect considerable research with this product. Several alterations of the basic protocol are being evaluated, however, much work is yet to be done, since field trials with CIDRs were limited during the FDA approval process. Caution is advised on the implementation of these protocols until thorough multi-site studies are conducted.

However, this product does effectively synchronize estrus with high pregnancy rates. With this product we may be able to encourage more producers to use estrus synchronization. When considering the reasons why producers don’t use AI reported in Table 1, these new protocols using the CIDR have utility (Table 12).

Table 12. Reason why producers don’t use AI and MGA/PGF\(_{2\alpha}\) and CIDR/PGF\(_{2\alpha}\) synchronization

<table>
<thead>
<tr>
<th>Reason for Not Using Synchronization</th>
<th>CIDR-Based Protocols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult to use</td>
<td>No</td>
</tr>
<tr>
<td>Valuable</td>
<td>Yes</td>
</tr>
<tr>
<td>Requires Extensive Time and Labor</td>
<td>No</td>
</tr>
<tr>
<td>High cost</td>
<td>No</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>High</td>
</tr>
<tr>
<td>Requires estrus detection</td>
<td>Limited</td>
</tr>
</tbody>
</table>
However, we must be careful so that producers don’t become confused or use a minimally tested protocol. Given the importance of consumer confidence to help drive beef demand, producers should act responsibly in implementation of synchronization programs.

Literature Cited


INCIDENCE OF POSTPARTUM ANESTRUS IN SUCKLED BEEF CATTLE: TREATMENTS TO INDUCE ESTRUS, OVULATION AND CONCEPTION

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Abstract

Early herd conception is limited by the proportion of cows that have resumed normal estrous cycles (cycling) before the beginning of the breeding season. Only 54% of 3,269 beef cows studied were cycling before onset of the breeding season. Parity, days postpartum, and body condition score (BCS) influenced the proportion of cows cycling as assessed by concentrations of blood progesterone. Compared to multiparous cows, fewer (P<0.01) primiparous cows were cycling, despite calving up to 3 wk earlier. Cycling activity increased linearly (P<0.001) from 9% (<30 d) to a peak of 70% 81 to 90 d postpartum. For every 10-d interval from <30 to >90 d, cycling activity increased (P<0.05) by 7.5 % 0.7%. As BCS increased from 3.5 to 6.0 (1 = thin and 9 = fat), the percentage of cows cycling increased linearly (P<0.001) by 18 % 2% for each unit increase in BCS. Ovulation was induced most successfully in noncycling cows after injection of GnRH or GnRH plus a progestin (7-d CIDR or 14-d feeding of melengestrol acetate [MGA] ending 12 d before GnRH). Ovulation induction was limited in primiparous cows until BCS were 5.0. In older cows, induction of ovulation increased linearly (P<0.05) with increasing BCS. As BCS increased from 3.5 to 6.0, expressed estrus increased (P<0.05), with more cycling than noncycling cows expressing estrus during the first week of the breeding season. Expression of estrus in cycling and noncycling cows was greatest after treatment with norgestomet for 7 d and GnRH and PGF2α when the implant was inserted and removed, respectively. Conception rates after timed AI (TAI) were greater for cows treated with the CIDR or norgestomet for 7 d if they were inserted or implanted just prior to GnRH injection and received PGF2α 7 d later at removal, with TAI occurring between 48 and 60 h later. Based on detected estrus, treatments with PGF2α were superior in primiparous cows, with no superior treatment identified for multiparous cows. Of the systems studied, anestrous suckled cows responded best to treatments that included GnRH plus a short-term progestin to maximize ovulation induction before PGF2α, and expression of estrus and conception after PGF2α.

(Key Words: Suckled Cows, Body Condition, Progestin, Postpartum, Anestrus, Ovulation.)

Introduction

The factor that most limits early conception of suckled cows is the proportion of cows that are not cycling (anestrus) at the beginning of the breeding season (Short et al., 1990). Other factors influencing the incidence of anestrus were reviewed (Stevenson et al., 1997). Continual presence of a suckling calf prolongs anestrus and delays the reinitiation of
estrous cycles (Williams, 1990). Insufficient energy and protein intake and insufficient body condition at calving are also limiting factors, but temporary or permanent weaning of the calf usually initiates estrus within a few days (Williams, 1990). Primiparous cows generally have a more prolonged anestrus because of their additional growth requirement (Short and Adams, 1988; Randel, 1990; Short et al., 1990).

Nutrients are used by cows according to an established priority (Short and Adams, 1988). The first priority is maintenance of essential body functions to preserve life. Once that maintenance requirement is met, remaining nutrients accommodate growth. Finally, lactation and the initiation of estrous cycles are supported. Older cows have no growth requirement, thus nutrients are more likely to be available for milk synthesis and initiation of estrous cycles. Because of this priority system, young, growing cows generally produce less milk and are anestrous longer after calving.

Since 1994, we have treated more than 3,269 beef cows with various hormonal treatments to synchronize estrus, ovulation, or both, in an attempt to achieve conception early in the breeding season and maximize the proportion of cows pregnant to genetically superior AI sires (Stevenson et al., 1997a,b; Thompson et al., 1999; Stevenson et al., 2000). As part of these studies, we measured the incidence of cyclicity at the beginning of the breeding season, both prior to hormonal injections and in response to these treatments. The major risk factors that limit a high rate of cyclicity at the beginning of the breeding season include age of cow, body condition, and days postpartum (Short et al., 1990).

The objectives of this report were to quantify the effects of body condition, parity, and days postpartum of suckled beef cows on the initiation of estrous cycles and to determine their influence on the proportion of cows cycling before and in response to hormonal treatments, as well as their effect on resulting fertility of such treatments.

**Materials and Methods**

Seven studies were conducted during spring 1994-2001 breeding seasons at five private ranches in Kansas and Minnesota, at the Kansas State University Purebred Beef Unit, and Agricultural Research Center–Hays.

**Study 1 (1994-1995)**

Purebred suckled cows (Simmental, Angus, and Hereford) at Kansas State University were used (n = 279). Controls received two injections of PGF$_{2\alpha}$ (2HPGF; 5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d ! 14 and 0, and were inseminated at estrus, or in the absence of estrus, at 80 h after the second injection of PGF$_{2\alpha}$. Treated cows received 25 mg of PGF$_{2\alpha}$ on d ! 14 and 0 plus 100 µg of GnRH (GnRH; 2 mL of Cystorelin, Merial Limited, Iselin, NJ) on d ! 7 and had a norgestomet ear implant (NORG, Syncro-Mate-B; Merial Limited, Iselin, NJ) in place for 8 d beginning on d ! 7. Treated cows were inseminated at 72 h after PGF$_{2\alpha}$ or 18 h after a second injection of GnRH given at 54 h after PGF$_{2\alpha}$.

**Study 2 (1996)**

Purebred suckled Angus, Gelbvieh, and Hereford cows and crossbred suckled cows (Simmental, Angus, and Hereford) on three private ranches were used (n = 890). Treatments are illustrated in Figure 1. Control cows received two injections of PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d ! 14 and 0 (2HPGF). A second group of
cows received GnRH on d 7 and PGF$_{2\alpha}$ on d 0 (GnRH; 2 mL of Cystorelin, Merial Limited, Iselin, NJ or 2 mL of Factrel, Fort Dodge Laboratories, Fort Dodge, IA). A third group was treated as the previous group plus an ear implant containing 6 mg of norgestomet (Syncro-Mate-B; Merial Limited, Iselin, NJ) for 7 d beginning on d 7 (NORG). Cows were inseminated after detected estrus. In addition, 164 purebred suckled cows received the NORG treatment at the Kansas State University Purebred Beef Unit. Cows were either inseminated after detected estrus (one-half) or at 48 h after PGF$_{2\alpha}$ and given 100 µg of GnRH at the time of AI.

**Study 3 (1997)**

Crossbred suckled cows (Simmental, Angus, and Hereford crosses) on two private ranches, plus purebred Simmental, Angus, and Hereford suckled cows at Kansas State University were used (n = 406). Treatments are illustrated in Figure 1. Cows were treated with 100 µg of GnRH (Cystorelin, Merial Limited, Iselin, NJ) on d 7 and PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d 0 (GnRH). They were inseminated after detected estrus, or in the absence of estrus, at 54 h after PGF$_{2\alpha}$ and given 100 µg of GnRH at the time of AI.

**Study 4 (1998)**

Purebred Angus, Simmental, and Hereford cows at Kansas State University were used (n = 187). Treatments are illustrated in Figure 1. All cows received 100 µg of GnRH (2 mL of Cystorelin, Merial Limited, Iselin, NJ) on d 7 and 25 mg of PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d 0 (GnRH). Half of the cows also received an intravaginal progesterone-releasing insert (CIDR-B, InterAg, Hamilton, NZ) on d 7, which was removed on d 0 (CIDR). All cows were inseminated at 48 h after PGF$_{2\alpha}$ and given 100 µg of GnRH at the time of AI.

![Figure 1. Experimental protocols used in the studies 2 through 7.](image-url)
Study 5 (1999)

Purebred Angus, Simmental, and Hereford cows at Kansas State University were used (n = 187). Treatments are illustrated in Figure 1. All cows received 100 µg of GnRH (2 mL of Cystorelin, Merial Limited, Iselin, NJ) on d 17 and 25 mg of PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d 0 (GnRH). Half also received an ear implant containing 6 mg of norgestomet (Syncro-Mate-B; Merial Limited, Iselin, NJ) on d 7 (NORG). It was removed on d 0. All cows were inseminated 48 h after PGF$_{2\alpha}$ and given 100 µg of GnRH at the time of AI.

Study 6 (2000)

Purebred suckled Angus, Charolais, and South Devon on one private ranch in Minnesota, purebred Angus cows at the North Central Research and Outreach Center, University of Minnesota, Grand Rapids, purebred Angus, Simmental, and Hereford cows at Kansas State University, and crossbred suckled cows (Simmental, Angus, and Hereford) on a private ranch in north central Kansas were used (n = 609). Treatments are illustrated in Figure 1. All cows received 100 µg of GnRH (2 mL of Cystorelin, Merial Limited, Iselin, NJ) on d 7 and 25 mg of PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d 0 (GnRH). Half of the cows also received an intravaginal progesterone insert (CIDR-B, InterAg, Hamilton, NZ) on d 7, which was removed on d 0 (CIDR), whereas the other half were fed 0.5 mg of melengestrol acetate (MGA; Pharmacia Animal Health, Kalamazoo, MI) for 14 d, ending 12 d before the first GnRH injection. All were inseminated at 48 h after PGF$_{2\alpha}$ and given 100 µg of GnRH at the time of AI.

Study 7 (2001)

Purebred Angus, Simmental, and Hereford cows at Kansas State University, and crossbred cows (Angus crossed primarily with Hereford, Simmental, and South Devon) at the Agricultural Research Center–Hays of Kansas State University were used (n = 359). Treatments are illustrated in Figure 1. All cows received 100 µg of GnRH (2 mL of Cystorelin, Merial Limited, Iselin, NJ) on d 7 and 25 mg of PGF$_{2\alpha}$ (5 mL of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) on d 0. In addition, all cows received an intravaginal progesterone-releasing insert (CIDR-B, InterAg, Hamilton, NZ) on d 7, which was removed on d 0 (CIDR). All were inseminated either at 48 or 60 h after PGF$_{2\alpha}$ and one-half of the cows within each timed insemination group received 100 µg of GnRH at the time of AI.

Definitions

Blood samples were collected prior to hormonal injections to determine if cows had a functional corpus luteum. At least two blood samples were collected between 7 and 11 d before each hormone injection. Progesterone was measured by radioimmunoassay (Skaggs et al., 1986). If either or both samples of blood serum contained concentrations of progesterone $\geq 1$ ng/mL, then the cows were assumed to have ovulated and resumed estrous cycles (cycling). If neither sample contained elevated concentrations of progesterone, then the cow was assumed to be anestrus (anestrus). Any cow classified as anestrus on d 7 that subsequently had elevated concentrations of progesterone on d 0 was assumed to have ovulated in response to GnRH administered on d 7 (induced ovulation). Body condition scores (BCS; 1 = thin, 9 = fat; Whitman, 1975) were assigned to cows on the first day of the
breeding season and days postpartum were calculated for each cow on the first day of the breeding season.

The onset of the calving season was defined to be when the third mature cow (Standardized Performance Analysis definition) or heifer calved and deviations of calving dates from that date for all other cows were calculated to create four calving periods of #21, 22 to 42, 43 to 63, and >63 d.

**Statistical Analyses**

Data were subjected to analyses of variance in which herd-year was used as the testing variance for effects of cyclicity, induced ovulation, incidence of estrus, and conception rate. Parity (primiparous vs. multiparous), treatments (PGF, GnRH, NORG, CIDR, or MGA), insemination timing (TAI or after detected estrus) or BCS and days postpartum (DPP) were independent variables included in models. Procedures GLM and MIXED (SAS, 1998) were used to generate regressions, variances, and estimate contrasts were constructed to test hypotheses of interest about the treatments using the within herd-year variance. Herd-year was declared to be random in each model.

The model used to estimate cycling status included year, herd within year, parity, parity within herd-year, DPP, BCS, DPP HBCS, DPP H parity within herd-year, BCS H parity within herd-year, and DPP HBCS H parity within herd-year.

The model used to estimate the incidence of induced ovulation in cows that were classified as anestrus before the onset of treatments included treatment H parity within herd-year, DPP, BCS, DPP HBCS, DPP H treatment within herd-year, DPP H parity within herd-year, BCS H treatment within herd-year, BCS H parity within herd-year, DPP H treatment H parity within herd-year, BCS H treatment H parity within herd-year, and DPP HBCS H treatment H parity within herd-year.

The model used to estimate the incidence of expressed estrus during 48 to 120 h after PGF$_{2\alpha}$ in those studies in which estrus was detected (Studies 1, 2, and 3) and conception rates in all studies included year, herd within year, treatment within herd-year, parity within herd-year, treatment H parity within herd-year, cycling status on d 0 (C), DPP, BCS, C H DPP, C HBCS, DPP HBCS, C HBCS H DPP, C H treatment within herd-year, C H parity within herd-year, C H treatment H parity within herd-year, DPP H treatment within herd-year, BCS H treatment within herd-year, DPP H parity within herd-year, BCS H parity within herd-year, DPP H treatment H parity within herd-year, BCS H treatment H parity within herd-year, and DPP H BCS H treatment H parity within herd-year. In addition, to when testing conception rates, occurrence of TAI or insemination after detected estrus was included in that model plus all possible interactions with the independent variables cited above.

**Results and Discussion**

**Cyclicity**

Similar percentages of heifers and cows calved during each 21-d interval after the onset of the calving season (Figure 2). In both parity groups, over 55% of the females calved during the first 21 d of the calving season, whereas <4% calved after 63 d. In all but one herd early breeding and calving of replacement heifers was carried out before that of the cow herd (22.9 ± 1.7 d; range: 12 to 36 d).
Despite the earlier calving of heifers in all but one herd, the percentage of primiparous cows (55%) that were cycling before the onset of the breeding season was less (P < 0.001) than that of the older cows (64%; Figure 3). The extra nutrient demand for growth clearly limits the proportion of primiparous cows that are cycling at the beginning of the breeding season (Short and Adams, 1988).

The percentage of cows cycling increased in a curvilinear fashion (P < 0.001) in all parities across days postpartum, reaching a peak at 81 to 90 d and tending to decrease thereafter (Figure 4). Average days postpartum were 86 ± 0.7 for primiparous and 68 ± 0.3 for multiparous cows. The decrease in percentage cycling for those >90 d postpartum may be partly a reflection of reduced cyclicity in primiparous cows, which comprised 85% of the cows this interval. For every 10-d interval since calving (from #30 to >90 d), the percentage of cows cycling increased (P < 0.01) by 7.5 ± 0.7%.

Figure 2. Within herd calving distribution for 3,269 suckled beef cows. Values in boxes are numbers of cows per category. Subsequent figure legends may display varying numbers of cows in boxes because of missing observations in each category.

Figure 3. Percentage of cows that had resumed estrous cycles (based on at least two estimates of concentrations of progesterone in blood collected 7 to 11 d before treatments) before initiation of treatments regressed on parity. Values in boxes are numbers of cows per category.
Similar to days postpartum, the percentage of cows cycling increased linearly ($P<0.001$) with increasing BCS (Figure 5) indicating that cows in lesser body condition were at risk for not cycling at the onset of the breeding season. An effect of parity was detected ($P=0.06$) as observed in Figure 3, but no interactions were detected between parity and BCS or parity and days postpartum. For every unit increase in BCS (range of 1 to 7), percentage of cows cycling increased ($P<0.01$) by 18 "2%. Beef cows should calve with a body condition score of at least 5 to prevent prolonged anestrus after calving (Short et al., 1990). Cows may gain or lose body condition between calving and the beginning of the breeding season, depending on postpartum nutritional conditions, early grass growth, and supplementation. Clearly, body condition scores are predictive of cycling activity.

Percentage of primiparous and multiparous cows cycling based on their within herd-year calving distribution is illustrated in Figure 6. For primiparous cows, the cycling status at the onset of treatments (7 d before the breeding season began) was not different among the first three 21-d intervals but greater ($P<0.05$) than those very late-calving cows (>63 days into the calving season). This supports the general recommendation to use a shorter breeding season for heifers to avoid problems getting late-calving heifers rebred. In contrast, cycling
rates were greater ($P<0.05$) for older cows that calved during the first 42 d of the calving season compared to those that calved after d 42. Thus, although early calving is critical because it allows more time for cows to resume estrous cycles before the breeding season, it seems to be more critical in older cows partially because they typically have 2 to 3 fewer weeks to resume estrous cycles before the breeding season than the primiparous cows.

**Induction of Ovulation**

Ability to induce ovulation in anestrous cows is key to the success of some of the most recently developed estrus- and ovulation-synchronization protocols in cattle (Stevenson et al., 2000). Based on the combined results of our seven studies, inducing ovulation was greatest in cows, regardless of parity, that received a combination of a progestin plus GnRH injection (Figure 7). A greater ($P<0.05$) percentage of cows treated with GnRH (plus PGF$_{2\alpha}$ 7 d later) and previously fed MGA for 14 d (Figure 1) or treated with a CIDR insert concurrent with the GnRH (plus PGF$_{2\alpha}$ 7 d later) were induced to ovulate in response to GnRH than the remaining treatments that included concurrent administration of another progestin (norgestomet), received either the treatments designated as GnRH or 2HPGF alone. The percentage response in the 2HPGF treatment reflects spontaneous ovulation because PGF$_{2\alpha}$ is incapable of inducing estrus in the absence of a corpus luteum.
The response to GnRH-induced ovulation was limited to less than 20% in primiparous cows until BCS were $5 at the onset of the breeding season and then as BCS increased, induction of ovulation increased in a parallel fashion (Figure 8). In contrast, induction of ovulation increased linearly in older cows as BCS increased. Because of the growth requirement of primiparous cows, and despite being further postpartum than older cows, induction of ovulation is limited until a minimal body condition is achieved.

Injections of GnRH induce release of both LH and FSH from the anterior pituitary gland resulting in ovulation if a mature ovarian follicle(s) is present. Injections of GnRH initiated turnover of large follicles (>10 mm) or induced ovulation of the dominant follicle (Crowe et al., 1993; Twagiramungu et al., 1995; Thompson et al., 1999). Recent studies, using transrectal ultrasonography or daily-collected blood samples to monitor concentrations of progesterone, showed that a single GnRH injection was quite effective (>80%) in inducing ovulation and formation of the first corpus luteum in late-calving (34±6 days postpartum), suckled anestrous cows (Thompson et al., 1999). Fewer anestrous cows ovulated in the presence of a progestin treatment, as observed with concurrent norgestomet implants at the time of GnRH injection in our study or short-term feeding of MGA concurrent with GnRH injection (D.J. Patterson, personal communication).

**Estrus Expression**

A greater ($P<.001$) proportion of cycling cows than anestrous cows (70 vs. 40%) showed behavioral estrus early in the breeding season. Included in these results are those cows that were induced to cycle after the initial GnRH injection and had elevated progesterone at the time of PGF$_{2\alpha}$ (d 0). Although the NORG treatment was less effective in inducing ovulation in anestrous cows than use of MGA or the CIDR insert, its inclusion increased ($P<0.05$) the proportion of cycling cows showing estrus compared to the GnRH + PGF or 2HPGF treatments alone (Figure 9).
The combination of norgestomet and GnRH increased \((P<0.05)\) the proportion of cows in estrus in both parity groups (Figure 10). This is consistent with the findings in cycling cows, anestrous cows at the time of \(\text{PGF}_2\alpha\) \((d 0)\) that had also failed to respond to GnRH on \(d 7\). Distribution of estrus and interval to estrus of cows in these treatments were reported earlier (Stevenson et al., 2000). Pretreatment of cows with norgestomet before GnRH increased the amount of GnRH-induced LH release (Thompson et al., 1999), increased the size of the dominant follicle (Garcia-Winder et al., 1987), and the proportion of GnRH-induced ovulations in noncycling, suckled cows (Troxel et al., 1993).

**Fertility**

Among primiparous cows, pregnancy rates after TAI were greater \((P<0.05)\) in those receiving the CIDR and NORG protocols than in those treated with the \(2\text{HPGF}\) protocol (Figure 11). Pregnancy rates in primiparous cows treated with the MGA and GnRH protocols were intermediate, but all protocols produced more pregnancies than that of \(2\text{HPGF}\) protocol. Among multiparous cows, the CIDR, MGA, and NORG protocols were superior \((P<0.05)\) to that of GnRH, whereas all protocols increased \((P<0.05)\) pregnancy outcomes above \(2\text{HPGF}\) alone.
In cows from three studies in which estrus was detected and inseminations were based on detected estrus, conception rates after the 2HPGF protocol were superior in primiparous cows to those after the GnRH protocol, with the NORG treatment being intermediate (Figure 12). No protocol tested in older cows was superior for cows detected in estrus. Although conception rates were greater in cows inseminated after detected estrus, pregnancy rates were not greater than those observed in protocols in which TAI was used because AI submission rates were 100% for TAI cows, whereas AI submission rates were <80% for cows detected in estrus (Stevenson et al., 2000).

Conception rates of cows according to when they calved during the calving season are illustrated in Figure 13. Those calving early tended to have the best fertility regardless of parity, despite having slightly less BCS. Only late calvings after d 63 of the calving season were associated with the lowest fertility, particularly for late-calving heifers.
In conclusion, incidence of anestrus in these herds over multiple years varied from 11 to 92% (0 = 62%). Resumption of estrous cycles was severely limited when BCS were <5 and cows were <63 d postpartum at the onset of the breeding season. Based on our observations, primiparous cows must calve during the first 60 d and older cows during the first 42 d of the calving season to reach a cycling status of 50% by the onset of the breeding season. Induction of ovulation prior to the breeding season was best achieved by either feeding MGA for 14 d before injecting GnRH 12 d later or inserting a CIDR at the time of a GnRH injection. These treatments were superior to the NORG or GnRH protocols (Figure 1). Further, success of ovulation induction in primiparous cows is limited unless BCS $5.0. Of those systems studied, expression of estrus by anestrous and cycling cows was better achieved with a progestin + GnRH treatment or 2\text{HPGF} protocols (Figure 1). Conception rates after TAI were superior for treatments that included MGA, NORG, or CIDR plus GnRH relative to GnRH + PGF or 2\text{HPGF}. Conception rates were greater in cows that calved during the first 63 d of the calving season, despite these having lower overall BCS than later calving cows.

**Implications**

Having more cycling cows at the beginning of the breeding season should maximize the proportion of cows that conceive to AI sires. More cows calving early during each successive calving season will enhance AI pregnancy rates, because of a year-to-year cumulative effect, thus increasing the number of cows that have initiated estrous cycles before the breeding season begins. In addition, winter supplementation programs must maintain cows in a body condition (minimum of BCS = 5) sufficient to resume estrous cycles before the breeding season in a cost-efficient manner. Overall pregnancy rates to a single timed insemination were superior for treatments that included MGA, NORG, or CIDR over GnRH + PGF or 2\text{HPGF} treatments (Figure 1), because in the presence of a progestin, no estrus occurs before PGF$_{2\alpha}$ and more estrus expression occurs after PGF$_{2\alpha}$.
Acknowledgments

The authors acknowledge the following colleagues and graduate students who assisted in these studies since 1994: J. A. Cartmill, L.R. Corah, S. Z. El-Zarkouny, C. R. Dahlen, W. L. Forbes, D. M. Grieger, B. A. Hensley, K. R. Harmoney, D. P. Hoffman, G. C. Lamb, T. J. Marple, M.A. Medina-Britos, and K. E. Thompson. In addition, we express appreciation to the following organizations (in order of contribution) for donation of product and(or) funding for these studies: Select Sires, Inc., Plain City, OH; Merial Limited, Iselin, NJ; Pharmacia Animal Health, Kalamazoo, MI; Fort Dodge Animal Health, Fort Dodge, IA, and Intervet, Inc., Millsboro, DE.

References


EFFECTS OF FAT SUPPLEMENTATION ON REPRODUCTION IN BEEF CATTLE

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Introduction

Adequate nutrition is critical for successful reproductive function. Inadequate dietary energy intake and poor body condition can negatively affect reproductive function. Supplemental lipids have been used to increase the energy density of the diet and avoid negative associative effects often experienced with cereal grains (Coppock and Wilks, 1991; Bowman and Sanson, 1996). Supplemental lipids may also have direct positive effects on reproduction in beef cattle independent of the energy contribution.

Sources

Several different fatty acid sources have been studied as they relate to direct actions on reproductive function. Plant derived oils appear to have the greatest impact on reproductive performance, several of the more common sources include: sunflower, safflower, cottonseed, rice hulls, and soybeans. Animal tallow and calcium salts of predominately saturated fatty acids escape rumen biohydrogenation to a greater extent and are incorporated into adipose tissue and milk. Effects on reproductive function appear to be more variable. Highly polyunsaturated fatty acids such as those found in fishmeal also bypass the rumen unaltered and have fewer effects on rumen fermentation. These have also been documented to affect reproductive processes.

Mechanism of Action

Potential mechanisms by which supplemental fat affects reproductive processes have recently been reviewed (Williams and Stanko, 1999; Mattos et al., 2000). Ruminal microflora hydrolyze triglycerides and phospholipids that contain polyunsaturated fatty acids. Fats of plant or animal origin contain the unsaturated fatty acids palmitoleic, oleic, linoleic, and α-linolenic acids. Linoleic acid predominates in seed and seed products, and α-linolenic predominates in forages.

The fatty acids are mostly metabolized in the rumen. However, some are spared and pass through the rumen unaltered. Fats are hydrolyzed to their polyunsaturated fatty acid constituents and glycerol. A high proportion of the fatty acids are then partially or completely hydrogenated and much of the glycerol is fermented to propionic acid, one of the major volatile fatty acids, that is a precursor for glucose. Feeding of supplemental fat increases propionic acid production and the propionate:acetate ratio. The potential for differences in the efficiency of energy utilization and energy partitioning exist when supplemental fat is provided. The consumption of polyunsaturated plant oils increases basal serum insulin concentrations in both dairy and beef cows. It is possible that increased serum concentrations of insulin may play a role in mediating increased...
follicular growth either directly or indirectly by modulating granulosa IGF-I (insulin-like growth factor – 1) production. Fat supplementation has also been shown to increase concentration of circulating growth hormone (Williams and Stanko, 1999).

Secretion of luteinizing hormone (LH) from the pituitary and follicular growth in cattle are regulated partially by the energy status of the animal. Energy provided by fat supplementation increases LH secretion in animals deficient in energy. A mechanism independent from energy by which dietary fatty acids affect LH secretion has not been established (Mattos et al., 2000). Feeding supplemental dietary fat also increased serum and follicular fluid cholesterol, serum progesterone, lifespan of induced corpus luteum (CL), the number of medium-sized follicles, and growth of the preovulatory follicle (Williams and Stanko, 1999).

Prostaglandins play an important role in reestablishing estrous cycles both immediately after parturition and thereafter until conception occurs. Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) is responsible for uterine involution after parturition. The greater the post-partum prostaglandin concentration, the faster the uterus involution. The uterus releases PGF$_{2\alpha}$ during each estrous to regress each new CL if the cow is not pregnant and initiate a new estrous cycle. During the period of CL regression, concentrations of PGF$_{2\alpha}$ and progesterone are inversely related. If the cow does conceive, release of PGF$_{2\alpha}$ from the uterus is prevented in order to preserve the CL and maintain pregnancy.

The fatty acid, linoleic acid is a substrate for the synthesis of PGF$_{2\alpha}$. Linoleic acid can be desaturated and elongated to form arachidonic acid (C20:4), which is a precursor for PGF$_{2\alpha}$. Regulatory enzymes for this conversion include delta six desaturase and cyclooxygenase. Linoleic acid can inhibit prostaglandin synthesis by competitive inhibition with these key enzymes. Arachidonic, and two fatty acids found in fishmeal, eicosapentaenoic (C20:5) and docosahexanoic (C22:6), have been shown to inhibit cyclooxygenase activity as well. It is important to note that linolenic acid (C18:3) was also present in the endometrial prostaglandin synthesis inhibitor isolated by Thatcher et al. (1994), and that linolenic acid has been shown to be a strong inhibitor of prostaglandin synthesis (Pace-Asciak and Wolfe, 1968). The amount and probably type of particular fatty acids reaching the target tissues likely influence whether prostaglandin synthesis is stimulated or inhibited (Thatcher and Staples, 2000).

**Production and Reproductive Responses**

Research with supplemental fat has been conducted on cows that have had one or more calves, and in replacement heifers. Fats have been fed before and after calving, and during the breeding season. Several response variables have been examined including: body weight and body condition score, age at puberty, postpartum interval, first service conception rates, pregnancy rates, calving interval, mammary gland development, milk yield and composition, calving difficulty, and calf birth and weaning weight. To determine potential mechanisms of action scientists have investigated changes in follicular and uterine development, hormonal profiles and changes, brain function, and embryonic development. Research on feeding supplemental fat has resulted in varied and inconsistent results as it relates to reproductive efficiency including positive, negative, and no apparent effect.

Elucidating mechanisms of action of how supplemental fat can influence reproductive function has been a difficult process. Animal response appears to be
dependent on body condition score, age (parity), nutrients available in the basal diet (pasture or range conditions), and type of fat supplement. The complexity of the reproductive system and makeup of fat supplements are often confounded by management conditions and forage quality both in research and in commercial feeding situations. This has contributed to inconsistencies in research findings.

**Research Summary**

Currently, research is inconclusive on exactly how to supplement fat to improve reproductive performance beyond the energy contribution of fat. Most studies have tried to achieve isocaloric and isonitrogenous diets, however, this can be challenging. Some studies only have sufficient animal numbers to detect very large differences in reproductive parameters such as conception and pregnancy rate. The following is a summary of several research trials investigating the effect of supplemental fat on reproductive performance in beef heifers and cows.

**Heifer development**

- Heifers fed safflower seeds (4.4% dietary fat) for 162 d had a tendency to have a greater percentage reaching puberty at the beginning of the breeding season than heifers fed no added dietary fat but had no difference in overall pregnancy rate. The diet x sire breed interaction suggested that the response to fat supplementation may be breed dependent. Heifers fed supplemental fat had greater cholesterol and progesterone concentrations than non-fat supplemented heifers. (Lammoglia et al., 2000).
- Supplementing soybean oil at 3% of the diet to a forage-based ration (hay plus oil as a top-dressed supplement) to pre-pubertal heifers for approximately 100 days increased feed efficiency in one experiment, but not in another, compared to heifers receiving a corn-based control supplement. Additionally, in the second experiment, but not the first, heifers tended to conceive earlier in the breeding season. In the above experiments, supplementing at a level of 6% soybean oil decreased feed efficiency compared to 3% added oil and did not change growth or reproductive performance compared to the other diets. No improvement in pregnancy rate was found among groups (Whitney et al., 2000).
- Feeding 2 lb (6-7 % total dietary fat) of whole sunflower seeds for either 30 or 60 d before AI did not improve estrous response, conception, or pregnancy rate in beef heifers (Funston et al., 2001).

**Pre-calving**

- Feeding 1 lb/day of protected fat (calcium salts of palm oil; 5% fat in diet) to well developed heifers (1,036 lb) from the beginning of the third trimester of pregnancy until the end of the their third estrus after calving increased the time from calving until first estrus. In this study fat had a negative effect on reproduction (Oss et al., 1993).
- Supplementing the diet of late gestation heifers (day 230 until calving) with safflower seeds at 1.5 lb/day (approximately 4.7% fat in the diet) increased subsequent pregnancy rate by 19% compared to control diets with similar energy and protein content (Lammoglia et al., 1997).
Supplementation with safflower seeds, soybeans, or sunflower seeds (4.7, 3.8 and 5.1% fat in diet, respectively) for the last 65 days before calving increased subsequent pregnancy rates (94%, 90%, and 91%, respectively) of first-calf heifers compared to controls (79%) receiving diets with equivalent energy (2.4% fat). In a second experiment, supplementing diets with sunflower seeds (6.5% fat in diet) the last 68 days before calving did not improve subsequent pregnancy rate compared to control diet (2.2% fat). The major difference between the two studies was forage availability. When adequate nutrients are available, the effects of supplemental fat may be masked (Bellows, et al., 2001).

First-calf heifers supplemented with 5% fishmeal 25 days before and through a 90-day breeding season tended to have a higher first service conception rate than heifers supplemented with corn gluten meal in a silage-based diet (Bonnette et al., 2001).

Two and three-year old cows were supplemented with fishmeal (1 lb/d) 25 days before and through the end of a 70-day breeding season while grazing on pasture. Synchronized estrous response, first service conception rates, AI pregnancy rates, and overall pregnancy rates did not differ between cows receiving the fat supplement and controls (no supplement). Plasma linolenic (LNA) and eicosapentaenoic acid (EPA) were similar at the start of the study, however, plasma LNA was higher in cows grazing pasture alone on d –7 and 70 of the breeding season, whereas plasma EPA was higher in cows supplemented with fishmeal on d –7, 45, and 70 of the breeding season. Fishmeal supplementation increased plasma EPA in cows grazing pasture but did not affect reproductive performance. It was stated that EPA and LNA have the ability to inhibit uterine prostaglandin synthesis, which may be the reason there were no differences in reproductive measures (Burns et al., 2002).

Mature crossbred cows were supplemented with safflower seeds (5% vs 2.5% fat for control diet) in two studies from 50-56 days before calving. Cows on high fat diet tended to have higher intake and gain more weight throughout the trial. Body weight and condition of cows were similar. Pregnancy rate, calf birth weight, and weaning weight were not affected by treatment. In both studies cows were in adequate (5-6) body condition and on a positive plane of nutrition throughout the experiment (Encinias et al., 2001).

Mature crossbred cows received a low fat milo-based supplement (6 lb; 2% fat; 18% CP) or a high fat sunflower-based supplement (3.5 lb; 26% fat; 18% CP) either prepartum (64 d) or postpartum (76 d). Reproductive response was not affected by type of supplement fed prepartum. In contrast, the proportion of cows cycling at the beginning of the breeding season and pregnancy rate to AI was greater for cows receiving the low fat supplement postpartum. Pregnancy rate at the end of the breeding season was not different between treatments (95%; Johnson, et al., 2001).

Feeding whole soybeans (WSB; 3.5 lb) before calving improved first service pregnancy rates in a 45-d natural service breeding period (WSB fed 45 d) and also in a synchronized AI program (WSB fed 30 d). No advantage was seen when supplementation was initiated at calving or 30 days before breeding (Graham et al., 2001).
Feeding high linoleate safflower seeds (5.3 % total dietary fat) 56 d before calving had no effect on weight gain, BCS, pregnancy rate, or postpartum interval. Calf birth weight, calving difficulty, and weaning weight were not affected by treatment; however, calf vigor was greater in calves born to heifers fed the high fat diet. Heifers were BCS 4.4 at the beginning of treatment and 5.8 at calving (Geary et al., 2002).

Performance of cows supplemented with safflower seeds (3.5 – 4% estimated total dietary fat) 49 d before calving on native range was influenced by calving season and cow age. Three-year-old cows calving in February and 5-year-old cows calving in April receiving a high fat supplement had greater pregnancy rates than those fed a low fat supplement. The opposite was found for 3-year-olds calving in April. There was no effect of supplement type on cows calving in June (Grings et al., 2001).

**Post-calving**

- Feeding 0.5 lb/day of protected fat (calcium salts of palm oil; 4.7% fat in diet) to first calf (BCS = 5) heifers for 30 days immediately after calving increased beneficial prostaglandin hormones after calving. However, no improvement in days to first estrus or pregnancy rate was found (Filley et al., 2000).
- Supplementing rice bran (5.2% fat in the diet) from day 1 to 50 after calving tended to improve pregnancy rate in mature cows compared to cows receiving a control diet (3.7% dietary fat; De Fries et al, 1998).
- Feeding 1.8 lb/day of rice bran (5.1% fat in diet) to BCS 6 cows starting one day after calving increased cumulative rate of return to estrus by day 60 after calving compared to cows consuming control diets of similar energy. However, diets containing rice bran plus lasalocid had lower reproductive performance (Webb et al., 2001).
- Supplementation with 3 lbs of whole cottonseed (5.5% fat in diet) 30 days before the breeding season increased the number of cows cycling at the start of the breeding season by 18%. Cow BCS was less than 5 (Wehrman et al., 1991).
- Supplementation with two different fat supplements (21 and 17% fat; 4 lb/d) improved estrous response in 2-year-old cows and first service conception rate in mature cows when fed for 51 and 45 d postpartum, respectively, compared to control supplement (3% fat; Bader et al., 2000).
- First-calf heifers were supplemented with two types (high oleate or linoleate; 5% fat in the diet) of cracked safflower seeds for 90 d postpartum. Type of supplement had no effect on length of postpartum interval, pregnancy rate, cow or calf weight change, calf weaning weight, and total or forage organic matter intake. Overall mean serum IGF-I was greater in heifers fed high linoleate safflower seeds compared to high oleate or control. Treatment did not affect growth hormone, glucose, or NEFA concentrations (Bottger et al., 2000).
- Cows calving with a BCS less than 4, and fed such that body weight and BCS do not increase, are unlikely to respond to short-term dietary fat supplementation (Ryan et al., 1994).
Feeding Considerations

Dose response studies indicate that the amount of added plant oil necessary to maximize positive ovarian effects is not less than 4% (Stanko, et al., 1997). This appears to be in agreement with most studies when a positive response to fat supplementation was seen, total dietary fat ranges from 4-6%. The duration of supplement feeding needed to elicit a positive response is not known, however, from the research that has been conducted, thirty to sixty days appears to be a reasonable duration of supplementation. The period of supplementation has varied from different times before breeding in heifer development, pre-calving, post-calving, and/or pre-breeding periods. The young, growing cow appears to be the most likely to respond to supplemental fat. An appropriate situation for fat supplementation may be when pasture or range conditions are limiting or are likely to be limiting before and during the breeding season. Feeding supplemental fat to well-developed heifers or cows in adequate body condition on pasture or range resources that are adequate may not provide any benefit beyond energy contribution to the diet.

Fats are highly digestible (approximately 80% digestible). However, high levels of fat in the diet have the potential to negatively impact fiber digestibility, decrease calcium absorption by formation of calcium salts of fatty acids, and increase vitamin E requirements (NRC Beef, 1996). In general, the amount of added fat in predominantly forage-based cattle diets should not exceed 6% of the total ration on a dry matter basis. Provide adequate calcium in the ration, and make sure the vitamin E content meets or exceeds requirements, especially when highly unsaturated oils are used.

Whole safflower seeds need to be processed to improve digestibility. Seeds should be processed (rolled) with enough pressure to crack about 90% of the seed hulls without extracting the oil (Lammoglia et al., 1999). Processed fats can be either liquid or solid at room temperature, therefore, transportation and storage of fats will differ. Some fats need to be melted before mixing with feed, especially in cold environments. It is important to keep moisture in the storage tank at less than 1.5% water so the fat does not become rancid (Zinn, UCD Research Report). Gossypol levels may be a concern when high levels of whole cottonseed are fed.

Implications

Improvements in reproduction reported in some studies may be a result of added energy in the diet or direct effects of specific fatty acids on reproductive processes. As is the case for any technology or management strategy that improves specific aspects of ovarian physiology and cyclic activity, actual improvements in pregnancy rates, weaned calf crop, or total weight of calf produced are dependent on an array of interactive management practices and environmental conditions. Until these interrelationships are better understood, producers are advised to strive for low cost and balanced rations. If a source of supplemental fat can be added with little or no change in the ration cost, producers would be advised to do so. Research investigating the role of fat supplementation on reproductive responses has been variable, therefore, adding fat when significantly increasing ration cost would be advised when the risk of low reproduction (young, growing, thin, and limited nutrients in the basal diet) is greatest.
References


TIMING OF VACCINATIONS IN
ESTROUS SYNCHRONIZATION PROGRAMS

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The development and implementation of an effective vaccination program requires the consideration of many factors such as nutritional status, age, stress, environment, colostral interference, and disease challenge. These factors can significantly alter the effectiveness of a vaccination program.

No vaccine provides 100 percent protection against disease. Thus, we recognize that in any population of animals there is a portion of those animals that remain susceptible to disease challenge. It is then important to consider not only the individual animal immunity, but also the population or the herd immunity as a whole. An effective vaccination program should focus on decreasing the number of susceptible animals within the herd and thereby increase the level of collective herd immunity. In doing so, we hopefully decrease the potential for a widespread outbreak of disease in the herd or, more importantly, if a disease pathogen is introduced into the herd, the effects of that pathogen are minimized so that the disease outbreak is mild or sub-clinical in nature.

One of the critical areas in developing an effective program is insuring adequate immunization of young stock. Vaccinations in replacement stock have two specific goals that need to be considered. The first is to prepare the calf against pathogens that cause disease problems and secondly, prepare the calf for entry into the adult herd with a good foundation of protection from which to build herd immunity (Cortese, 1999).

Given the factors mentioned above, it is a common recommendation made by many veterinarians that replacement stock receive initial vaccinations against the major diseases that cause reproductive losses and reduced reproductive performance in cattle beginning at or before weaning followed by appropriate boosters in yearlings prior to first breeding. The common diseases included in these vaccination protocols are Leptospira, Campylobacter (Vibrio), bovine virus diarrhea (BVD), bovine herpesvirus type-I (IBR), and optionally bovine trichomoniasis and hemophilus.

Both modified live and killed virus vaccines are commercially available for IBR and BVD. Controversy still surrounds the efficacy and safety of modified live versus killed IBR and BVD vaccines and both have strong advocates and opponents.

The reproductive effects of IBR and BVD in the pregnant animal are well documented. However, reproductive effects in the non-pregnant animal are less well defined. Reduced fertility resulting from a necrotizing oophoritis has been reported in animals infected with IBR virus either from natural infection or vaccination with modified live IBR vaccine (Chiang, 1990; Smith, 1990; Miller, 1991). The most severe effect described was damage to the corpus luteum during the first three to four days post ovulation (Smith, 1991). As a result of luteal dysfunction, the estrous cycle was severely
disrupted. This effect appears short lived. In most heifers, the subsequent estrous cycle occurred on schedule and was normal.

Ovarian pathology has also been reported as a result of natural infection or vaccination with modified live virus BVD vaccine (Grooms et al., 1998). Although these studies showed oophoritis associated with BVD virus, effects on fertility were not evaluated. Also, sero-negative, virus negative animals were used in these studies. The authors point out that in previously immunized animals, modified live vaccines may have no effect on the ovaries (Grooms et al., 1998).

One common characteristic of many of the current synchronization protocols for beef cattle is the necessity of putting the cattle through the chute multiple times to complete the protocol. Commercial cow-calf producers often combine as many procedures as possible to minimize time and labor. This may result in vaccinations being given too close to breeding with potential negative effects to the success of the synchronization and AI breeding program. An example would be vaccinating at the time that prostaglandin is injected.

To offset any potential negative effect from vaccination, veterinarians should insure that their clients have an understanding regarding timing of vaccinations in relationship to breeding. A commonly recommended vaccination protocol is to allow a minimum of 30 days and preferably 45 to 60 days between vaccination and breeding. Vaccination can be incorporated into a pre-breeding examination performed 45 to 60 days prior to breeding. This allows evaluation of the animals far enough in advance so that potential problems identified can be corrected and concerns relating to vaccination can be minimized.

**Literature Cited**


7-11 SYNCH

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Prologue

Precise control of estrous cycles in cattle requires the synchronization of follicular growth, and synchronized luteal regression. The “7-11 Synch” protocol (Figure 1) that was designed to: 1) shorten the treatment period from the 14-17 d or 14-19 d melengestrol acetate (MGA)-prostaglandin F$_{2\alpha}$ (PG) programs without reducing fertility; and 2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development. This review includes three published studies involving the 7-11 Synch protocol including: 1) “Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 Synch” (Kojima et al., 2000); 2) “A fixed-time AI program for beef cows with 7-11 Synch” (Kojima et al., 2002); and 3) “Comparison of melengestrol acetate-based estrus synchronization protocols in yearling beef heifers” (Kojima et al., 2001).

References


Development of 7-11 Synch

- Short-term MGA system (7-11 Synch) was designed to synchronize CL life span, follicular development, and estrus without reducing fertility

- If cows are not cycling, MGA (7 days) can induce estrus cyclicity

- If cows are cycling, PG regresses CL

- GnRH induces Ovulation

- GnRH-induced CL

Figure 1. Illustration of 7-11 Synch protocol. MGA = melengestrol acetate; PG = prostaglandin F$_{2\alpha}$. 
DEVELOPMENT OF AN ESTRUS SYNCHRONIZATION PROTOCOL FOR BEEF CATTE WITH SHORT TERM FEEDING OF MELENGESTROL ACETATE 7-11 SYNCH

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Abstract

An estrus synchronization protocol (7-11 Synch) was developed to synchronize the first follicular wave and timing of ovulation in postpartum beef cows. In Exp. 1, follicular development and timing of ovulation in response to the following protocol were evaluated. Beef heifers (n=12) and cows (n=6), at random stages of the estrous cycle, were fed melengestrol acetate (MGA: .5 mg·cow⁻¹·d⁻¹) for 7 d and injected with PGF₂α (PG: 25 mg) on the last day of MGA. A second injection of PG was administered 11 d after cessation of MGA. After the second injection of PG, estrus was synchronized in 6/12 heifers and 3/6 cows. The interval to estrus in heifers and cows was 54 and 64 h, respectively (P > .10). All animals exhibiting estrus ovulated first wave follicles. Animals that failed to respond to the second injection of PG were in estrus later than 6 d after cessation of MGA and had corpora lutea that were unresponsive to the injection of PG. Based on the variation in interval to estrus following the first PG injection on the last day of MGA feeding in Exp. 1, an injection of GnRH (100 µg) was added to the protocol 4 d after the cessation of MGA to ensure ovulation or luteinization of dominant follicles and synchronization of first wave follicular development. This revised protocol was termed “7-11 Synch”. In Exp. 2, two estrus synchronization protocols were compared. Multiparous beef cows were stratified by breed and postpartum interval and randomly assigned to the 7-11 Synch (n = 44) or Select Synch protocols (GnRH injection followed by PG injection 7 d later: n=45). Timing of estrus after the last PG injection (0 h) ranged from 42 to 102 h in the 7-11 Synch group and –30 to 114 h in the Select Synch group. Eight cows (18 %) in the Select Synch group exhibited estrus 30 h before to 18 h after PG. Synchronized estrus peaked between 42 to 66 h after the last PG injection with a maximum number of cows in estrus at 54 h for both treatment groups. Synchrony of estrus from 42 to 66 h was greater (P < .05) in 7-11 Synch (91 %: 41/44) than for Select Synch.

1 Contribution from the Missouri Agricultural Experiment Station Journal Series Number 12987. The authors thank Brad Belew, Phil Brooks, and Eric Sholljegerdes for managing the cattle used in this experiment and for helping with data collection. The authors also thank Jay Daniel, Chris Morrison, Chad Hale, and Yass Kobayashi for their help with data collection. We gratefully acknowledge Pharmacia and Upjohn, Inc., Kalamazoo, MI, for providing the Lutalyse Sterile Solution and Merial, Islin, NJ, for providing Cystorelin, and KABA/Select Sires, Inc., for financial support of this research.

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Synch treated cows (69 %: 31/45). AI pregnancy rate from 42 to 66 h was greater ($P < .05$) in the 7-11 Synch group (66 %: 29/44) than in the Select Synch group (40 %: 18/45). 

In summary, the 7-11 Synch protocol improved synchrony of estrus without reducing fertility. This protocol has potential future application for fixed-time AI in beef cattle production systems.

**Keywords:** Estrus Synchronization, Artificial Insemination, Beef Cows

**Introduction**

Precise control of estrous cycles in cattle requires the synchronization of follicular growth, and synchronized luteal regression. Feeding melengestrol acetate (MGA) for 14 days ($0.5$ mg·head$^{-1}$·d$^{-1}$) followed by an injection of PGF$_2$α (PG) 17 days after MGA feeding (14/17-d MGA/PG protocol) is an effective method of estrous cycle control in heifers (Brown et al., 1988; Patterson and Corah, 1992). Recently, an increase in estrus response, synchronized conception, and pregnancy rates, and fecundity in the postpartum cow was reported among cows treated with the 14/17-d MGA/PG protocol when compared to PG alone (Patterson et al., 1995; Fralix et al., 1996). The advantages of using MGA for estrus synchronization include ease of administration and reasonable cost; however, length of the treatment protocol creates a need for increased management and, in some cases, extends the duration of the treatment period beyond practical limits.

Short-term feeding of MGA (5 or 7 d) combined with an injection of PG has been shown to be effective in synchronizing estrus in a high percentage of cattle when compared to untreated controls, PG or MGA alone, but fertility was reduced (Moody et al., 1978; Patterson et al., 1986; Beal et al., 1988; Chenault et al., 1990). A short-term MGA protocol, however, offers more flexibility in management of breeding animals compared to the currently available 14/17-d MGA/PG program (Patterson et al., 1989; Odde, 1990). Another advantage is that cows are further postpartum at the time of MGA treatment. Incorporation of MGA into an estrus synchronization program provides the opportunity to induce estrous cyclicity in peripubertal heifers (Imwalle et al., 1998) and anestrous postpartum beef cows (Fralix et al., 1996). Therefore, the objective of the present study was to develop an estrus synchronization program for beef cattle with short-term feeding of MGA that precisely synchronizes development of first wave follicles and timing of ovulation.

**Materials and Methods**

**Experiment 1**

**Experimental Protocol.** Crossbred beef heifers (n=12) and non-lactating beef cows (n=6), at random stages of the estrous cycle, were used to characterize changes that occur in cows and heifers that were treated with the new estrus synchronization protocol. Animals were fed MGA ($0.5$ mg·head$^{-1}$·d$^{-1}$) in a carrier pellet (CATTLE CHARGE, MFA Inc., Columbia, MO) for 7 d and injected with PG (25 mg Lutalyse Sterile Solution; Pharmacia and Upjohn, Inc., Kalamazoo, MI) on the last day of MGA feeding. A second injection of PG was given 11 d after cessation of MGA feeding (Figure 1).
Treatment days and blood collections

**Figure 1.** Illustration of the treatment schedule and events associated with both Experiment 1 and 2. MGA = melengestrol acetate, and PG = prostaglandin F\(_2\alpha\). Arrows indicating the time blood samples were collected (solid arrow = 7-11 Synch, and gray arrow = Select Synch).

**Estrus Detection and Ultrasonography.** Behavioral estrus was observed every 6 h for 6 d following the last feeding of MGA and for 5 d following the second injection of PG. Development of first wave follicles and corpora lutea (CL) were monitored by real-time ultrasonography (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 7.5-MHz linear array transducer. Ultrasonography was performed every other day beginning at the last feeding of MGA through the second injection of PG, and every 12 h thereafter for 5 d, to determine the timing of ovulation.

**Blood Collection and RIA.** Blood samples were collected via jugular venipuncture at 10 and 2 d before the start of MGA feeding. Additional blood samples were collected on the last day of MGA feeding, 5, 7, and 9 d after MGA feeding, and daily for 5 d following the second injection of PG. Samples were collected into EDTA-treated tubes and placed immediately on ice. Within 4 h of collection, plasma was harvested by centrifugation and stored at -20°C until concentrations of progesterone were quantified by RIA within a single assay (Kirby et al., 1997: COAT-A-COUNT, Diagnostic Products Corporation, Los Angeles, CA). Intraassay coefficient of variation was 6.9% and assay sensitivity was 0.2 ng/mL.

**Statistical Analysis.** Intervals to the onset of estrus after MGA feeding and after the last injection of PG, diameter of dominant follicles, and timing of ovulation were analyzed by ANOVA using General Linear Models procedure of SAS (1988). Duncan’s new multiple range test (Steel and Torrie, 1980) was used for mean separation.

**Experiment 2**

**Experimental Protocol.** Based on the results from Exp. 1, an injection of 100 µg GnRH (Cystorelin; Merial, Islin, NJ) was included in the treatment protocol 4 d following the last day of MGA feeding to ensure ovulation or luteinization of dominant
follicles and synchronization of the first follicular wave. This revised protocol was termed “7-11 Synch” (Figure 1). In Exp. 2, 89 multiparous beef cows with calves were stratified by breed and postpartum interval (range 15-77 d) and were randomly assigned to two estrus synchronization treatments, 7-11 Synch (n = 44) or Select Synch (n = 45). The Select Synch protocol was chosen to compare with 7-11 Synch because of similarity in the treatment schedules for injections of GnRH and PG (Figure 1).

All cows were fed a carrier pellet as described in Exp. 1, starting 3 d prior to the initiation of MGA treatment. The first day of MGA feeding was defined as Day 1 (d 1) of the experiment (Figure 1). Cows in the Select Synch group continued to receive carrier pellets for an additional 7 d (from d 1 to 7). Cows in the 7-11 Synch group were fed MGA (.5 mg\cdot head^{-1} \cdot d^{-1}) in the carrier pellet for 7 d (from d 1 to 7) and received an injection of PG on the last day of MGA feeding (d 7). Four days after the last MGA feeding (d 11), GnRH was administered and a second injection of PG was given 11 d after the last day MGA was fed (d 18). Cows in the Select Synch group received an injection of GnRH (d 11), and then an injection of PG 7 d later (d 18).

Estrus Detection and AI. Cows were observed three times a day (0600, 1200, and 1900 h) for signs of behavioral estrus after the GnRH injection and for 7 d following the last injection of PG (from d 11 to 25). KAMAR heatmountTM detectors (KAMAR, Inc., Steamboat Springs, CO) were used to aid in detection of estrus. Timing of behavioral estrus was recorded for each cow. Body condition scores (BCS; 1 to 9 scale, 1 = emaciated, and 9 = obese) were recorded on all cows before insemination. Cows were artificially inseminated 12 h after detection of behavioral estrus by one of two experienced technicians and semen from three bulls was used (Select Sires, Inc., Plain City, OH). The number of cows in each treatment group that were inseminated by an individual technician was approximately equal. Cows were exposed for a 60-day natural service period beginning on d 39. Conception rate to AI or natural service was determined by a single technician by transrectal ultrasonography approximately 60 d after the last AI (approximately d 85). Pregnancy status was confirmed by rectal palpation approximately 120 d later and calving record.

Blood Collection and RIA. Blood samples were collected on d 1 and 7 from the 7-11 Synch group and on d 4 and 11 from the Select Synch group to determine postpartum status (cyclic or anestrus). The blood collection schedule is shown in Figure 1. Cows were considered cyclic when concentrations of progesterone were greater than 1 ng/mL in at least one sample collected 7 d apart. Additional samples were collected on the day of GnRH injection (d 11) for the 7-11 Synch group and on the day of second PG injections (d 18) for both groups to determine presence of luteal activity. Sample collection was performed as described in Exp. 1. Concentrations of progesterone were quantified by RIA within a single assay as described in Exp. 1. Intra-assay coefficient of variation was 7.3% and assay sensitivity was 0.2 ng/mL.

Statistical Analysis. Synchrony of estrus, conception rate, and pregnancy rates during both the AI period (d 11 to 25) and peak response period were analyzed using Chi-square analysis, CATMOD procedure, of SAS (1988) including the fixed effects of treatment, breed, AI technician, AI sire, cyclicity status, BCS, number of days postpartum, and the interactions of each with treatment. The Fisher’s exact test (Steel and Torrie, 1980) was used for mean separation. Maximum estrus response occurred at 54 h for both treatment groups; therefore, “peak response” period was defined as 12 h before
(42 h) and 12 h after (66 h) the maximum estrus response (54 h). During the 24-h peak response period (42 to 66 h), 14, 73, and 5% of the cows in the 7-11 Synch group and 20, 36, and 13% of the cows in the Select Synch group exhibited estrus at 42, 54, and 66 h, respectively. Degree of synchrony was analyzed by ratio of variance (F-test) of mean time interval to onset of estrus. No breed x treatment interaction was observed (P > .10); therefore, all data from different breeds within a treatment group were pooled for analysis. No effect (P > .10) of AI technician, BCS, or number of days postpartum on pregnancy rates were observed, and those were removed from the model. Three AI sires were used in this experiment and were assigned to equal numbers of cows for each estrus synchronization protocol. Overall AI pregnancy rates of three AI sires, regardless of treatment, were not different (75, 52, and 76%; P > .10). Because no significant differences occurred in pregnancy rate among sires, AI sire was removed from the model.

Results

Experiment 1

The number of cattle exhibiting estrus within 7 d after MGA treatment and the interval to estrus were 10/12 and 96 ± 4.4 h (mean ± SE), respectively, in heifers, and 4/6 and 84 ± 7.0 h, respectively, in cows. Timing of estrus did not differ (P > .10) between heifers and cows. After the second injection of PG, 6/12 heifers and 3/6 cows were synchronized. The interval to estrus was not different (P > .10) between heifers (54 ± 6.2 h) and cows (64 ± 4.0 h). All cattle exhibiting estrus after the second injection of PG ovulated first wave follicles and timing of ovulation did not differ (P > .10) between heifers (80 ± 7.4 h) and cows (96 ± 4.0 h). Cattle that failed to respond to the second injection of PG had either a cystic follicle (n = 1) or early developing CL that were not responsive to PG (n = 8). Early developing CL were the result of delayed estrus and ovulation after the last feeding of MGA. Diameter of dominant follicles at the time of the second PG injection tended to be larger (P < .07) in cows that were successfully synchronized compared to non-responders (16.0 ± 1.0 mm and 8.3 ± 2.8 mm, respectively). Among heifers, mean diameter of the dominant follicle did not differ (P > .10) between synchronized heifers and non-responders (14.1 ± 0.4 mm and 11.5 ± 2.2 mm, respectively).

Experiment 2

Average postpartum interval at the first PG injection on d 7 (7-11 Synch) or the GnRH injection on d 11 (Select Synch), and BCS before AI were not different (P > .10) between the 7-11 Synch (56 ± 2.4 d and 5.4 ± 0.04, respectively) and Select Synch groups (60 ± 2.3 d and 5.4 ± 0.05, respectively). The proportion of cows that were anestrous or cyclic at the GnRH injection was not different (P > .10) between the 7-11 Synch (34 %: 15/44, and 66 %: 29/44, respectively) and Select Synch groups (38 %: 17/45, and 62 %: 28/45, respectively).

Timing of estrus after the last PG injection (0 h) ranged from 42 to 102 h in the 7-11 Synch group (60-h period) and –30 to 114 h in the Select Synch group (144-h period; Figure 2). Synchrony of estrus during the 14-d AI period did not differ (P > .1) between 7-11 Synch (95 %: 42/44) and Select Synch treated groups (96 %: 43/45). Eight cows (18 %) in the Select Synch group exhibited estrus from –30 h to 18 h after PG injection. Synchronized estrus peaked at 54 h after the last PG injection for both the 7-11 Synch (73
%: 32/44) and the Select Synch groups (36 %: 16/45). As indicated by the lower variance for mean interval to estrus analyzed by F-test, degree of estrus synchrony was greater ($P < .0001$) in the 7-11 Synch treated cows (111.6, df = 41) than for Select Synch treated cows (768.7, df = 42).

Overall AI pregnancy rates during the 14-d AI period (after the GnRH injection and for a 7-d period following the last injection of PG: from d 11 to 25) and overall pregnancy rates during the breeding season (14-d AI period followed by 60-d natural service) did not differ ($P > .10$) between treatments (7-11 Synch: 70%: 31/44, and 89%: 39/44, respectively; Select Synch: 69%: 31/45, and 91%: 41/45, respectively).

Synchrony of estrus during the peak response period (42 to 66 h) was greater ($P < .05$) in 7-11 Synch (91%: 40/44) than in Select Synch treated cows (69%: 31/45; Figure 3). Consequently, AI pregnancy rate during the peak response period was greater ($P < .05$) in the 7-11 Synch group (68%: 30/44) than in the Select Synch group (47%: 21/45; Figure 4). During the peak response period, greater synchrony of estrus ($P < .05$) was observed in cyclic cows treated with the 7-11 Synch protocol than with the Select Synch protocol, resulting in greater ($P < .05$) AI pregnancy rates; anestrous cows responded similarly ($P > .10$) to these treatments (Figures 3 and 4).

![Figure 2. Distribution of estrous response in cows treated with either the 7-11 Synch or Select Synch protocols (Hour 0 = time of prostaglandin F$_{2\alpha}$ [PG] administration). Dash-line box indicated 24-h peak response period (42 to 66 h). Cows were observed three times a day (0600, 1200, and 1900 h) for behavioral estrus.](image-url)
Figure 3. Estrous response (number of cows detected in estrus / total number of cows treated) of the 7-11 Synch or Select Synch treated cows during the 24-h (42 to 66 h) peak response period. * = Estrous response was greater ($P < .05$) for total and cyclic cows treated with the 7-11 Synch protocol compared with the Select Synch protocol.

Figure 4. AI pregnancy rates (number of cows AI / total number of cows treated) of the 7-11 Synch or Select Synch treated cows during the 24-h (42 to 66 h) peak response period. * = Pregnancy rates were greater ($P < .05$) for total and cyclic cows treated with the 7-11 Synch protocol compared with the Select Synch protocol.

At the time of the last PG injection, three observations were made based on blood samples for progesterone: 1) cows with concentrations of progesterone greater than 1 ng/mL, indicating presence of a functional CL; 2) concentrations of progesterone ranging
from 0.3 to 1 ng/mL, suggesting the possible presence of luteinized follicles; and 3) no detectable concentrations of progesterone (< 0.2 ng/mL; below the assay sensitivity). Sixty-eight percent of cows in the 7-11 Synch group had concentrations of progesterone > 1 ng/mL at the time of the last PG injection (30/44: anestrus 10/15 and cyclic 20/29), and 32% had concentrations of progesterone between 0.3-1 ng/mL (14/44: anestrus 5/15 and cyclic 9/29). For cows in the Select Synch group, 58% had concentrations of progesterone > 1 ng/mL (26/45: anestrus 9/17 and cyclic 17/28); 22% had concentrations of progesterone between 0.3-1 ng/mL (10/45: anestrus 6/17 and cyclic 4/28); and 20% had no detectable concentrations of progesterone (9/45: anestrus 2/15 and cyclic 7/28). All cows in the 7-11 Synch group had detectable progesterone concentrations (> 0.3 ng/mL) compared with 80% (36/45) in the Select Synch group. These data suggest that the 7-11 Synch protocol successfully induced CL or resulted in the formation of luteinized follicles capable of responding to PG.

Discussion

Precise control of estrous cycles in cattle requires the synchronization of follicular growth, and synchronized luteal regression. The new treatment tested in these experiments was designed to: 1) shorten the treatment period compared to a 14/17-d MGA/PG program without reducing fertility; and 2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development compared to currently available estrus synchronization protocols.

A high percentage of cattle can be expected to exhibit estrus 3 to 5 d after short-term feeding of MGA (Moody et al., 1978; Patterson et al., 1986; Beal et al., 1988; Chenault et al., 1990). These cattle would be on days 6 to 8 of the estrous cycle at the time of the proposed second injection of PG 11 d later (Exp. 1). Consequently, CL should respond to the injection of PG and the existing dominant follicles should ovulate. Cattle that failed to respond to the second PG injection in Exp. 1 had either a cystic follicle (n = 1) or early developing CL (n = 8). Early developing CL were caused by delayed estrus and ovulation after the last feeding of MGA. Although a decline in concentrations of progesterone was observed after the last day of MGA feeding and the first PG injection, timing of estrus and ovulation was delayed in cows that did not respond to this treatment. Variation in expression of estrus following MGA feeding may be related to differences in clearance of MGA among individual cattle (Kojima et al., 1995). In fact, MGA can be stored in adipose tissue and released at different rates for individual cattle after withdrawal of MGA from the feed (Neff, 1983); therefore, body condition and/or amount of MGA consumed would affect clearance of MGA and consequently the timing of estrus and ovulation after the last day of MGA feeding. Another possibility for delayed estrus is that some animals may have been in the latter portion of the follicular wave at the end of MGA feeding at the time PG was administered. Hence, initiation of the new follicular wave occurred after the first injection of PG, which resulted in delayed expression of estrus.

An injection of GnRH was added to this estrus synchronization protocol 4 d after the last day of MGA feeding to ensure ovulation or luteinization of dominant follicles and synchronization of first wave follicular development, and to determine whether synchrony of estrus could be improved (Exp. 2). Timing of the GnRH injection was determined by the expected day of MGA clearance based on results from Exp. 1 and
previous literature (Moody et al., 1978; Patterson et al., 1986; Beal et al., 1988; Chenault et al., 1990).

The relationship between follicular development and timing of GnRH injection in estrus synchronization protocols may differ between anestrous and cyclic cows; however, both cyclic and anestrous cows responded equally well to the 7-11 Synch protocol. Although the number of anestrous cows in the present study was small, timing of estrus, estrus response, and pregnancy rates of anestrous cows were similar to those of cyclic cows.

The 7-11 Synch protocol resulted in a higher degree of estrus synchrony and greater AI pregnancy rates during a 24-h peak response period (42 to 66 h) compared to the Select Synch protocol. The interval to peak estrus response (42 to 66 h) in 7-11 Synch treated cows was similar to that observed in Exp. 1. These results agree with previous studies when cyclic cattle were injected with PG during the early (d 5 to 7) portion of the estrous cycle (Tanabe and Hann, 1984; Watts and Fuquay, 1985). These data indicate that the 7-11 Synch protocol effectively synchronized the first wave of follicular development resulting in a fertile estrus in both anestrous and cyclic cows.

Concentrations of progesterone at the time of the last injection of PG demonstrated that the injection of GnRH in the 7-11 Synch protocol successfully resulted in ovulation or luteinization of dominant follicles followed by initiation of new follicular waves. Either CL or luteinized follicles induced by GnRH would be capable of responding to PG; therefore, a relationship may exist between concentrations of progesterone before PG and improved synchrony of estrus for cows treated with the 7-11 Synch protocol. Consequently, follicles from the first wave ovulated after the second injection of PG, resulting in an earlier estrus response compared to the interval typically observed in cows injected with PG during the mid- to late estrous cycle (approximately 70 to 75 h: Tanabe and Hann, 1984; Watts and Fuquay, 1985). Although a decreased estrus response was reported in earlier studies when cattle were injected with PG during d 5 to 7 of the estrous cycle (Tanabe and Hann, 1984; Watts and Fuquay, 1985), cows assigned to the 7-11 Synch protocol demonstrated excellent synchrony of estrus when PG was administered on approximately d 7 of the estrous cycle. Further study is necessary to confirm effectiveness of the 7-11 Synch protocol in anestrous cows and peripubertal heifers. It would appear that 7-11 Synch may offer the potential for fixed-time AI programs because of the high degree of estrus synchrony exhibited by cows on this treatment.

In general, GnRH-PG based protocols are economical and less labor intensive compared to other protocols currently available (Twagiramungu et al., 1992; Pursley et al., 1995). The drawback of these protocols is that approximately 5 to 15% of the cyclic cows exhibit estrus prior to and immediately after the time PG is administered, resulting in the need for increased length of time to detect estrus or decreased response during the synchronized period after PG injection (Pursley et al., 1995; Twagiramungu et al., 1995). In Experiment 2, 9% (4/45) of the cows in the Select Synch group exhibited estrus after the GnRH injection and before PG (-30 to 0 h), and another 9% (4/45) exhibited estrus immediately after the injection of PG (0 to 18 h), necessitating a prolonged period of estrus detection and AI. Additionally, these cows did not have detectable concentrations of progesterone at the time of PG; of which 4 cows (1 anestrous and 3 cyclic) exhibited estrus after GnRH and before PG (-30 to 0 h). Another 4 cows (4 cyclic) exhibited estrus
immediately after injection with PG (0 to 18 h), and one anestrous cow did not respond to the treatment. These observations indicate that those cows did not respond to the injection of GnRH and exhibited estrus regardless of the treatment. This is in agreement with the previous report that Select Synch treated cows that exhibit estrus early are in the late portion of the estrous cycle (d15 to 17) at the time GnRH is administered (Downing et al., 1998).

The advantages of MGA for synchronization of estrus are ease of administration and low cost. Furthermore, MGA recently received clearance from FDA for use in reproductive classes of beef cattle and dairy heifers (Federal Register, 1997); therefore, research of estrus synchronization methods involving MGA bears increased significance and marked relevance to current industry needs. However, other progestin treatments (i.e. Controlled Intravaginal Drug Release [CIDR], Progesterone Releasing Intravaginal Device [PRID] or Norgestomet implants [as in SYNCRO-MATE-B treatment]) could be used in place of MGA feeding in the 7-11 Synch system, offering a variety of alternatives to fit individual needs. In summary, the 7-11 Synch protocol improved synchrony of estrus in both cyclic and anestrous cows without reducing fertility. This protocol provides potential future application in estrus synchronization and fixed-time AI programs for use in beef cattle production systems.

**Implications**

The advantages of the 7-11 Synch protocol compared to a 14/17-d MGA/PG program include: 1) shorter treatment period; and 2) improved synchrony of estrus. Improved synchrony of estrus should reduce labor costs associated with estrus detection and offset the increased treatment cost of this protocol compared to other estrus synchronization protocols currently available. The drawback associated with 7-11 Synch is that cattle need to be worked 4 times to successfully administer the treatment and AI the cows. The 7-11 Synch protocol, however, provides potential future application in estrus synchronization and fixed-time AI programs for use in beef cattle production systems.

**Literature Cited**


A FIXED-TIME AI PROGRAM FOR BEEF COWS WITH 7-11 SYNCH\textsuperscript{1,2}

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University of Missouri, Columbia, MO\textsuperscript{3}

Abstract

The 7-11 Synch protocol for synchronization of estrus in beef cows results in a tightly synchronized estrus response that generally peaks at 54 h following treatment. The objectives of this study were: 1) to determine the potential for fixed-time AI of beef cows using 7-11 Synch; and 2) to determine whether the addition of GnRH at AI improves pregnancy rates resulting from fixed-time AI. Field trials were conducted at three locations (location 1, n = 90; location 2, n = 120; and location 3, n = 171). Cows were managed at each location in two or three separate herds (herd 1A, 1B, 2A, 2B, 2C, 3A, 3B, or 3C) based on cow age. Cows at each location and within each herd were stratified by age, days postpartum, and body condition score, and randomly assigned to one of two treatments at AI. All cows were synchronized with the 7-11 Synch and were fed melengestrol acetate (MGA: 0.5 mg•hd\textsuperscript{-1}•d\textsuperscript{-1}) for 7 d followed by injections of prostaglandin F\textsubscript{2α} (PG: 25 mg Lutalyse\textsuperscript{®}) on d 7 of MGA, GnRH (100 µg Cystrelin\textsuperscript{®}) on d 11, and PG on d 18. Fixed-time AI was performed 60 h after the last PG with or without GnRH at the time of AI. AI pregnancy rate was determined by ultrasonography 40 to 60 d after AI. Data were analyzed for each herd separately based on the interaction (P < 0.05) among location, herd, age, and AI sire on AI pregnancy rate. There was no difference (P > 0.10) in pregnancy rate resulting from fixed-time AI based on whether or not cows received GnRH at AI. AI pregnancy rates with or without GnRH at AI for each herd were: 1A, 70 % (28/40) and 60 % (24/40); 1B, 44 % (4/9) and 33 % (3/9); 2A, 65 % (22/34) and 73 % (24/33); 2B, 64 % (7/11) and 80 % (8/10); 2C, 53 % (8/15) and 35 % (6/17); 3A, 68 % (15/22) and 43 % (9/21); 3B, 55 % (22/40) and 41 % (16/39); and 3C, 31 % (8/26) and 48 % (11/23), respectively. These data indicate that 7-11 Synch provides significant opportunity to AI cows at a fixed time with resulting high fertility, eliminating the need to detect estrus. Further research is needed to more precisely determine the appropriate time of AI and the necessity of administering GnRH at AI.

Key words: Beef Cows, Estrus Synchronization, Fixed-time AI

Introduction

Genetic improvement of economically important traits in beef cattle can be achieved most rapidly through selection of genetically superior sires and widespread use of artificial insemination (AI). Currently, however, less than 5 % of cow-calf operations in the United States practice AI or utilize any form of estrus synchronization (NAHMS, 1994). The lack of time/labor was the most common reason for not utilizing AI and estrus

\textsuperscript{1} Research supported by USDA-NRI 00-35203-9175.
\textsuperscript{2} The authors gratefully acknowledge Pharmacia Animal Health, Kalamazoo, MI, for providing the Lutalyse® Sterile Solution and Merial, Athens, GA, for providing Cystorelin® - for this research.
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synchronization (NAHMS, 1994). In order to facilitate use of AI and estrus synchronization by cow-calf operations, the development of effective estrus synchronization protocols that allow fixed-time AI without need of estrus detection and resulting high fertility is required.

We previously developed the 7-11 Synch protocol for beef cows which improved synchrony of estrus by effectively synchronizing the first wave of follicle development and ovulation (Kojima et al., 2000). Estrus response following this protocol generally peaks at 54 h (ranging from 42 to 66 h) and more than 90% of beef cows expressed estrus during this period (Kojima et al., 2000). Because of the tight synchrony of estrus, fixed-time AI at 60 h following the 7-11 Synch protocol may not require an injection of GnRH at the time of AI, whereas all available fixed-time AI protocols require an injection of GnRH at AI.

The objectives of this study were: 1) to determine the potential for fixed-time AI of beef cows using the 7-11 Synch protocol; and 2) to determine whether the addition of GnRH injection at AI improves pregnancy rates resulting from fixed-time AI at 60 h following the 7-11 Synch protocol.

Materials and Methods

Experimental Design. Field trials were conducted during the 2001 fall breeding season at three locations (location 1, n = 98; location 2, n = 120; and location 3, n = 171). Angus-based crossbred cows were managed at each location in two or three separate herds (herd 1A, 1B, 2A, 2B, 2C, 3A, 3B, or 3C) based on cow age. Cows at each location and within each herd were stratified by age, days postpartum (day of treatment initiation), and body condition score (BCS; 1 to 9 scale, 1 = emaciated, and 9 = obese), and randomly assigned to one of two treatments at AI (Table 1).

<table>
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<tr>
<th>Herd</th>
<th>Cows (n)</th>
<th>Age</th>
<th>Days postpartum</th>
<th>BCS</th>
</tr>
</thead>
<tbody>
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<td>1A</td>
<td>80</td>
<td>5.4 ± 0.1</td>
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<td>18</td>
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</tr>
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<td>67</td>
<td>7.0 ± 0.4</td>
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<tr>
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</tr>
<tr>
<td>3C</td>
<td>49</td>
<td>6.5 ± 0.3</td>
<td>51.5 ± 1.4</td>
<td>5.8 ± 0.1</td>
</tr>
</tbody>
</table>

*a Means reflect days postpartum for cows in each herd on the first day of melengestrol acetate (MGA) feeding.
*b,c,d,e Unlike superscripts within a column differ (P < 0.05).

All cows synchronized with the 7-11 Synch protocol, were fed melengestrol acetate (MGA: 0.5 mg•hd⁻¹•d⁻¹) in a grain carrier for 7 d (from d 1 to d 7), and received an injection of prostaglandin F₂α (PG: 25 mg Lutalyse® Sterile Solution, Pharmacia Animal Health, Kalamazoo, MI) on the last day of MGA feeding (d 7). Four days after the last MGA feeding (d 11), injection of GnRH (100 µg Cystrelin®, Merial, Athens, GA)
was given, and a second injection of PG was given 11 d after the last MGA feeding (d 18). Fixed-time AI was performed 60 h after the last injection of PG with or without an injection of GnRH at the time of AI (Figure 1).

Cows at each location and within each herd were stratified by age, days postpartum, BCS, and treatment groups, and randomly assigned to one of two experienced AI technicians. Number of cows in each treatment group at each location and within each herd that were inseminated by an individual technician was approximately equal. Number of AI sires used in these trials was 2, 6, and 4 at location 1, 2, and 3, respectively. Pregnancy rate to AI was determined by ultrasonography (Aloka 500V equipped with 5.0 MHz linear-array transducer, Aloka, Wallingford, CT) 40 to 60 d after AI by a single technician.

**Statistical Analyses.** Effects of treatment on fixed-time AI pregnancy rates were analyzed by Chi-square analysis, and the interaction between variables (location, herd, age, days postpartum, BCS, and AI sire) on AI pregnancy rate was also analyzed (StatView®, SAS institute Inc., Cary, NC). The AI pregnancy rates were defined as the percentage of animals pregnant at 40 to 60 d after the fixed-time AI.

**Results and Discussion**
There were differences (P < 0.05) in age of cows, days postpartum, and BCS within location and between herds (Table 1). Data were analyzed for each herd separately based on the interaction (P < 0.05) among location, herd, age, and AI sire on fixed-time AI pregnancy rate. When data were analyzed for each herd separately, fixed-time AI pregnancy rates were not different (P > 0.10) between treatments (with GnRH or without GnRH: Table 2). Pregnancy rates to AI with GnRH resulted in 57.9 % (114/197) and ranged from 31 % to 70 %, while pregnancy rates to AI without GnRH resulted in 52.6 % (101/192) and ranged from 33 % to 80 %.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Cows (n)</th>
<th>with GnRH</th>
<th>without GnRH</th>
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<tr>
<td>1A</td>
<td>80</td>
<td>70 % (28/40)</td>
<td>60 % (24/40)</td>
</tr>
<tr>
<td>1B</td>
<td>18</td>
<td>44 % (4/9)</td>
<td>33 % (3/9)</td>
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<td>2A</td>
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<td>65 % (22/34)</td>
<td>73 % (24/33)</td>
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<td>64 % (7/11)</td>
<td>80 % (8/10)</td>
</tr>
<tr>
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<td>32</td>
<td>53 % (8/15)</td>
<td>35 % (6/17)</td>
</tr>
<tr>
<td>3A</td>
<td>43</td>
<td>68 % (15/22)</td>
<td>43 % (9/21)</td>
</tr>
<tr>
<td>3B</td>
<td>79</td>
<td>55 % (22/40)</td>
<td>41 % (16/39)</td>
</tr>
<tr>
<td>3C</td>
<td>49</td>
<td>31 % (8/26)</td>
<td>48 % (11/23)</td>
</tr>
<tr>
<td>Total</td>
<td>389</td>
<td>58 % (114/197)</td>
<td>53 % (101/192)</td>
</tr>
</tbody>
</table>

**Table 2.**
Pregnancy rates to fixed-time AI at 60 h after the 7-11 Synch with or without GnRH at the time of AI in each herda.

---

a Interaction among location, herd, and AI sire on AI pregnancy rate (P < 0.05).
The 7-11 Synch protocol has resulted in the tightest estrus synchrony (peaking at 48 to 60 h after the last PG) among all estrus synchronization protocols we have tested (Kojima et al., 2000; Stegner et al., 2001). Because of the tight synchrony of estrus this treatment provides, 7-11 Synch is perhaps the best available alternative to use with fixed-time AI. Increased use of AI and rapid genetic improvement in U.S. beef herds could be facilitated if reliable fixed-time AI programs were developed. Results from the present study demonstrate the potential for the development of fixed-time AI programs based on the 7-11 Synch protocol.

The 7-11 Synch protocol provides significant opportunity to AI cows at a fixed time with resulting high fertility, eliminating the need to detect estrus. Further research is needed to more precisely determine the appropriate time of AI and the necessity of administering GnRH at AI.

**Implications**

The 7-11 Synch protocol provides significant opportunity to AI cows at a fixed time with resulting greater fertility, eliminating the need to detect estrus. Further research is needed to more precisely determine the appropriate time of AI and the necessity of administering GnRH at AI.

**Literature Cited**


COMPARISON OF MELENGESTROL ACETATE-BASED ESTRUS SYNCHRONIZATION PROTOCOLS IN YEARLING BEEF HEIFERS

University of Missouri, Columbia, MO

Introduction

Feeding melengestrol acetate (MGA) at 0.5 mg·hd⁻¹·d⁻¹ for 14 d followed by an injection of prostaglandin F₂α (PG) 17 d after MGA withdrawal was found to be an effective method of estrous cycle control in beef heifers (Brown et al., 1988; Patterson and Corah, 1992). Studies show that synchrony of estrus and total estrus response were improved in beef heifers when PG was administered 19 days, instead of 17 days, after MGA withdrawal (Nix et al., 1998; Deutscher et al., 2000; Lamb et al., 2000). Addition of GnRH to the 14-19 d MGA-PG protocol further improved estrus response, estrus synchrony, and ovulation in beef heifers (Wood et al., 2001). Recently, GnRH was added to a short-term progestin-PG protocol (7-11 Synch) and effectively synchronized estrus and ovulation in postpartum beef cows (Kojima et al., 2000). The objective of this study was to identify estrus synchronization protocols that offer potential for use in fixed-time AI programs for replacement beef heifers.

Materials and Methods

Three MGA-based protocols were compared in yearling Angus heifers (n = 345) at the Circle A Ranch Heifer Development Center, Lineville, IA. Heifers were assigned to one of three treatments by reproductive tract score (RTS: Anderson et al., 1991; Patterson et al., 2000), age, and weight 2 wk prior to the initiation of treatments (Table 1: n = 115/treatment). Treatments were: 1) MGA (0.5 mg·hd⁻¹·d⁻¹) for 14 d followed by PG (25 mg Lutalyse® Sterile Solution, Pharmacia Animal Health, Kalamazoo, MI) 19 d after MGA withdrawal (MGA-PG: Nix et al., 1998; Deutscher et al., 2000; Lamb et al., 2000); 2) addition of GnRH (100 µg Cystorelin®, Merial, Athens, GA) on d 26 of the MGA-PG protocol (MGA® Select: Wood et al., 2001); and 3) MGA for 7 d, PG on the last day of MGA, GnRH 4 d after PG, and a second injection of PG 11 d after the last day of MGA (7-11 Synch: Kojima et al., 2000). Figure 1 illustrates treatment schedules for MGA-PG, MGA® Select, and 7-11 Synch. Heifers were monitored for signs of behavioral estrus by the HeatWatch® estrus detection system (DDx, Inc., Denver, CO) for 7 d beginning on the day PG was administered. AI was performed by a single technician 12 h after onset of estrus. A single AI sire was used in this study. The natural service breeding season began

1 Contribution from the Missouri Agriculture Experiment Station. This research was supported by USDA-NRI 00-35203-9175. The authors gratefully acknowledge Pharmacia Animal Health, Kalamazoo, MI, for providing the Lutalyse® Sterile Solution; Merial, Athens, GA, for providing Cystorelin®; and KABA/Select Sires, Inc., for financial support of this research.

14 d after the last AI for 45 d. Pregnancy diagnosis was performed by ultrasonography (Aloka 500V: Aloka, Wallingford, CT) by a single technician at 35 d after the last AI and also at 40 d after the end of breeding season. Estrus response, synchronized conception rate, synchronized pregnancy rate, and final pregnancy rate were analyzed by Chi-square analysis. Synchrony of estrus was analyzed by ratio of variance (F-test) for mean time interval to onset of estrus.

**Results**

Estrus response did not differ (P > 0.10) among treatments. Estrus synchrony was greater (P < 0.05) for 7-11 Synch treated heifers (331.9) than for MGA-PG (667.1) or MGA® Select treated heifers (539.3: Figure 2 and Table 2). Synchronized conception rate and synchronized pregnancy rate were greater (P < 0.05) for MGA-PG (63 % and 54 %) than MGA® Select (45 % and 39 %) or 7-11 Synch (47 % and 37 %) treated heifers (Table 3). There was an effect of reproductive maturity/cycling status of heifers, indicated by RTS, on estrus response, synchronized conception rate, and synchronized pregnancy rate regardless of treatment. Estrus response (93 % and 81 %), synchronized conception rate (66 % and 46 %), and synchronized pregnancy rate (62 % and 37 %) were all greater (P < 0.01) among cycling compared to non-cycling heifers.

**Summary**

Estrus response, synchronized conception rate, and synchronized pregnancy rate were all lower than previous trials (Wood et al., 2000, 2001). Pregnancy rates were high among heifers assigned to the MGA-PG treatment, however, the variance for interval to estrus was greater. The variance for interval to estrus was lowest among the 7-11 Synch treated-heifers; indicating better estrus synchrony. Reproductive maturity/cycling status significantly influenced response to synchronization treatment and subsequent conception and pregnancy rate in yearling beef heifers.

### Table 1. Age and weight of heifers at the 1st day of MGA feeding.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of heifers</th>
<th>Age (day)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-PG</td>
<td>115</td>
<td>405 ± 1.7a</td>
<td>286 ± 2.0</td>
</tr>
<tr>
<td>MGA® Select</td>
<td>115</td>
<td>404 ± 1.5a</td>
<td>286 ± 1.9</td>
</tr>
<tr>
<td>7-11 Synch</td>
<td>115</td>
<td>422 ± 1.4b</td>
<td>286 ± 1.8</td>
</tr>
</tbody>
</table>

*a, b Numbers with different superscript within column differ (P < 0.01).
* Initiation of MGA feeding was 15 days later in 7-11 Synch group.

### Table 2. Estrus response, interval to estrus, and variance for interval to estrus (estrus synchrony).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estrus response</th>
<th>Interval to estrus (h)</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-PG</td>
<td>100/115 (87 %)</td>
<td>54.7 ± 2.6</td>
<td>667.1a</td>
</tr>
<tr>
<td>MGA® Select</td>
<td>100/115 (87 %)</td>
<td>52.0 ± 2.4</td>
<td>593.3a</td>
</tr>
<tr>
<td>7-11 Synch</td>
<td>91/115 (79 %)</td>
<td>52.1 ± 1.9</td>
<td>334.9b</td>
</tr>
</tbody>
</table>

*a, b Numbers with different superscript within column differ (P < 0.01).
Table 3. Synchronized conception, synchronized pregnancy, and final pregnancy rates.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Synchronized conception</th>
<th>Synchronized pregnancy</th>
<th>Final pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGA-PG</td>
<td>61/100 (61 %)</td>
<td>61/115 (53 %)</td>
<td>96/115 (83 %)</td>
</tr>
<tr>
<td>MGA® Select</td>
<td>46/100 (46 %)</td>
<td>46/115 (40 %)</td>
<td>101/115 (88 %)</td>
</tr>
<tr>
<td>7-11 Synch</td>
<td>44/91 (48 %)</td>
<td>44/115 (38 %)</td>
<td>96/115 (83 %)</td>
</tr>
</tbody>
</table>

a, b Numbers with different superscript within column differ (P < 0.08).
c, d Numbers with different superscript within column differ (P < 0.05).

Figure 1. Illustration of the treatment schedule for MGA-PG, MGA® Select, and 7-11 Synch. MGA = melengestrol acetate, and PG = prostaglandin F$_{2\alpha}$.

Figure 2. Distribution of estrus response in heifers treated with MGA-PG, MGA® Select, and 7-11 Synch (Hour 0 = time of prostaglandin F$_{2\alpha}$ [PG] administration).
References


RESYNCHRONIZATION OF ESTRUS IN CATTLE OF UNKNOWN PREGNANCY STATUS USING ESTROGEN, PROGESTERONE, OR BOTH

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*Northwest Research and Extension Center, Kansas State University, Colby
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Abstract

Our objectives were to develop a treatment applied to cattle of unknown pregnancy status that would synchronize the repeat estrus of nonpregnant females. In Exp. 1, previously inseminated dairy and beef heifers were assigned randomly to three treatments at 13 d after AI: 1) no further treatment (controls; n = 44); 2) 0.5 mg of estradiol cypionate (ECP) was given i.m. on d 13 and 20 at the time of insertion and removal of a used intravaginal P4-releasing insert (CIDR-B; P4+ECP; n=44); and 3) same as P4+ECP without the injections of ECP (P4; n = 42). The P4+ECP (>90%) and P4 (>75%) protocols effectively synchronized repeat periods of estrus to 2 d and did not harm established pregnancies, but tended to reduce conception rates of heifers inseminated after CIDR removal. In Exp. 2, treatments were similar to those in Exp. 1 were applied to previously inseminated beef heifers in which feeding 0.5 mg of melengestrol acetate (MGA) replaced the CIDR as a source of progestin and it was fed on d 13 through 19 after AI. Reinsemination rates exceeded 86% in all treatments but conception at the resynchronized estrus was reduced (P<0.05) to 61 to 71% of controls. In Exp. 3, previously inseminated lactating beef cows at four locations were assigned within herd to three treatments according to days postpartum, parity, breed, (primiparous vs. multiparous), and previous synchronization treatment used before their initial AI: 1) no further treatment (control; n = 307); 2) as in Exp. 1, but P4 + 1 mg of estradiol benzoate on d 13 and 20 (P4+EB; n = 54); and 3) as in Exp. 1, P4+ECP (n=148). At two locations, insertion of the used CIDRs and first estradiol (EB or ECP) injections occurred on d 11 after timed AI (TAI) and CIDR removal and second estradiol injections on d 18. Treatments with P4 +ECP did not reduce pregnancy rates in pregnant cows at any location, but increased (P<0.05) the 20- to 23-d return rate from 29% in controls, to 86% in P4+EB, and 65% in P4+ECP cows. Conception rates were not reduced when treatments occurred between d 13 and 20. Overall 23-d pregnancy rates after two inseminations and embryo survival between d 29-33 and 54-61 of pregnancy did not differ among treatments. In Exp. 4, lactating beef cows were assigned as in Exp. 3 to three treatments: 1) no further treatment(controls; n = 51); 2) P4 +ECP (n = 47); and 3) a single injection of ECP on d 13 (n = 48). Although established pregnancies were not harmed, return rates of nonpregnant cows were not different among treatments and the single injection of ECP caused more than 30% of the returns to occur after 8 d and more than 60% on 11 to 12 d after ECP injection. In both heifers and lactating beef cows, the resynchronization treatments increased synchronized return rates in cases where estrus-detection rates were poor, seemed to have no negative effects on established pregnancies, but seemed to reduce conception rates in heifers but not cows at the resynchronized estrus.
Introduction

Pregnancy outcome after synchronization of estrus and ovulation is unknown until cows return to estrus (19 to 24 d after AI) or after diagnosis of pregnancy. In such cases, the full extent of the advantages for synchronizing estrous cycles is not realized. Because the cycles of most nonpregnant cows are closely synchronized, resynchronization for a subsequent AI is possible. From an economic standpoint, if the second estrus can be resynchronized with little additional cost because of the existing synchrony from the first synchronization, costs associated with the first estrus can be spread out over a second breeding. New and previously used progestin-releasing inserts or implants have been used successfully to reduce the variation in returns to service after AI in previously synchronized cows and heifers (Stevenson and Mee, 1991; Favero et al., 1993, 1995; Van Cleeff et al., 1996; Purvis and Whittier, 1997). The variability in fertility results from these studies may imply that the synchrony of follicular growth could be improved. Reinsemination of nonpregnant females at the first eligible estrus can be facilitated by resynchronization of estrus (Van Cleeff et al., 1996) and may increase conception at the resynchronized estrus (Stevenson and Mee, 1991). Reinsertion of a progestin insert or implant between 13 and 20 d after AI in addition to an injection of estradiol benzoate (EB) given at insertion and removal of the progestin treatment increased the probability of identifying nonpregnant cows within 2 d after the second EB injection (Macmillan et al., 1997). Moreover, treatment of EB 13 d after the first insemination was effective in turning over the dominant follicle without affecting CL function or pregnancies established at a previous AI (Macmillan et al., 1997; Burke et al., 2000).

The only estrogen product available in the U.S. for use in the bovine is estradiol cypionate (ECP). It has multiple label indications including one “to correct anestrus [absence of heat period] in the absence of follicular cysts” at doses of 3 to 5 mg. Esterification of estradiol-17β to produce EB or ECP increases the half-life of the estrogen compared with metabolism of the parent estradiol product following hydrolysis (Vynckier et al., 1990). Studies using 10 mg of ECP produced peak concentrations of plasma estradiol at 20 h that remained elevated for up to 5 d (Vynckier et al., 1990). Administration of 2 mg of ECP was sufficient to cause ovariectomized heifers to display estrus (Lefebvre and Block, 1992). It has been demonstrated that 1 or 0.5 mg of ECP induces an LH surge in lactating dairy cows (Pancarci et al., 2002; Stevenson et al., 2002) and dairy heifers (Lopes et al., 2000) when given 24 h after a luteolytic dose of PGF2α.

The present study was comprised of four experiments to determine if resynchronization of estrus was feasible in beef cattle. The objectives were to determine whether treatments would: 1) reduce pregnancy rates in previously inseminated cattle whose pregnancy status was unknown at the onset of treatments; 2) increase AI resubmission rates with subsequent normal fertility; and 3) increase cumulative pregnancy rates after two inseminations. The first experiment utilized progesterone (P4; via a used CIDR-B insert) and P4 + ECP as tools to resynchronize estrus in dairy and beef heifers compared to controls. The second experiment tested MGA as the progestin plus or minus ECP with controls in beef heifers. The third experiment tested P4 + either EB or ECP with controls in suckled beef cattle. The fourth experiment tested P4 + ECP and ECP alone with controls in suckled beef cattle.
Materials and Methods

Experiment 1

Previously inseminated Holstein heifers (n = 68) were studied between September 2000 and August 2001 in six replications and purebred Angus, Simmental, and Hereford heifers (n = 62) during the spring 2001 breeding season. Heifers were assigned randomly within breed to three treatments on d 13 (range: 11 to 15 d) after first insemination (Figure 1): 1) no further treatment (controls; n = 44); 2) 0.5 mg of ECP (Pharmacia Animal Health, Kalamazoo, MI) was given i.m. on d 13 and 20 at the time of insertion and removal of a used intravaginal progesterone-releasing insert (CIDR-B; P4+ECP; n = 44); and 3) same as P4+ECP without the injections of ECP (P4; n = 42).

Beef heifers were observed visually for signs of estrus at multiple times in daylight hours during periods of expected returns to estrus (d 18 to 26). Dairy heifers were fitted with electronic estrus-detection devices (HeatWatch, DDx Inc., Denver, CO) for detection of standing estrus. Once estrus was detected, inseminations were performed 8 to 14 h later by one technician for dairy heifers and three technicians for beef heifers. Pregnancy was diagnosed once between 27 and 34 d after AI by transrectal ultrasonography to visualize fluid, embryo, or both.

Blood samples were collected from all heifers via puncture of a coccygeal vessel on d 13 and 20 after the initial AI corresponding to when the CIDR was inserted and removed. Progesterone was measured in blood sera using a specific validated radio-immunoassay (Skaggs et al., 1986). The inter- and intra-assay coefficients of variation were 11.3% and 8.6%, respectively, for two assays.

Characteristics of estrus for dairy heifers were calculated from HeatWatch data (duration of estrus, number of standing events, duration of all standing events, and duration of individual standing events) for the first post-insemination estrus (18 to 26 d). Conception rates at the initial insemination and those at the resynchronized estrus, concentrations of progesterone on d 20 when CIDR inserts were removed, percentages of heifers with low (<1 ng/mL) or high ($1 ng/mL) concentrations of progesterone on d 20, 26-d pregnancy rate (proportion pregnant after two inseminations), interval between inseminations, and percentage of nonpregnant heifers detected in estrus (AI resubmission rate for second service after resynchronization) were analyzed using procedure GLM (SAS, 2001). The model consisted of treatment, location (dairy vs. beef), and its interaction. Because AI technicians and sires were unique to each location, those effects were confounded with location. Means were separated by orthogonal contrasts (control vs. both P4 treatments and P4 vs. P4 + ECP) or by LSD tests when associated with a protected F-test ($P \#0.05$) in the GLM procedure. Levene's test for heterogeneity of variance (Milliken and Johnson, 1984) was used to analyze the variability of return-to-estrus patterns after resynchronization treatments.
Figure 1. Experimental protocols employed in each of four experiments. Blood samples were collected on d 13 and 20 after first insemination when CIDR inserts were placed intravaginally and removed, respectively. Experiment 1 consisted of three treatments: CIDR (progesterone), progesterone + 0.5 mg of ECP (estradiol cypionate), and control. Experiment 2 consisted of three treatments: MGA (melengestrol acetate was fed on d 13 through 19), MGA + 0.5 mg of ECP (estradiol cypionate), and control. Experiment 3 consisted of three treatments: CIDR, CIDR + 1 mg of EB (estradiol benzoate), and CIDR + 0.5 mg of ECP. Experiment 4 consisted of three treatments: CIDR + 0.5 mg of ECP, single injection of 0.5 mg of ECP on d 13 (not shown), and control. Females were observed for estrus and inseminated during MGA feeding (Exp. 2) through d 33 or on or after CIDR inserts removal (d 20). CIDR = controlled internal drug release insert that is placed intravaginally through which progesterone is released.

**Experiment 2**
This experiment was conducted at Losey Land and Cattle, Agra, KS with yearling Angus and Hereford and Angus crossbred heifers. Heifers used in this study were previously synchronized with a standard MGA + PGF\(_{2\alpha}\) protocol; 0.5 mg of MGA fed per head per day for 14 d and 25 mg of PGF\(_{2\alpha}\) (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 19 d after the last feeding of MGA. Heifers were inseminated based on the AM-PM rule until 72 h after PGF\(_{2\alpha}\), at which time all heifers that had not shown estrus were inseminated. The average day of insemination was considered to be d 0 of the current study. A majority (431 of 439) were inseminated from 1.5 d before to 1.5 d after d 0, and the remaining heifers had been time inseminated but then were observed in estrus 2 to 2.5 d later and were reinseminated. Immediately following the initial AI, heifers were returned to new pens based on the time they were inseminated. Five days before resynchronization treatments were begun, each pen of heifers was gate cut to divide the heifers into three treatments. Heifers that returned to estrus before sorting (d 3 to d 8) into resynchronization treatments were excluded from the experiment. Treatments for this experiment (Figure 1) were: 1) no further treatment (control; n = 87); 2) MGA (n = 176) fed at 0.5 mg\(\text{hd}^{-1}\text{d}^{-1}\) from d 13 (d 0 = mean day of previous insemination) through d 19; and 3) MGA + ECP (n = 176), which was the same as the previous treatment plus 0.5 mg (i.m.) of ECP administered on d 13 and 20.
Heifers were observed for estrus at least twice daily from d 0 to d 33 and were reinseminated according to the AM-PM rule.

Pregnancy was determined on d 33 (check of initial insemination) and d 59 (check of resynchronized insemination) by transrectal ultrasonography to visualize embryo, fluid or both. Data were analyzed with the mixed models procedure (SAS, 2001). Treatment and type of first AI (estrus AI or TAI) were considered fixed effects and sire and technician were considered random effects.

Experiment 3

This experiment was conducted at four locations: University of Minnesota Research and Outreach Center, Grand Rapids (81 Angus cows); DarLynn Ranch, Pierz, MN (149 Angus, Hereford, and South Devon cows); Kansas State University Purebred Beef Unit, Manhattan (161 Angus, Hereford, and Simmental cows); and Thielen Ranch, Dorrance, KS (218 cows consisting of a three-way rotational cross of Angus, Hereford, and Simmental). Cows were inseminated previously in another experiment (Stevenson et al., 2002) and assigned within herd to three treatments according to days postpartum, parity (primiparous vs. multiparous), and previous synchronization treatment used before their initial TAI (Figure 1): 1) no further treatment (control; n = 307); 2) a previously used CIDR insert was reinserted 13 d after TAI for a period of 7 d and 1 mg of estradiol benzoate (EB; Sigma Chemical, St. Louis, MO) diluted in sesame oil was administered i.m. on d 13 when the CIDR was inserted and on d 20 when it was removed (P4 + EB; n = 154); and 3) as in the previous treatment but 0.5 mg of ECP was injected at insertion and removal of the used CIDR (P4 + ECP; n = 148). At the two Minnesota locations, insertion of the used CIDR's and first estradiol (EB or ECP) injections occurred on d 11 after TAI and CIDR removal and second estradiol injections on d 18. Blood samples were collected at insertion and removal of the CIDR insert as in Exp. 1 and assayed for progesterone (Skaggs et al, 1986) in six assays with intra- and interassay CV's of 6.4 and 7.8%, respectively.

Cows were observed for estrus two or three times daily from d 19 to 23 d after TAI at the Kansas locations and from d 17 to 21 at the Minnesota locations. Cows in estrus were inseminated 8 to 14 h after first detected estrus. Sires and inseminators were distributed equally among treatments and were confounded within herd. Pregnancy was diagnosed 29 to 33 d after the initial TAI and again 54 to 61 d after TAI (or 35 to 42 d after the second AI that followed resynchronization treatments) by transrectal ultrasonography to visualize fluid, embryo, or both.

All variables (described in Exp. 1) were analyzed using procedure GLM (SAS, 2001) using a model that consisted of resynchronization treatment, location (MN vs. KS), cycling status at the time of the first AI (based on previous blood collection [Stevenson et al.,2002]), parity (primiparous vs. multiparous), all two-way interactions with treatment, with days postpartum and body condition score (BCS; 1 = thin and 9 = fat; Whitman, 1975) as regression variables. Least-square means or unadjusted mean percentages were separated using LSD tests (SAS, 2001) when associated with a protected F-test (P #0.05) in procedure GLM or by orthogonal contrasts (control vs. both CIDR treatments; and P4 +ECP vs. P4 + EB).
**Experiment 4**

This experiment was conducted at the Kansas State University Purebred Unit with 146 previously inseminated purebred Angus, Hereford, and Simmental lactating cows used in a previous experiment (Stevenson et al., 2002). After the initial timed insemination, cows were assigned randomly to three resynchronization treatments based on breed, parity (primiparous vs. multiparous), days postpartum, and previous synchronization treatment used before TAI (Figure 1): 1) no further treatment (control; n = 51); 2) a previously used CIDR insert was reinserted on d 13 d after TAI for a period of 7 d and 0.5 mg of ECP was injected at its insertion and removal (P4 + ECP; n = 47); and 3) a single 0.5 mg injection of ECP on d 13 (ECP; n = 48). Blood samples were collected as in Exp. 1 and assayed for progesterone (Skaggs et al, 1986) in two assays with inter- and intraassay CV’s of 7.5 and 6.8%, respectively.

Cows were observed twice daily for estrus after the initial TAI and were reinseminated as in Exp. 3. Pregnancy was diagnosed as described above at 35 to 36 d after the TAI and after second AI that followed resynchronization treatments. All variables (described for Exp. 1 and 3) were analyzed in procedure GLM (SAS, 2001) in a model including resynchronization treatments, parity (primiparous vs. multiparous), breed, and cycling status at the initial TAI (based on previous blood collection [Stevenson et al., 2002]). All two-way interactions with treatment were included with days postpartum and BCS as regression variables. Least-square means or adjusted mean percentages were separated using LSD tests (SAS, 2001) when associated with a protected F-test ($P \leq 0.05$) in procedure GLM or by orthogonal contrasts (control vs. P4+ECP and ECP; and P4+ECP vs. ECP).

These studies were conducted while availability of new CIDR inserts was limited so used CIDR inserts were employed. The used CIDR’s contained either 1.38 or 1.9 g of progesterone when new and had been used once or twice previously in lactating cows. We have conducted previous studies with new CIDR inserts under the authorization of the U.S. Food and Drug Administration Investigational New Animal Drug 6450. The intent of the current treatments was to test the efficacy of supplying progesterone to prevent premature occurrence of repeat estrus during the resynchronization treatment period rather than testing the used CIDR insert itself, which could not be done without the concurrent administration of new CIDR inserts as controls. Application of the previously used CIDR inserts in no way implies that we endorse their reuse. Although precautions were taken to clean and sanitize the used CIDR inserts prior to their reuse, no guarantee of their purity, potency, or sterility can be made.

**Results and Discussion**

**Experiment 1**

Distribution of estrus after CIDR removal for the nonpregnant dairy (visual observations plus electronic estrus-detection system) and beef heifers (visual observation) is illustrated in Figure 2. Most of the heifers in the P4 + ECP (64%) treatment were in estrus the day after CIDR removal. The majority of the controls came into estrus before (25%), on the day of CIDR removal (25%), or 4 or more d after CIDR removal (19%). On the day following the second ECP injection (d 1), more ($P < 0.05$) P4+ECP heifers (64%) were in estrus than in either of the other treatments (P4 = 33%; control = 13%). In fact, the P4+ECP treatment produced nearly as many heifers in estrus on d 1 (64%) as the P4 treatment did for...
2 d (d 1 and 2; 76%). The variability of the return pattern was less (P<0.01) in both P4 treatments than in that of the control.

Figure 2. Distribution of repeat estrus in previously inseminated heifers relative to CIDR insert removal for those treated with CIDR inserts for 7 d beginning on d 13 after AI; CIDR inserts + estradiol cypionate (ECP); or controls. The pattern of return was less (P<0.01) variable in both resynchronization treatments that employed the CIDR compared to controls (Exp. 1).

Table 1 summarizes the reproductive characteristics of all heifers to which the resynchronization treatments were applied. Based on elevated ($\geq 1$ ng/mL) concentrations of progesterone on d 20 (CIDR removal), more (P < 0.05) P4-treated heifers had high progesterone than controls. However, concentrations of progesterone for the P4 treatment tended (P = 0.09) to be greater than those of the P4+ECP treatment, suggesting that the prior ECP injection on d 13 may have reduced progesterone secretion. Serum concentrations of progesterone were less (P<0.05) in beef than in dairy heifers on d 13 (4.1 $\pm$ 0.5 vs. 5.5 $\pm$ 0.2 ng/mL) and 20 (3.4 $\pm$ 0.4 vs. 4.5 $\pm$ 0.4 ng/mL). Although the proportion pregnant varied from 47 to 60%, the resynchronization treatments seemed to have no adverse effect on the proportion of heifers that conceived to the previous insemination before their application. Of those heifers that failed to conceive to the first insemination, only numerically fewer controls were detected in estrus following resynchronization treatments than after the P4 or P4+ECP treatments. But 84 to 90% of our nonpregnant heifers were detected in estrus after resynchronization compared to 75% of the heifers that were resynchronized with a CIDR alone for 5 d (d 17 to 22; Van Cleeff et al., 1996). Although limited numbers of heifers were reinseminated, the P4 and P4+ECP treatments tended (P = 0.13) to suppress conception at the repeat service compared to controls. The 26-d pregnancy rate, those conceiving after either the first or second insemination, did not differ among treatments. Further, the interval between first and second AI did not differ among treatments, confirming earlier observations (Stevenson and Mee, 1991; Van Cleeff et al., 1996).
Table 1. Reproductive characteristics of dairy and beef heifers (Exp. 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>P4</th>
<th>P4 + ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High P4 on d 20 after first AI, %</td>
<td>68.2c (30/44)</td>
<td>88.1 (37/42)</td>
<td>81.8 (36/44)</td>
</tr>
<tr>
<td>Concentration of P4 on d 20, ng/mL</td>
<td>3.6 ± 0.4 (44)</td>
<td>4.7 ± 0.5d (42)</td>
<td>3.6 ± 0.4 (44)</td>
</tr>
<tr>
<td>Pregnancy rate after first AI, %</td>
<td>53.2 (25/47)</td>
<td>46.7 (21/45)</td>
<td>60.4 (29/48)</td>
</tr>
<tr>
<td>Returned to estrus 18-26 d after initial AI, %</td>
<td>72.7 (16/22)</td>
<td>84.0e (21/25)</td>
<td>89.5 (17/19)</td>
</tr>
<tr>
<td>Conception of repeat AI, %</td>
<td>60.0f (9/15)</td>
<td>33.3 (7/21)</td>
<td>35.2 (6/17)</td>
</tr>
<tr>
<td>26-d pregnancy rateb, %</td>
<td>72.3 (34/47)</td>
<td>60.0 (27/45)</td>
<td>72.9 (35/48)</td>
</tr>
<tr>
<td>Interval between 1st and 2nd AI, d</td>
<td>22.4 ± 0.7 (16)</td>
<td>22.6 ± 0.6 (21)</td>
<td>21.8 ± 0.6 (17)</td>
</tr>
</tbody>
</table>

aSee Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
bHeifers conceiving after two inseminations.
cDifferent (P < 0.05) from both P4 treatments.
dDifferent (P < 0.09) from P4 + ECP and control.
eOne pregnant heifer was detected in estrus after CIDR removal.
fDifferent (P = 0.13) from both P4 treatments.

Sexual behavioral characteristics of dairy heifers whose estrus was resynchronized were summarized in Table 2. No significant differences among treatments were detected for the duration of standing estrus, total number and duration of all standing events, or duration of individual standing events (Table 2). However, the P4+ECP treatment had the greatest number of standing events and the duration of total standing time. Variability of these traits were not different among treatments.

All nonpregnant heifers in the P4+ECP treatment were observed visually in estrus compared to less than 64% of heifers in the P4 and control heifers. The ECP injection may have enhanced the proportion of heifers visually detected in estrus because of a tendency for more estrual activity induced by ECP (Lefebvre and Block, 1992). Further, because no significant differences were detected, one could conclude that the 0.5 mg dose of ECP used is apparently producing a normal physiological estrus. Estradiol-17β (2 mg) administered 24 h after removal of the progestin caused 98 to 100% of cows to show estrus during a 48-h period (Wiltbank et al., 1971). Similar findings were observed after 0.5 mg of ECP in dairy heifers (Lopes et al., 2000). In the latter study, 100% of heifers treated with ECP were synchronized, whereas 89% of the estrous periods of controls were synchronized. Administration of EB 24 h after CIDR removal seemed to increase the number of heifers exhibiting estrus (Hanlon et al., 1996).
Table 2. Sexual behavioral characteristics of dairy heifers whose estrus was resynchronized based on the HeatWatch system (Exp. 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>P4</th>
<th>P4 + ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td>7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Duration of standing estrus, h</td>
<td>14.0 &quot; 1.9</td>
<td>11.2 &quot; 1.5</td>
<td>11.1 &quot; 1.6</td>
</tr>
<tr>
<td>Total number of standing events</td>
<td>28.6 &quot; 9.5</td>
<td>28.4 &quot; 7.6</td>
<td>40.6 &quot; 7.9</td>
</tr>
<tr>
<td>Total duration of standing events, s</td>
<td>59.2 &quot; 22.5</td>
<td>70.1 &quot; 18.1</td>
<td>94.9 &quot; 19.1</td>
</tr>
<tr>
<td>Duration of individual standing events, s</td>
<td>2.1 &quot; 0.4</td>
<td>2.9&quot; 0.3</td>
<td>2.3 &quot; 0.3</td>
</tr>
<tr>
<td>Observed visually, %</td>
<td>57.1</td>
<td>63.6</td>
<td>100</td>
</tr>
</tbody>
</table>

*See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.

**Experiment 2**

Resynchronization treatments had no negative effect on the pregnancy rates resulting from the initial insemination; 58/87 (67%), 132/176 (75%) and 112/176 (64%) for control, MGA, and MGA + ECP, respectively. Mean hours from the second injection of ECP to estrus tended (P<0.07) to be less for control (54 " 9) than for MGA (72 " 6) or MGA+ECP (74 " 5). Further, the variance of the interval to estrus was greater (P<0.05) for control than MGA or MGA+ECP (1160, 819 and 912, respectively). Greater (P<0.05) proportions of control than MGA or MGA+ECP treated heifers were in estrus before and during MGA feeding (Table 3).

Feeding MGA seemed to delay estrus in the MGA and MGA+ECP treated heifers because 14% of heifers in each treatment were in estrus more than 25 d after the first insemination, whereas none of the controls were in estrus during this period (Figure 3). Distribution of estrus after the second ECP injection shows no clear peak for the MGA or MGA+ECP treatments. Conception rates were greater in control than MGA (P<0.05) or MGA + ECP (P<0.09) treated heifers (Table 3). These differences seem to be due to lower conception rates during the targeted resynchronization period (d 20 to 25) for MGA and MGA + ECP heifers than control heifers (Table 3). Total pregnancy rates resulting from the first and second AI did not differ among treatments. If only a 5-d period of detected estrus had been employed for the second insemination, the 25-d pregnancy rate would not show an advantage for the resynchronization treatments.
That resynchronization had no effect on pregnancy rates to the first insemination agrees with previous studies (Purvis and Whittier, 1997). Despite this, more MGA-treated heifers were in estrus beyond d 25 suggesting that embryonic loss occurred in these heifers. The total percentage of heifers reinseminated was similar among treatments, so perhaps MGA "spared" embryos for a short period of time, but were eventually lost.

Conception rate of beef heifers to a second insemination following resynchronization with MGA was not different from controls, but was numerically lower in MGA-treated heifers (Purvis and Whittier, 1997). Lower conception rates in the MGA and MGA + ECP heifers indicated that some persistent follicles might have developed in heifers assigned to these treatments (Chenault et al., 1990). Based on previous results with estradiol benzoate (Burke et al., 1999) and ECP (El-Zarkouny and Stevenson; 2002), it was expected that the ECP injection on d 13 would initiate a new wave of follicular growth, if the majority of heifers had two follicular waves. A greater variation in cycle duration in yearling heifers may make attempts more difficult to resynchronize estrus, partially because a greater percentage of heifers may have three follicular waves and the existing dominant follicle on d 13 after AI may not be LH-dependent and thus respond to the loss of LH pulses (turn over and lose its dominance) after an estrogen injection (Burke et al., 1999).
### Table 3. Reproductive traits of heifers after resynchronization with MGA or MGA plus ECPa (Exp 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>MGA</th>
<th>MGA+ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>87</td>
<td>176</td>
<td>176</td>
</tr>
<tr>
<td>No. pregnant to first insemination</td>
<td>58</td>
<td>132</td>
<td>112</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>67</td>
<td>75</td>
<td>64</td>
</tr>
<tr>
<td>No. not pregnant</td>
<td>29</td>
<td>44</td>
<td>59</td>
</tr>
<tr>
<td>No. reinseminated (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before MGA (&lt; d 13)</td>
<td>3 (10.3)</td>
<td>1 (2.2)</td>
<td>6 (10.2)</td>
</tr>
<tr>
<td>During MGA (d 13 to 19)</td>
<td>13 (31.0)</td>
<td>3 (6.8)</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>After MGA (d 20 to 25)</td>
<td>10 (34.5)</td>
<td>29 (65.9)</td>
<td>34 (57.6)</td>
</tr>
<tr>
<td>After MGA (&gt;d 25)</td>
<td>0</td>
<td>6 (13.6)</td>
<td>8 (13.6)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (89.7)</td>
<td>39 (88.6)</td>
<td>51 (86.4)</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before MGA (&lt;d 13)</td>
<td>3/3</td>
<td>1/1</td>
<td>4/6 (66.7)</td>
</tr>
<tr>
<td>During MGA (d 13 to 19)</td>
<td>9/13 (69.2)</td>
<td>2/3 (66.7)</td>
<td>2/3 (66.7)</td>
</tr>
<tr>
<td>After MGA (d 20 to 25)</td>
<td>7/9 (77.8)</td>
<td>10/29 (34.5)</td>
<td>16/34 (47.1)</td>
</tr>
<tr>
<td>After MGA (&gt;d 25)</td>
<td>0</td>
<td>5/6 (83.3)</td>
<td>6/8 (75)</td>
</tr>
<tr>
<td>Total</td>
<td>19/25 (76)</td>
<td>18/39 (46)b</td>
<td>28/52 (55)c</td>
</tr>
</tbody>
</table>

aSee Figure 1. MGA was fed for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed. Day 0 = mean day of first insemination.
bMean differs (P<0.05) from Control.
cMean differs (P<0.09) from Control.

### Experiment 3

Timing of administration of resynchronization treatments affected the distribution of returns to estrus (Figure 4) between the Kansas and Minnesota herds. Only one pregnant cow was detected in estrus as a consequence of the P4+EB treatment and she was eliminated from further analyses. In the Minnesota herds, no incidence of return to estrus was observed before CIDR removal in either estrogen treatment (Figure 4; lower panel). In contrast, some Kansas controls returned to estrus on d 1 and 0 (d 19 and 20 after TAI; Figure 4; upper panel). The P4+EB treatment concentrated estrus on d 1 and 2, with the majority on d 1. In contrast, the P4+ECP treatment concentrated estrus on d 2 and 3, but mainly on d 2.

In the Minnesota herds, the second injection of estrogen may not have been as effective in inducing estrus and the LH surge because subsequent estrus was distributed across more than 3 d. In both cases, the second injections of EB and ECP were given on d 18 after TAI when endogenous concentrations of progesterone may yet have been elevated sufficiently to block the LH surge. In either P4+estrogen combination, return to estrus was uniformly distributed over a 3-d period. Similar distribution patterns of estrus on d 1 (43%) and d 2 (42%) after CIDR removal were reported in dairy cattle when a single injection of EB was administered on d 13 (CIDR insertion) and on d 20 at CIDR removal (Macmillan et al., 1997).
That estrus activity in the P4+EB treatment was concentrated and occurred earlier than that of the P4+ECP treatment is consistent with the half-lives of the two forms of estradiol as well as their rates of absorption and conversion to estradiol-17β. Plasma estradiol-17β reached supraphysiologic concentrations 1 to 23 h after EB treatment and remained elevated for 20 to 30 h (Vynckier et al., 1990; Lammoglia et al., 1998). A pronounced increase in the peak of plasma estradiol-17β does not occur after ECP injection. Rather, maximum concentrations are observed 13 to 31 h after treatment and remain elevated for 170 h before decreasing steadily (Vynckier et al., 1990). Therefore, differences in the pattern of estrus distribution may occur either because EB produces adequate concentrations of estradiol-17β to induce follicular atresia earlier than ECP (d 13 injection) or because of prolonged concentration of estradiol-17β after the ECP injection (d 20 injection). Asynchronous emergence or delay of a new follicular wave may occur because of prolonged elevated concentrations of estradiol-17β (Bo et al., 2000). The difference in dosage between EB (1 mg) and ECP (0.5 mg) also may have influenced emergence of the new follicular wave.

More (P<0.05) cows treated with P4+estrogen than controls had elevated concentrations of progesterone on d 20 after TAI when the CIDR insert was removed (Table 4). However, average concentrations of progesterone were only greater (P<0.05) in the P4+ECP treatment compared to controls, with the P4+EB treatment being intermediate. As expected, fewer (P<0.001) cows (53%; n = 62) that were anestrous just prior to the TAI had elevated serum progesterone on d 20 than cycling cows (87%; n = 526), which is reflected in average concentrations of progesterone (anestrous = 2.0 " 2.0 ng/mL; n = 62 vs. cycling = 3.9 " 2.5 ng/mL; n = 526). For each unit increase in BCS, concentrations of progesterone on d 20 were increased by 0.7 " 0.2 ng/mL.

No harm occurred to pregnancies established in cows when resynchronization treatments were applied (Table 4). Pregnancy rates varied from 44 to 52% across treatments, with fewer (P<0.05) anestrous cows conceiving (33%; n = 66) than cycling cows (52%; n = 543).
Table 4. Reproductive characteristics of suckled cows exposed to resynchronization treatments (Exp.3)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>P4 + EB</th>
<th>P4 + ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High P4 on d 20 after initial AI, %</td>
<td>78.1c (228/292)</td>
<td>86.1d (130/151)</td>
<td>90.3d (131/145)</td>
</tr>
<tr>
<td>Concentration of P4 on d 20, ng/mL</td>
<td>3.1 &quot; 0.3c (292)</td>
<td>3.4 &quot; 0.4c,d (151)</td>
<td>4.1 &quot; 0.4d (145)</td>
</tr>
<tr>
<td>Pregnancy rates after initial AI, %</td>
<td>51.8 (159/307)</td>
<td>44.4 (70/153)</td>
<td>51.7 (77/149)</td>
</tr>
<tr>
<td>Returned to estrus 20-23 d after initial AI, %</td>
<td>29.1c (43/148)</td>
<td>83.5d (71/85)</td>
<td>65.3d (47/72)</td>
</tr>
<tr>
<td>23-d pregnancy rateb, %</td>
<td>54.7 (168/307)</td>
<td>60.1 (92/153)</td>
<td>68.5 (102/149)</td>
</tr>
<tr>
<td>Embryo survival, %</td>
<td>86.1 (99/115)</td>
<td>90.0 (45/50)</td>
<td>94.8 (55/58)</td>
</tr>
</tbody>
</table>

aSee Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus either 1 mg of estradiol benzoate (EB) or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
bCows conceiving after two inseminations.
c,dMeans with uncommon superscript letters differ (P<0.05).

The total proportion of nonpregnant cows returning to estrus between d 1 and 3 after CIDR removal (d 0) was 2.2 to 2.9 times greater (P<0.05) after both P4+estrogen treatments than in controls (Table 4). No difference was detected between estrogen treatments, but proportions of primiparous cows (41%; n = 59) that returned to estrus were less (P<0.05) than those of multiparous cows (56%; n = 246).

Intervals between TAI and the repeat estrus were influenced by a treatment Hlocation interaction (P<0.05; Table 5). Because of the earlier administration of the resynchronization treatments in the Minnesota herds, concentrations of serum progesterone on d 18 may have blocked the ability of the second injection of estradiol to induce estrus and the LH surge. Intervals to returned estrus among treatments were not different. In contrast, in the Kansas herds in which resynchronization treatments were administered between d 13 and 20, the P4+ECP treatment prolonged (P < 0.05) the average interval to estrus compared to controls and P4+EB.

Table 5. Interval to estrus and conception rate after resynchronization of estrus at Kansas and Minnesota locations (Exp.3)

<table>
<thead>
<tr>
<th>Item</th>
<th>Location</th>
<th>Control</th>
<th>P4 + EB</th>
<th>P4 + ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval between 1st and 2nd AI, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>20.8 &quot; 0.3b (26)</td>
<td>21.2 &quot; 0.2b (48)</td>
<td>22.4 &quot; 0.2e (31)</td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>21.6 &quot; 0.3 (17)</td>
<td>21.6 &quot; 0.2 (23)</td>
<td>22.3 &quot; 0.3 (16)</td>
<td></td>
</tr>
<tr>
<td>Conception rate of repeat AI, %</td>
<td>KS</td>
<td>65.4 (26)</td>
<td>52.1 (48)</td>
<td>64.5 (31)</td>
</tr>
<tr>
<td>MN</td>
<td>52.9d (17)</td>
<td>17.4e (23)</td>
<td>50.0de (16)</td>
<td></td>
</tr>
</tbody>
</table>

aSee Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus either 1 mg of estradiol benzoate (EB) or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
bTreatment Hlocation interaction (P<0.05)
c,dTreatment Hlocation interaction (P=0.07).
Interval to estrus: DPP per 10 d = 7.9 " 3.5% (P<0.05). CR: BCS per unit = 17.1 " 7.2% (P<0.05).
As a consequence, conception rates also were affected differently between locations (P < 0.05). No difference among treatments were observed for the Kansas herds, but for the Minnesota herds, the P4+EB treatment tended (P=0.07) to reduce conception rates compared to controls.

The 23-d pregnancy rates tended (P = 0.15) to be greater after both P4+estrogen treatments, with the biggest difference between the P4+ECP and controls (Table 4). The 23-d pregnancy rate was greater in cycling cows (62%; n = 543) than in anestrous cows (41%; n = 66). This relationship is substantiated by the fact that for each 10-d increase in days since calving at the time of first AI, a 2.8 \( \times \) 1.1% increase (P<0.01) in 23-d pregnancy rates was detected. Pregnancy rate is a product of the rates of detected estrus or AI submission rate and conception rates. Therefore, because both P4+estrogen treatments increased return rates and did not affect conception rates, it is not surprising that pregnancy rates tended to improve relative to controls. In another study, the combination of EB+CIDR effectively synchronized returns to service of nonpregnant cows, and as a consequence, overall pregnancy rates increased (Macmillan et al., 1997). Our results further indicated that the P4+ECP treatment was as effective as EB+CIDR in synchronizing returns to estrus and subsequently increasing total 23-d pregnancy rates. In fact, nearly 69% of the cows treated with P4+ECP were pregnant after two inseminations. Based on the fact that the only estrogen available in the U.S. market is ECP, this study opens the possibility for its use in mature or lactating beef cattle for resynchronization of estrus.

Embryo survival in pregnant cows after the initial pregnancy diagnosis between d 29 and 33 after the TAI was affected (P< 0.05) by herd. Although survival was numerically greater in the P4+ECP cows, it did not differ from the other two treatments. Most embryo deaths seem to occur between d 8 and 16 or 18 (Roche, 1981). Pregnant cows have elevated concentrations of progesterone when compared to nonpregnant and cycling cows from d 6 to d 14 or 18 after estrus, which ensures a quiescent uterus during early stages of embryo development (Robinson et al., 1989). Luteal insufficiency is considered to be one cause of embryonic death. Interestingly, the proportion of cows in each treatment that had elevated progesterone on d 20 (Table 5) paralleled the percentages of embryo survival (Table 4). Supplementing exogenous progesterone may prevent low concentrations of progesterone from occurring in the maternal circulation and prevent embryo losses (Stevenson and Mee, 1991; Van Cleeff et al., 1996). In dairy cows, providing supplemental progesterone with a progesterone-releasing intravaginal device (PRID) increased pregnancy rates when treatments were initiated no earlier than d 3 after AI (Robinson et al., 1989; Van Cleeff et al., 1991), but not consistently (Stevenson and Mee, 1991). Supplemental progestin during the luteal phase tended to increase conception rates (Wilmut et al., 1986) or calving rates of beef heifers (Faveria et al., 1993). In Exp. 3, the CIDR was inserted between d 13 and 20 (Kansas herds) or d 11 and 18 (Minnesota herds) after TAI, thus covering that time interval when embryo loss is reported to be greatest. It can be assumed that supplemental progesterone from the CIDR might prevent early embryo death when combined with the appropriate form of estradiol, but further observations are warranted.
**Experiment 4**

Distribution of estrus after a single injection of ECP on d 13 or the 7-d CIDR plus injections of ECP on d 13 and 20 is illustrated in Figure 5. Nearly 67% of the P4+ECP cows were in estrus on d 2 after removal of the CIDR, with the pattern of distribution of estrus after the single ECP injection skewed to the right of the figure. More than 62% of the ECP-treated cows were in estrus on or after d 4 ($d_2$ 24 or $d_1$ 11 d after ECP injection). Our results don’t dispute previous observations that estrus occurred 9 to 10 d after a single injection of EB was administered on d 13 of the estrous cycle (Burke et al., 2000). However, the apparent bimodal distribution of return to estrus among cows treated with only ECP on d 13 suggests the possibility that perhaps cows may respond differently depending on whether or not they have a dominant follicle that is responsive to estrogen (i.e., larger second-wave follicle or a smaller third wave follicle).

Results of Exp. 4 are summarized in Table 6. Treatments did not affect the proportion of cows with elevated progesterone or the concentration of progesterone on d 20. Pregnancy rates of cows exposed to these resynchronization treatments were not different, but as in Exp. 3, anestrous cows at the time of first AI had lower (P<0.05) pregnancy rates (20%; $n=10$) than cycling cows (46%; $n=136$). Rates of return to estrus between 0 and 5 d after CIDR insert removal (20 to 25 d after TAI) were unaffected by treatment. Intervals between inseminations tended (P=0.09) to be longer in P4+ECP and ECP-treated cows compared to controls. Conception rates and 25-d pregnancy rates after resynchronization did not seem to be affected by treatments, although an insufficient number of cows were tested in this experiment to detect differences.

![Figure 5. Distribution of repeat estrus in previously inseminated lactating beef cows relative to CIDR insert removal for those treated with CIDR inserts for 7 d beginning on d 13 after AI + estradiol cypionate (ECP) on d 13 and 20; ECP on d 13; or controls (Exp. 4).](image-url)
Table 6. Reproductive characteristics of suckled cows exposed to resynchronization treatments (Exp.4)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>P4 + ECP</th>
<th>ECP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High P4 on d 20 after initial AI, %</td>
<td>60.8 (31/51)</td>
<td>61.7 (29/47)</td>
<td>62.5 (30/48)</td>
</tr>
<tr>
<td>Concentration of P4 on d 20, ng/mL</td>
<td>2.0 ± 1.0</td>
<td>2.4 ± 0.7</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>Pregnancy rates after initial AI, %</td>
<td>41.2 (21/51)</td>
<td>42.6 (20/47)</td>
<td>47.9 (23/48)</td>
</tr>
<tr>
<td>Returned to estrus 20-25 d after initial AI, %</td>
<td>73.0 (22/30)</td>
<td>75.0c (21/28)</td>
<td>61.5c (16/26)</td>
</tr>
<tr>
<td>Interval between 1st and 2nd AI, d</td>
<td>20.1 ± 1.8d (22)</td>
<td>22.3 ± 1.7 (21)</td>
<td>25.1 ± 1.3 (16)</td>
</tr>
<tr>
<td>Conception rate of repeat AI, %</td>
<td>81.0 (17/21)</td>
<td>65.0 (13/20)</td>
<td>53.3 ( 8/15)</td>
</tr>
<tr>
<td>25-d pregnancy rateb, %</td>
<td>74.5 (38/51)</td>
<td>70.2 (33/47)</td>
<td>62.5 (30/48)</td>
</tr>
</tbody>
</table>

*aSee Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed or a single injection of ECP was given on d 13.

bCows conceiving after two inseminations.

cOne cow in each treatment showed estrus and was reinseminated when pregnant.

dDifferent (P=0.09) from P4 + ECP and ECP. Breed × treatment interaction (P<0.01).

General Discussion

Recently, it was suggested that the combination of estradiol and progesterone may be useful to manipulate ovarian follicular development and may have important implications for use of AI (Bo et al., 1995). Our results confirm those conclusions and suggest that either P4+EB or P4+ECP are effective in synchronizing returns to estrus in lactating cattle. Perhaps because of high rates of return and possible reduced conception rates in Exp. 1 and 2, resynchronization treatments may not be needed or cost effective for heifer. One purpose of these studies was to determine if the first eligible estrus after insemination could be resynchronized without harming established pregnancies. It seems clear from all four experiments that administering either estrogen alone or in combination with a 7-d treatment with progesterone via the CIDR insert or MGA that established pregnancies were unaffected. This finding was true for heifers and lactating cows. Established pregnancies likewise were unaffected. This finding was true for heifers and lactating cows. Established pregnancies likewise were unaffected when injections of EB were administered on d 12, 13 or 14 after AI (Macmillan et al, 1997). In addition, injections of 1 mg of EB or 0.5 mg of ECP administered at insertion and at removal of a used CIDR did not compromise the ability of the CL to maintain pregnancies already established in our studies. Some caution is warranted in interpreting the lack of harm of resynchronization protocols attempted. In none of the individual experiments were there sufficient observations to avoid making a type II error. However, collectively, the data suggest that no harm was caused to ongoing pregnancies.

Our results show that administering progesterone via the CIDR was also effective in preventing occurrence of spontaneous estrus before its removal. The combination of estradiol and progesterone were effective in inducing atresia of dominant follicles regardless of their age or diameter (Burke et al., 1999). Further, they suggested that duration of progesterone treatment must be sufficient to prevent occurrence of estrus until complete spontaneous
luteolysis has occurred. Occurrence of estrus in control cows in the Kansas herds in Exp. 3 on d 1 and 0 was consistent with occurrence of luteolysis before d 19 of the estrous cycle. In contrast, because detection of estrus in the Minnesota herds started on d 17, and no estrus was observed in control cows until d 19 (d 1 after CIDR removal), then the duration of progesterone treatment must have been sufficient. Control heifers in Exp. 2 came into estrus consistently during the resynchronization treatments and even a few MGA treated heifers were in estrus during MGA feeding. Ensuring adequate consumption of MGA in a resynchronization treatment or synchronization protocol is a limitation to its use.

Concentrating the distribution of estrus into a short, predictable time frame provides advantages for an AI program. Detection of estrus is both time consuming and labor intensive, especially for repeat estrus periods after a failed AI because the interval to estrus is more variable than in noninseminated females (Van Cleeff et al., 1996). Our results with either EB or ECP plus P4 confirm previous observations that injections of estrogen on d 13 and 20 of the estrous cycle corresponding to insertion and removal, of a used CIDR respectively, synchronized returns to estrus of nonpregnant cows (Macmillan et al., 1997).

The purpose of the first estrogen injection is to initiate a new follicular wave so that the timing of the new dominant follicle corresponds to withdrawal of progesterone when the CIDR is removed. The progesterone released via the CIDR likewise can turnover dominant follicles and initiate a new follicular wave (Kang et al., 1999). Exogenous estradiol is normally luteolytic when administered early in the estrous cycle (Wilbank and Kasson, 1968). Although estrogen is an integral component of the natural luteolytic mechanism, the effect of exogenous estrogen is variable and it should not be considered equipotent to the putative luteolysin, PGF$_2$α (Burke et al., 1999).

The purpose of the second injection of estrogen was to induce a LH surge and subsequently reduce the period necessary to detect estrus. Further, the second injection of EB reduced the time to the next eligible estrus so that more nonpregnant cows returned to estrus sooner (Macmillan et al., 1997). Thus, in the present experiments, in both P4 + estrogen treatments, most of the returns to estrus occurred earlier than expected, probably due to the second injection of estrogen. In cattle, an increased titer of endogenous estradiol normally promotes the preovulatory LH surge by stimulating the number of GnRH receptors in the anterior pituitary while concentrations of progesterone are basal (Hansel and Convey, 1983). Therefore, administration of exogenous estrogen after luteolysis may induce a LH surge (Fike et al., 1997; Lammoglia et al., 1998). A dose of 1 mg of EB has been shown to be sufficient to elicit behavioral signs of estrus in anestrous cows (Fike et al., 1997). It has been demonstrated that 1 or 0.5 mg of ECP induces an LH surge in lactating dairy cows (Pancarci et al., 2002; Stevenson et al., 2002) and dairy heifers (Lopes et al., 2000) when given 24 h after a luteolytic dose of PGF$_2$α. Administration of EB at CIDR removal on d 20 of the cycle has been used to reduce variability in timing of the LH surge (Hanlon et al., 1996). When administering EB 24 h after CIDR removal, an LH surge occurred approximately 24 h later, with females exhibiting estrus and ovulating earlier than those with no EB injection after insert removal (Hanlon et al., 1996). Based on our results, a greater percentage of returns to estrus occurred 8 d after EB injection on d 13, which was one day earlier than predicted based on the latter report. We suggest that the initial injection of ECP probably induced emergence of a new wave 1 d later than EB, consistent with the delayed estrus in ECP vs. EB-treated cows in Exp. 3. Therefore the expected day of estrus occurred 10 to 11 d after ECP administration in Exp. 3 and 4.
(following a single injection of ECP on d 13). However, peak estrus occurred on d 9 after ECP or 2 d after CIDR removal when ECP injection was combined with the CIDR insert.

One report indicated that the EB + CIDR treatments increased fertility of dairy cows as a consequence of promoting three follicular waves (Macmillan et al., 1997). This was evident when conception rates were less in cows in which the fertilized oocyte was derived from the second (58%) compared to the third (95%) follicular wave of the estrous cycle in beef (Ahmad et al., 1997) and dairy cows (30 vs. 68%; Townson et al., 2002). Even though our findings are not consistent with the previous report where fertility was enhanced, the fact that neither estrogen treatment in lactating cows, compromised conception at the resynchronized estrus may have important implications for increasing use of AI in the beef cattle industry. However, given the current data, resynchronization of estrus may not be productive for heifers.

The final and most important practical goal of estrus-synchronization programs is to facilitate and increase the usage of AI. Unfortunately, less than 6% of beef cows in the U.S. are inseminated annually (NAHMS, 1997). According to that survey of cattle producers, the most common reasons for not utilizing estrus-synchronization programs and AI include lack of time and labor (37%), too complicated (20%), other (20%), cost (13%), lack of facilities (8%) and do not work (2%). Resynchronization treatments based on estrogen and the used CIDR tended to increase the pregnancy rates after two inseminations in lactating cows during the early breeding season, and also were inexpensive to apply. This may represent a strategic tool to increase the utilization of AI and increase profits on cow-calf operations.

**Implications**

Resynchronization of estrus beginning 13 d following insemination by feeding MGA, inserting a CIDR, or CIDR + estrogen injection increased the synchrony of estrus and visual detection of estrus (when ECP was added) of nonpregnant heifers. In lactating beef cows, incorporation of either estradiol benzoate or estradiol cypionate and a CIDR insert to resynchronize repeat estrus increased AI resubmission rate, tended to increase 25-d pregnancy rates, and reduced the period needed for detection of estrus. In both heifers and lactating beef cows, the resynchronization treatments had no negative effects on established pregnancies, but tended to reduce conception rates after resynchronization in heifers. Resynchronization provides another tool for cow-calf producers enterprises to facilitate the use of AI and make genetic progress.

**Acknowledgements**

We acknowledge Troy J. Marple and the assistance of student workers at the KSU Purebred Beef Unit and animal technicians at the KSU Dairy Teaching and Research Center for their care of cattle used in these studies. We thank owners of Losey Land and Cattle, Agra, KS (Exp. 2) and Darlynn Ranch, Pierz, MN and Thielen Ranch, Dorrance, KS (Exp. 3) for cooperation and use of their cattle. We express appreciation to Betty A. Hensley for her expert laboratory assistance.

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Vynckier, L., M. Debackere, A. De Kruijf, and M.Cory. 1990. Plasma estradiol-17β concentrations in the cow during induced estrus and after injection of estradiol-17β


USE OF BULLS WITH ESTROUS SYNCHRONIZATION

“Bull-Sync”

Peter J. Chenoweth,
College of Veterinary Medicine, Kansas State University

Introduction

Despite its potential for accelerating genetic progress, AI/Synchronization (AI/Sync) programs have been slow to be adopted by the beef industry. This relatively slow acceptance may be attributed to a number of factors, including the lack of acceptable procedures for appointment breeding. Also, producers may balk at the costs and effort of instituting an AI/Sync program, especially when good results are far from assured.

Early work with Bull/Sync programs was directed at a better understanding of the reproductive capabilities of bulls. With subsequent research, it became evident that Bull/Sync represented a viable management option in itself. Although natural breeding and estrous-synchronization may not appear to be natural bed-fellows, there are situations where this combination may be advantageous. For example, synchronization in conjunction with natural breeding (Bull/Sync) offers an interim alternative to AI/Sync with lesser demands on management, facilities, labor and expense. Here, a number of managerial aspects can be honed, and eligible female groups assembled, with less risk of disaster than which might occur with the sudden imposition of a full AI/Sync Program. Such programs can be successful using “normal” bull-to-female ratios (BFRs), providing the bulls employed are active and fertile (Pexton et al., 1990).

Another application of Bull-Sync is to concentrate breeding and calving periods in select groups, such as heifers. Here it can be advantageous both for replacement heifer selection, and for managing the calving period. Bull-Sync has also proven to be a useful management tool for mixed enterprises, which want to get both breeding and calving over within a manageable, predictable period.

Most of the earlier studies were conducted with the use of either PGF or SMB as estrus synchronization agents. Here, results showed little difference between these two methods in terms of pregnancy rates achieved. These, in turn, were generally comparable with results obtained in well-run AI programs (Pexton et al., 1989). Bull-Sync has also been employed successfully with both MGA/PGF and Select Synch protocols. It is not recommended to be used with the CoSynch or OvSynch protocols, as, here, females do not show heat signs.
A number of advantages of Bull/Sync are indicated below:

<table>
<thead>
<tr>
<th>Advantages of Bull/Synch</th>
</tr>
</thead>
<tbody>
<tr>
<td>☀ Synchrony of breeding/calving</td>
</tr>
<tr>
<td>☀ Interim step to AI/Sync</td>
</tr>
<tr>
<td>☀ Less effort than AI</td>
</tr>
<tr>
<td>☀ Less need for special facilities</td>
</tr>
<tr>
<td>☀ Less “risk” than AI &quot;Sync</td>
</tr>
<tr>
<td>☀ Flexible (less management and facilities)</td>
</tr>
<tr>
<td>☀ Equal or better fertility than AI &quot;Sync</td>
</tr>
<tr>
<td>☀ Possible “biostimulation” effects</td>
</tr>
</tbody>
</table>

However, Bull/Sync also poses some disadvantages. Firstly, it requires additional managerial time and effort compared with natural breeding with non-synchronized females. This includes a higher level of management than that associated with normal natural breeding. Females need to be selected and synchronized. Bulls need to have passed a Breeding Soundness Exam. Some monitoring of the intensive breeding period (2-5 days initially) is recommended.

Secondly, Bull-Sync has less potential to create genetic progress than does AI when this is conducted using bulls of superior EPDs. However, if due care is taken, the results obtainable with Bull-Sync are comparable with those obtained with good, well-run AI programs, and sometimes better (Farin et al., 1989).

<table>
<thead>
<tr>
<th>Disadvantages of Bull/Sync</th>
</tr>
</thead>
<tbody>
<tr>
<td>☀ Bull(s) ! (danger, facilities, inconvenience)</td>
</tr>
<tr>
<td>☀ Less potential genetic progress than AI</td>
</tr>
<tr>
<td>☀ Bull infertility/veneral disease</td>
</tr>
<tr>
<td>☀ Misadventure</td>
</tr>
</tbody>
</table>

An example of a Bull/Sync program, using different combinations of PGF (1 and 2 injection) as well as the associated effect of biostimulation (using androgenized cows) is shown in Tables 1a and 1b. Here, in all synchronized groups, over 80% of “eligible” females (i.e. those considered to be cycling) became pregnant at the synchronized first cycle, compared with 50% of controls (non-synchronized). Prior biostimulation appeared to benefit heifer response to synchronization in this study by increasing net pregnancy rate (Chenoweth and Lennon 1984).
Table 1a. Breeding Group Data

<table>
<thead>
<tr>
<th>Response</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Heifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>93</td>
<td>97</td>
<td>99</td>
<td>92</td>
<td>381</td>
</tr>
<tr>
<td>1 x PGF</td>
<td>279</td>
<td>284</td>
<td>292</td>
<td>282</td>
<td>287 NS</td>
</tr>
<tr>
<td>2 x PGF</td>
<td>4.0</td>
<td>3.9</td>
<td>3.8</td>
<td>3.8</td>
<td>3.9 NS</td>
</tr>
<tr>
<td>2 x PGF Plus Biost*</td>
<td>81.6</td>
<td>71</td>
<td>71.9</td>
<td>80.0</td>
<td>76.1 NS</td>
</tr>
</tbody>
</table>

* “Biostimulation” = 5 androgenized cows for 14 day pre-breeding
+ Palpable follicle(s) and/or CL(s)
NS = non-significant.
BCS = body condition score (scale of 1 to 5)
PGF = prostaglandin F2*

Table 1b. Responses in Heifer Groups

<table>
<thead>
<tr>
<th>Response (%)</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>% with paint marks (5 d)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>after 1 PGF</td>
<td>67.0a</td>
<td>52.6b</td>
<td>63.0a</td>
<td>-</td>
<td>60.8</td>
</tr>
<tr>
<td>after 2 PGF</td>
<td>64.5</td>
<td>71.1</td>
<td>61.1</td>
<td>64.1</td>
<td>65.4</td>
</tr>
<tr>
<td>Control</td>
<td>48.4a</td>
<td>56.7a</td>
<td>53.5a</td>
<td>33.7c</td>
<td>48.3</td>
</tr>
<tr>
<td>Gross PR (GPR)</td>
<td>75.0b</td>
<td>79.7c</td>
<td>86.9d</td>
<td>52.5a</td>
<td>73.9</td>
</tr>
</tbody>
</table>

In Colorado trials, comparisons were made of different BFRs (bull-to-female ratios) as well as different bull ages (Table 2) (Pexton et al., 1990). Here, yearling bulls obtained lower results than older bulls, despite exceeding breeding soundness standards, and despite comparable (if not higher) sexual activity with synchronized females. In these trials, BFRs were comparable across the different age groups.
Table 2. Mating Performance as Affected by Age of Hereford and Angus Bulls

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>One</th>
<th>Two</th>
<th>Three</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. bulls</td>
<td>29</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>No. mounts</td>
<td>207.1</td>
<td>120.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>85.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. services</td>
<td>54.5</td>
<td>37.6</td>
<td>40.5</td>
</tr>
<tr>
<td>Mounts : services</td>
<td>6.6:1</td>
<td>5.4:1</td>
<td>4.5:1</td>
</tr>
<tr>
<td>Serviced/estrus</td>
<td>69.4</td>
<td>73.8</td>
<td>72.0</td>
</tr>
<tr>
<td>Pregnant/serviced</td>
<td>39.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnant/estrus</td>
<td>30.2</td>
<td>40.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>50.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total pregnancy rate</td>
<td>30.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>49.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>c,d,e</sup> Means differ (P < .05).  Pexton et al. (1990)

An empirical comparison of results obtained in different trials employing *Bos Taurus* or *Bos indicus* bulls showed some interesting differences (Table 3) (Williams et al., 1988; Pexton et al., 1989). Here, although *Bos indicus* bulls were apparently less sexually active than *Bos taurus* bulls (i.e. they completed less services), they achieved comparable results (such as females served and pregnancy rates in those females) during the synchronized breeding.

Table 3. Comparison of Different Bull Synchronization Trials with *Bos taurus* and *Bos indicus* Cattle

<table>
<thead>
<tr>
<th></th>
<th><em>Bos taurus&lt;sup&gt;a&lt;/sup&gt;</em></th>
<th><em>Bos indicus&lt;sup&gt;b&lt;/sup&gt;</em></th>
<th>SMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. groups</td>
<td>39</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>BFR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:7 to 51</td>
<td>1:15 to 20</td>
<td></td>
</tr>
<tr>
<td>Females in estrus (%)</td>
<td>90.8</td>
<td>78.3</td>
<td>77.2</td>
</tr>
<tr>
<td>Served/estrus (%)</td>
<td>73.3</td>
<td>70.4</td>
<td>72.0</td>
</tr>
<tr>
<td>Total females served (%)</td>
<td>66.1</td>
<td>55.1</td>
<td>55.7</td>
</tr>
<tr>
<td>Avg. services per bull</td>
<td>45.1</td>
<td></td>
<td>23.6</td>
</tr>
<tr>
<td>Pregnant/estrus (%)</td>
<td>42.4</td>
<td>41.0</td>
<td>40.6</td>
</tr>
<tr>
<td>Pregnant/served (%)</td>
<td>56.4</td>
<td>56.1</td>
<td>57.3</td>
</tr>
<tr>
<td>Pregnant/total (%)</td>
<td>41.3</td>
<td>42.7</td>
<td>32.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pexton et. al. (1989)
<sup>b</sup>Williams (1988)
<sup>c</sup>BFR=bull to female ratio
Bull/Sync involves intense bull sexual activity within a contracted period. A number of considerations are pertinent to the success of such programs, as shown below.

**Bull-Sync Guidelines:**

- Prior assess bulls
- Use young (2-4 years old), agile, active bulls
- Conduct breeding in small pen or yard
- Use “normal” BFRs (1:15-1:25)
- Use single-sires if possible
- Monitor breeding (2-5 days)
- Provide adequate R & R (2-3 weeks plus) before re-use

**References**


Introduction

Today’s producer has an increasing number of options available to use estrous synchronization and artificial insemination (AI) to incorporate the desired genetics into the herd. Low cost production is and will continue to be vital for survival in the beef industry. So understanding the costs of producing pregnancies via various methods and the associated value is important. For some, the fact that you have to do something other than turn a bull out will be enough analysis for them to not consider AI. Others will take a broader view of the issue and may find that AI is a tool that can improve profitability.

This paper will look at costs associated with producing pregnancies via natural service and various synchronization systems. For some parts of the process it will be relatively easy to assign costs and make comparisons, for others assigning economic values will be much more difficult. As always, to make the most informed decisions, producers need to know their own costs of production.

Cost of Natural Service

Understanding the costs associated with natural service breeding is a good place to begin. The original purchase price, bull to cow ratio and years of use are all important factors that affect breeding costs. Table 1 shows annual bull ownership costs and estimated costs per pregnancy for a range of bull purchase prices ($1,500 to $3,000) and bull to cow ratios (1:15 to 1:50). For reference, the American Angus Association reported the average price of Angus bulls sold for fiscal years 2000 and 2001 as $2,292 and $2,267, respectively. Annual bull costs were calculated using Kansas Cow-Calf Enterprise Budget cost estimates (Foglemen and Jones, 2001) and annual bull costs were separated using the method of Kasari et al., 1996. Assumptions include use for four breeding seasons, 10% death loss, 9% interest rate, and a 94% pregnancy rate. Annual feed costs for cows have been shown to vary by as much as $200 per head (Stryker, 2001) among producers, and this same variability would be expected in feed costs for bulls as well. Increasing annual feed costs by $100, increases cost per pregnancy $7.41 for light bull use (15 cows/yr) and $2.22 for heavy use (50 cows/yr) given a $2,000 purchase price.

Producers who use breeding pastures with carrying capacities less than the serving capacity of the bull, will naturally drive up cost per pregnancy. Whereas producers who can correctly identify highly fertile bulls and increase the number of females exposed over more conservative recommendations can greatly reduce their costs per pregnancy.
Table 1. Annual bull costs based on purchase price and associated cost per pregnancy.

<table>
<thead>
<tr>
<th>Purchase price</th>
<th>$1,500.00</th>
<th>$1,700.00</th>
<th>$2,000.00</th>
<th>$2,300.00</th>
<th>$2,500.00</th>
<th>$3,000.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salvage value</td>
<td>$860.00</td>
<td>$860.00</td>
<td>$860.00</td>
<td>$860.00</td>
<td>$860.00</td>
<td>$860.00</td>
</tr>
<tr>
<td>Summer pasture</td>
<td>$104.13</td>
<td>$104.13</td>
<td>$104.13</td>
<td>$104.13</td>
<td>$104.13</td>
<td>$104.13</td>
</tr>
<tr>
<td>Crop residue</td>
<td>$7.50</td>
<td>$7.50</td>
<td>$7.50</td>
<td>$7.50</td>
<td>$7.50</td>
<td>$7.50</td>
</tr>
<tr>
<td>Hay</td>
<td>$90.61</td>
<td>$90.61</td>
<td>$90.61</td>
<td>$90.61</td>
<td>$90.61</td>
<td>$90.61</td>
</tr>
<tr>
<td>Protein, mineral</td>
<td>$25.00</td>
<td>$25.00</td>
<td>$25.00</td>
<td>$25.00</td>
<td>$25.00</td>
<td>$25.00</td>
</tr>
<tr>
<td>Labor</td>
<td>$50.00</td>
<td>$50.00</td>
<td>$50.00</td>
<td>$50.00</td>
<td>$50.00</td>
<td>$50.00</td>
</tr>
<tr>
<td>Vet</td>
<td>$21.00</td>
<td>$21.00</td>
<td>$21.00</td>
<td>$21.00</td>
<td>$21.00</td>
<td>$21.00</td>
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<tr>
<td>Repairs</td>
<td>$31.00</td>
<td>$31.00</td>
<td>$31.00</td>
<td>$31.00</td>
<td>$31.00</td>
<td>$31.00</td>
</tr>
<tr>
<td>Misc</td>
<td>$7.00</td>
<td>$7.00</td>
<td>$7.00</td>
<td>$7.00</td>
<td>$7.00</td>
<td>$7.00</td>
</tr>
<tr>
<td>Interest</td>
<td>$15.13</td>
<td>$15.13</td>
<td>$15.13</td>
<td>$15.13</td>
<td>$15.13</td>
<td>$15.13</td>
</tr>
<tr>
<td>Total variable</td>
<td>$351.37</td>
<td>$351.37</td>
<td>$351.37</td>
<td>$351.37</td>
<td>$351.37</td>
<td>$351.37</td>
</tr>
<tr>
<td>Deprec on bull</td>
<td>$160.00</td>
<td>$210.00</td>
<td>$285.00</td>
<td>$360.00</td>
<td>$410.00</td>
<td>$535.00</td>
</tr>
<tr>
<td>Interest on bull</td>
<td>$212.40</td>
<td>$230.40</td>
<td>$257.40</td>
<td>$284.40</td>
<td>$302.40</td>
<td>$347.40</td>
</tr>
<tr>
<td>Death loss</td>
<td>$15.00</td>
<td>$17.00</td>
<td>$20.00</td>
<td>$23.00</td>
<td>$25.00</td>
<td>$30.00</td>
</tr>
<tr>
<td>Total fixed</td>
<td>$399.79</td>
<td>$469.79</td>
<td>$574.79</td>
<td>$679.79</td>
<td>$749.79</td>
<td>$924.79</td>
</tr>
<tr>
<td>Total cost/yr</td>
<td>$751.16</td>
<td>$821.16</td>
<td>$926.16</td>
<td>$1,031.16</td>
<td>$1,101.16</td>
<td>$1,276.16</td>
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<table>
<thead>
<tr>
<th>Purchase price</th>
<th>$1,500.00</th>
<th>$1,700.00</th>
<th>$2,000.00</th>
<th>$2,300.00</th>
<th>$2,500.00</th>
<th>$3,000.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows Per Year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>$53.27</td>
<td>$58.24</td>
<td>$65.69</td>
<td>$73.13</td>
<td>$78.10</td>
<td>$90.51</td>
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<tr>
<td>20</td>
<td>$39.96</td>
<td>$43.68</td>
<td>$49.26</td>
<td>$54.85</td>
<td>$58.57</td>
<td>$67.88</td>
</tr>
<tr>
<td>25</td>
<td>$31.96</td>
<td>$34.94</td>
<td>$39.41</td>
<td>$43.88</td>
<td>$46.86</td>
<td>$54.30</td>
</tr>
<tr>
<td>30</td>
<td>$26.64</td>
<td>$29.12</td>
<td>$32.84</td>
<td>$36.57</td>
<td>$39.05</td>
<td>$45.25</td>
</tr>
<tr>
<td>35</td>
<td>$22.83</td>
<td>$24.96</td>
<td>$28.15</td>
<td>$31.34</td>
<td>$33.47</td>
<td>$38.79</td>
</tr>
<tr>
<td>40</td>
<td>$19.98</td>
<td>$21.84</td>
<td>$24.63</td>
<td>$27.42</td>
<td>$29.29</td>
<td>$33.94</td>
</tr>
<tr>
<td>50</td>
<td>$15.98</td>
<td>$17.47</td>
<td>$19.71</td>
<td>$21.94</td>
<td>$23.43</td>
<td>$27.15</td>
</tr>
</tbody>
</table>

Cost of AI and Estrous Synchronization

A partial budget is a good tool to gain an overview of potential differences between an AI program and natural service. Compared to natural service, increased costs of an AI program will result from synchronization products, labor to synchronize and inseminate, time for planning, and perhaps improvements in facilities. Costs associated with these items are relatively easily calculated and usually are the first to come to mind in this type of discussion. On the decreased returns side, income from the sale of cull bulls will likely be reduced because fewer bulls will be purchased in most cases. Depending on the size and management of the operation, costs could be decreased by having fewer bulls to purchase, maintain and keep out of trouble, less time and labor for calving in a shorter calving season, and fewer pulled calves from high-accuracy, low-calving-difficulty bulls. Income will increase as a result of more older, heavier calves at
weaning. Producers with good marketing skills will also increase returns from a more uniform calf crop and by producing genetics that are in demand. If replacement heifers are generated from within the herd, long-term benefits may accrue from selection for traits such as milk production or longevity. The beneficial items in our budget (i.e. quality genetics, more concentrated calving season) are much more difficult to place a value on, and some might not be captured by producers without additional marketing efforts. Nevertheless, in a marketplace that is increasingly value driven, the opportunity to capture this genetic value will expand in the future.

Table 2. Partial budget for estrous synchronization

<table>
<thead>
<tr>
<th>Budget Effect</th>
<th>Source</th>
<th>Budget Effect</th>
<th>Source</th>
</tr>
</thead>
</table>
| Increased returns | Average age of calves is older, producing heavier calves  
Quality of genetics (calves and replacement females)  
Uniformity of Calf Crop (shorter calving season) | Decreased returns | Fewer cull bulls to sell |
| + + $$         |                                                                        | - $$          |                                  |
| Decreased costs | Fewer bulls to purchase and maintain  
More concentrated calving season  
More predictable calving ease | Increased costs | Planning & management for AI & estrous synchronization  
Synchronization products and supplies  
Labor  
Improved facilities? |

One way to estimate the value of genetics is to look at the data in Table 3. It summarizes boxed beef values from Angus sires with 10 or more carcass data records into the top 10% and the bottom 10% for carcass value. The difference in carcass value was $206 per head for sires in the two groups. Clearly you could pay a few more dollars in breeding costs to produce a product worth $206 more at harvest. Because the industry has been operating on selling commodity cattle with an average value for so long, it is hard for the average producer to market calves in such a way that he is more nearly paid for the true value of the genetics produced. Currently, these value differences are more readily observed at harvest than weaning, but the trend is toward identifying and rewarding known genetics earlier in the production process. Excellent marketing was cited as one of four key advantages of producers with high returns on assets for cow/calf enterprises in the Northern Great Plains (Dunn, 2000). As the beef industry continues to shift from a commodity market to a value based market, differences in costs and returns for various breeding systems may be more readily calculated. At this point producers may ask themselves: If the cost per pregnancy is higher for a particular method of breeding, what are the chances I can recoup that cost by achieving higher marketing returns on the superior genetics?
Table 2. Average boxed beef values for Angus sires with 10 or more carcass data records*

<table>
<thead>
<tr>
<th>Trait</th>
<th>Top 10%</th>
<th>Bottom 10%</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Progeny</td>
<td>2728</td>
<td>1751</td>
<td></td>
</tr>
<tr>
<td>No. Sires</td>
<td>109</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>% Prime</td>
<td>7.7</td>
<td>0.7</td>
<td>+7.0</td>
</tr>
<tr>
<td>% CAB</td>
<td>47.4</td>
<td>.7</td>
<td>+46.7</td>
</tr>
<tr>
<td>% Choice &amp; above</td>
<td>93.7</td>
<td>48.1</td>
<td>+45.6</td>
</tr>
<tr>
<td>% Select</td>
<td>6.1</td>
<td>35.0</td>
<td>-28.9</td>
</tr>
<tr>
<td>% Standard</td>
<td>0.2</td>
<td>16.9</td>
<td>-16.7</td>
</tr>
<tr>
<td>% YG 1&amp;2</td>
<td>60.0</td>
<td>38.2</td>
<td>+21.8</td>
</tr>
<tr>
<td>% YG 4&amp;5</td>
<td>1.4</td>
<td>18.2</td>
<td>-16.8</td>
</tr>
<tr>
<td>Carcass Price/cwt</td>
<td>$110.19</td>
<td>$94.15</td>
<td>$16.04</td>
</tr>
<tr>
<td>Carcass Value/hd</td>
<td>$822.27</td>
<td>$616.36</td>
<td>$205.91</td>
</tr>
</tbody>
</table>


Whole herd cost of pregnancy

To evaluate the breeding costs under different breeding systems, methods used by Loseke, 1989, were updated to reflect current conditions. Briefly, a survey was taken of beef producers using artificial insemination in Nebraska. From that survey, regression equations were estimated for total labor hours required for various AI programs.

Nonsynchronized program:

\[ TM = 19 + 0.036(CD) \]

\[ R^2 = 0.83 \]

Lutalyse synchronization program:

\[ TM = 2.65(CD)^{0.5} \]

\[ R^2 = 0.60 \]

Syncro-Mate-B synchronization program:

\[ TM = 2.53(CD)^{0.5} \]

\[ R^2 = 0.87 \]

Where TM – Total hours of labor required for AI program
C – Total number of cows and heifers being bred AI
D – Total number of days in AI program

The equation for the Syncro-Mate-B system was used for all the estrous synchronization systems in this report. Breeding systems were evaluated for various size cowherds. Breeding herds of 35, 116, and 348-head allowed for culling of nonpregnant and physically impaired cows to yield 30-, 100-, and 300-head calving herds. For the current model, costs were estimated over a range of AI pregnancy rates. Pregnancy rate was multiplied by number of cows, and the product was divided by an average conception rate of 70% to get the number of cows in estrus. Cows and heifers not pregnant to AI were exposed to bulls for the remainder of the breeding season. Total breeding season
pregnancy rate was 94%. The number of bulls required for clean-up was calculated based on the outcome of the AI program. One bull was used per 30 nonpregnant females. Variable and fixed costs for AI are shown in Table 4. The annual interest rate charged for cash costs was 9%. The labor rate used was $10.77 per hour (Fogleman, 2002). Annual bull costs ($2,000 purchase price) were $926 per bull as described in the previous section. Budget items from the partial budget in Table 2 that are not accounted for in this model include value of AI-sired replacement heifers, more concentrated calving season, more predictable calving ease, and any facility improvements.

### Table 4. Artificial insemination costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Cost per unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen</td>
<td>$13 – straw</td>
</tr>
<tr>
<td>Prostaglandin F 2α</td>
<td>$2.00 – dose</td>
</tr>
<tr>
<td>GnRH</td>
<td>$3.00 – dose</td>
</tr>
<tr>
<td>CIDR</td>
<td>$8.00 - dose</td>
</tr>
<tr>
<td>Supplies</td>
<td>$.50 – insemination</td>
</tr>
<tr>
<td>Fixed costs⁴</td>
<td>176.30</td>
</tr>
</tbody>
</table>

⁴Semen tank, carrying case, pipette gun, thaw box, liquid nitrogen

Cost per pregnant female as calculated in this model reflects both AI and natural service pregnancies. In this case, pregnancy rate to AI impacts the cost per pregnant female in two ways. As AI pregnancy rate is reduced without changing the number of bulls required for natural service, cost per pregnancy actually decreases because of lower costs for semen and interest for a system involving heat detection and AI. While this reduction would mean fewer AI-sired calves, the impact of that reduction would depend on how well the producer capitalizes on the genetic value of the calves and is not reflected in the cost per pregnant female. When pregnancy rate increases to a point where the operation can get along with one less bull, then the reduced bull costs significantly lower cost per pregnancy with little change in the pregnancy rate. As seen in Table 5, an additional bull for natural service adds from $8.27 per pregnant female for herds of 100 head and only $2.61 for herds of 300 head. As the AI pregnancy rate increases, the percentage of costs due to semen expense increases and those attributed to the bull decrease. At what might be considered typical AI pregnancy rates, approximately 50%, bull costs easily represent the largest share of costs followed by semen costs. The importance of annual bull costs to the total cost of the breeding system would be further emphasized with bulls with a higher initial purchase price. The percentage of total costs attributed to bulls reflects how bull costs change based on the number of cows pregnant to AI. In reality, a decision on how many bulls to place with the cows after AI has to be made before knowing the AI pregnancy rate. Successfully identifying bulls that can reliably service more than the 30 cows used in this example would be extremely valuable. If four bulls are used rather than 5 bulls for the 300-head herd when the pregnancy rate is 65%, the cost per pregnant female is reduced $2.83.

A better evaluation of breeding systems would be to account for the proportion of pregnancies from AI or natural service in each system. To do this, calves with AI sires were assigned a value of $25 per head greater than those born to natural service. The AI sired calves would be on average 10 days older and 20 pounds heavier at weaning thus...
increasing the return at weaning by $20 if the additional weight is worth $1/cwt. An extra $5 per head was assigned for “genetic” value. This is a fairly conservative estimate compared to the $25 per head bonus for calves that fit the Laura’s Lean specifications (genetic and management requirements) and an average of $10-15 per head bonus on carcass performance (Charlie Peters, personal communication). So for this model, calves sired by AI were valued at $525 per head, and natural service sired calves were valued at $500 per head. To compare breeding system costs and returns, a standardized production scale was generated. Breeding system cost per exposed female was reduced for any increased revenue from AI sired calves and expressed as a 500 lb equivalent weaned calf breeding cost per hundred (cwt). A weaned calf crop of 82% was assumed.

Table 5. Effect of changing pregnancy rate on breeding cost per pregnant female in a Select Synch protocol.

<table>
<thead>
<tr>
<th>Calving herd size</th>
<th>AI pregnancy rate</th>
<th>No. of bulls for natural service</th>
<th>Breeding cost per pregnant female</th>
<th>% of total cost attributed to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 hd</td>
<td>75%</td>
<td>1</td>
<td>$40.90</td>
<td>Bulls 21% Semen 38% Labor 20% Treatments 13%</td>
</tr>
<tr>
<td></td>
<td>74%</td>
<td>2</td>
<td>$48.28</td>
<td>35% 30% 17% 11%</td>
</tr>
<tr>
<td></td>
<td>55%</td>
<td>2</td>
<td>$44.92</td>
<td>38% 25% 18% 12%</td>
</tr>
<tr>
<td></td>
<td>49%</td>
<td>2</td>
<td>$43.58</td>
<td>39% 23% 19% 12%</td>
</tr>
<tr>
<td></td>
<td>48%</td>
<td>3</td>
<td>$51.85</td>
<td>49% 19% 16% 10%</td>
</tr>
<tr>
<td>300 hd</td>
<td>66%</td>
<td>4</td>
<td>$37.13</td>
<td>30% 36% 13% 14%</td>
</tr>
<tr>
<td></td>
<td>65%</td>
<td>5</td>
<td>$39.74</td>
<td>36% 34% 12% 13%</td>
</tr>
<tr>
<td></td>
<td>57%</td>
<td>5</td>
<td>$37.95</td>
<td>37% 31% 12% 14%</td>
</tr>
<tr>
<td></td>
<td>56%</td>
<td>6</td>
<td>$40.56</td>
<td>42% 28% 11% 13%</td>
</tr>
<tr>
<td></td>
<td>55%</td>
<td>6</td>
<td>$40.33</td>
<td>42% 28% 12% 13%</td>
</tr>
<tr>
<td></td>
<td>49%</td>
<td>6</td>
<td>$38.99</td>
<td>44% 26% 12% 14%</td>
</tr>
<tr>
<td></td>
<td>48%</td>
<td>7</td>
<td>$41.60</td>
<td>48% 24% 11% 13%</td>
</tr>
</tbody>
</table>

Breeding system costs and the standardized cost per hundred for various breeding systems assuming equivalent AI pregnancy rates (50%) are in Table 6. Looking at the breeding system cost per pregnant female, natural service followed by MGA/PGF and MGA-Select or Select Synch were the least expensive depending on herd size; CO-Synch+CIDR was most expensive. On a standardized production scale, 500 lb equivalent weaned calf breeding cost per hundred, many systems have costs nearly equal to or less than natural service. These include MGA/PGF, MGA Select, and Select Synch for all herd sizes and include 7-11 Synch and CIDR+PGF for a herd size of 300. So, decisions based strictly on cost and not the returns generated by those costs, may be erroneous. Systems with the highest standardized cost per hundred involve CIDRs and or timed AI. The difference in cost per hundred between MGA/PGF and natural service was $2.23/cwt and $1.71/cwt for herd sizes of 300 and 30, respectively. The difference in cost per hundred between natural service and MGA/PGF indicates the amount the breakeven price for weaned calves would need to change to account for differences in breeding system costs and number of AI pregnancies. Therefore, the weaning breakeven price would need to be $2.23/cwt higher for a natural service breeding system than one using...
MGA/PGF to generate equal returns given all else was equal. The CO-Synch+CIDR system standardized cost per hundred was $2.10 and $2.13 more than natural service for herd sizes of 30 and 100, respectively. The common factors among those systems with the lowest standardized costs seem to be low treatment costs, heat detection and estrus AI, and relatively higher labor costs. A comparison in this manor assumes that additional labor to facilitate the heat detection and AI is either readily available or can be hired. If competent help can be hired to complete the task, then that would seem to be the most economical method to use. Some cannot or will not hire outside help, in which case the opportunity cost of the time spent on AI may be perceived to be too great compared to other farming or ranching activities.

In comparing a timed insemination system such as CO-Synch to Select Synch where cows are inseminated after an observed estrus, the standardized costs per hundred are lower with the Select Synch system, and the difference is greatest for the largest herd size. So, while in most cases estrus AI may produce more pregnancies with less overall expense, the additional cost for timed AI may allow a producer to use AI who would not have considered AI if heat detection was necessary because of herd size or a pasture too large for efficient heat detection, or if labor was unavailable. This type of producer may have greater ability to recover the additional cost of timed AI in the value received for the genetics produced.

A further examination of the Select Synch and CO-Synch systems at varying labor and semen costs is shown in Table 7. Combinations of semen and labor costs do exist where standardized costs for the CO-Synch system are less than the Select Synch system. Costs per hundred for CO-Synch at a 60% pregnancy rate are $.22/cwt less than Select Synch when semen cost is $3 per unit and labor is $15.77 per hour in the 100 head herd size (Table 7). For a herd size of 30, the breeding costs per hundred are less for CO-Synch than Select Synch at low semen costs and medium to high labor costs and at the highest semen and labor costs and at an AI pregnancy rate of 60%. For a herd size of 300, at equal pregnancy rates, there are no combinations where the costs are less for CO-Synch. Averaged across all herd sizes and AI pregnancy rates, and at the highest labor cost, the standardized cost for Select Synch is $0.53/cwt less than CO-Synch and this increases to $1.35/cwt at low labor costs. At the lowest semen cost, averaged across all herd sizes and AI pregnancy rates, the advantage of Select Synch over CO-Synch is only $0.18 and increases to $1.70/cwt at high semen costs.

<table>
<thead>
<tr>
<th>System</th>
<th>Preg Rate</th>
<th>Labor Cost ($/hour)</th>
<th>$3/unit</th>
<th>$13/unit</th>
<th>$23/unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO-Synch</td>
<td>40%</td>
<td>$5.77</td>
<td>$10.77</td>
<td>$15.77</td>
<td>$5.77</td>
</tr>
<tr>
<td>CO-Synch</td>
<td>50%</td>
<td>$5.36</td>
<td>$10.97</td>
<td>$15.47</td>
<td>$5.77</td>
</tr>
<tr>
<td>CO-Synch</td>
<td>60%</td>
<td>$4.84</td>
<td>$10.77</td>
<td>$15.77</td>
<td>$5.77</td>
</tr>
<tr>
<td>Select Synch</td>
<td>40%</td>
<td>$7.04</td>
<td>$10.28</td>
<td>$14.30</td>
<td>$9.75</td>
</tr>
<tr>
<td>Select Synch</td>
<td>50%</td>
<td>$7.41</td>
<td>$10.48</td>
<td>$14.58</td>
<td>$9.75</td>
</tr>
<tr>
<td>Select Synch</td>
<td>60%</td>
<td>$4.33</td>
<td>$10.61</td>
<td>$14.72</td>
<td>$9.75</td>
</tr>
</tbody>
</table>

Table 7. 500 lb equivalent weaned calf breeding costs per cwt for a herd size of 100 at various labor and semen costs.
Pregnancy rates to AI will vary based on a variety of factors and the effect of changing pregnancy rate on the standardized cost per hundred was calculated within each system (Table 8). Notice that for a herd size of 30 using CO-Synch, the cost per exposed female remains the same despite differences in AI pregnancy rates. This is because all animals are treated and inseminated, one bull is still needed for clean up and total number of cows pregnant at the end of the entire breeding season is the same. The benefit of more AI pregnancies is reflected in the standardized production scale.

Table 8 allows a comparison of systems at different AI pregnancy rate outcomes. For example, this allows us to see that if heat detection is a problem and reduces the pregnancy rate to 40% in a Select Synch system, that the pregnancy rate to timed AI in the CO-Synch system would need to be somewhere between 50-60% to yield similar costs per hundred for a herd size of 300. In larger herds where heat detection may really present a challenge, this could easily be true.

Comparing Select Synch to Select Synch+CIDR, the CIDR allows for 2 fewer days of heat detection and should increase pregnancy rates over Select Synch, particularly in anestrous cows. However, even at a 60% pregnancy rate for the Select Synch+CIDR, the cost per hundred is still less for a Select Synch system yielding a 40% pregnancy rate. MGA-Select requires one additional injection of GnRH and one more day of labor than MGA/PGF. Costs per hundred for MGA/PGF at a 40% pregnancy rate are slightly less than a 50% pregnancy rate with MGA/Select (300 head). CO-Synch and MGA-CO-Synch have very similar costs and returns, because there is little added cost with the MGA-CO-Synch in this model. This is based on the assumption that there is no additional labor cost to deliver the MGA, and the MGA carrier is part of the normal ration. A comparison of giving PGF on the day before CIDR removal or at CIDR removal (CIDR+PGFd7) indicates that the CIDR+PGFd7 system reduces cost from $0.84 to $0.27 per exposed female for herd sizes of 30 to 300, respectively and reduces cost per hundred $0.21 to $0.07.

Economies of scale are evident in these results, however breeding costs are just part of the picture. Both Kansas SPA and Farm Management databases indicate that small producers are just as likely to be profitable as large producers.

Pregnancy rates to AI

The costs and returns based on various AI pregnancy rates and synchronization systems have been shown. The question then becomes, what pregnancy rate can be expected from various systems in my herd? The differential response of postpartum cows based on factors such as age, body condition, and days postpartum is thoroughly described in the paper of Stevenson et al., found earlier in these proceedings. Table 9 depicts a summary of recent published data in beef cows of which only one study has less than 75 cows per treatment; most represent large herds and multiple locations. Where available, information on body condition score, days postpartum and proportion cycling has been provided. Most of the herds used in these trials represent average or above average management. This table may serve as a guide to the level and variability of published results.

Excluding the Bos Indicus data, the average pregnancy rate reported for Select Synch is 48% and for CO-Synch, 46%. Other systems have fewer reported values, so averages across studies have not been made. Where those limited observations would
fall within a normal distribution of pregnancy rates for that system is hard to know. Very limited data is available providing head-to-head comparisons of various systems with large numbers of animals at multiple locations. Without this type of data it is hard to sell any one system as so much better than another.

In most cases the pregnancy rates reported in these studies represent an accurate assessment of AI pregnancies because authors reported waiting at least 10 days after AI to turn in bulls for clean-up and an early pregnancy diagnosis ensured clear distinction between AI and natural service pregnancies. Caution must be used when evaluating field reports of pregnancy rates from various systems when they are reported in conditions other than those described above because the results can be distorted. This caution should also be noted for individuals purchasing animals that are supposedly pregnant to AI or have AI-sired calves because the conditions used to assure and identify those pregnancies vary. Another possible distortion is that in some cases only part of the herd (mature cows or early calvers) is synchronized. This may be a wise and practical way to implement an AI program, but the results will likely be different than when the entire herd is synchronized.

One thing is clear, there are now reliable systems to generate AI pregnancy rates near 50% or better, and that result can be achieved with a single timed insemination. Producers who refine their synchronization preparation and procedures, identify highly fertile bulls for both AI and natural service, and have a gradually increasing percentage of cows calving early will find even better results over time.

Conclusions

While costs of a breeding system are important, a system that can be implemented correctly and efficiently within a given production environment may be equally important. The length or complexity of a system may make it a bad choice for certain situations even though it looks good on paper. The model described here does not account for such things as the likelihood that the right treatment will be given on the right day or that the facilities are adequate to allow estrous detection and sorting.

Results indicate that synchronization systems that involve considerable animal handling and heat detection can generate a return greater than natural service. Given all the demands on the CEO’s of today’s beef business, hiring highly skilled, specialized people to perform AI and estrous synchronization seems to make good sense. Particularly for someone just starting an estrous synchronization program, experienced help may be worth a lot to the success of a program. The planning required to schedule help is a problem for some, but should be a priority.

Researchers have been working hard to improve responses to timed AI because of the unwillingness of producers to commit time to heat detection. If labor is available and heat detection is feasible, cost analysis would indicate that AI after estrus rather than timed AI would produce greater returns. Some timed insemination systems have standardized costs similar to natural service at a 50% pregnancy rate and lower costs at 60% depending on herd size. For producers who can further increase returns for AI-sired calves, this benefit would be even greater.
References


Table 6. Breeding system costs and 500 lb equivalent weaned calf breeding cost per hundred.

<table>
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<tr>
<th>Herd Size</th>
<th>Days worked</th>
<th>Preg rate</th>
<th>Total labor hours</th>
<th>No. of Bulls</th>
<th>Cost per pregnant female</th>
<th>500 lb equivalent weaned calf breeding cost per hundred</th>
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*Descriptions of these systems can be found elsewhere in these proceedings
**Assumes 40% of cows bred based on observed estrus (no GnRH at AI)
*Diff= difference between natural service and breeding system, $/cwt
Table 8. Breeding system costs and 500 lb equivalent weaned calf breeding cost per hundred at various AI pregnancy rates.

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<tr>
<th>Days Worked</th>
<th>Preg Rate</th>
<th>No. of bulls</th>
<th>Cost per pregnant female</th>
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*Diff= difference between natural service and breeding system, $/cwt*
Table 9. Summary of cow studies

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<td>31%</td>
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<td>CO-Synch+progestin</td>
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<td>CO-Synch + Calf Removal</td>
<td>46%</td>
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<td>CO-Synch (hCG)</td>
<td>34%</td>
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<tr>
<td>CO-Synch (hCG) + Calf Removal</td>
<td>35%</td>
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<td>CO-Synch + horn bred</td>
<td>42%*</td>
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<td>Osynch</td>
<td>52%</td>
<td>57%*</td>
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<tr>
<td>Osynch+Calf Removal</td>
<td>54%*</td>
<td>61%</td>
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<td>MGA+CO-Synch</td>
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<td>7-11 Synch +GnRH &amp; 48-60 h TAI</td>
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<td>MGA</td>
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<td>Bos Indicus</td>
<td>CIDR</td>
<td>CIDR</td>
<td>Norg</td>
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<td>5 Griege et al., 1998</td>
<td>9 Geary &amp; Whitter, 1998</td>
<td>13 Medina-Britos et al., 2001</td>
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<td>10 Johnson et al., 2000</td>
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<td>11 Lamb et al., 2001</td>
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<td>4 Lemaster et al., 2001</td>
<td>8 Geary et al., 2001b</td>
<td>12 Stevenson et al., 2000a</td>
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</table>

†at start of MGA, ††At start of breeding season, †††M=Multiparous, P=primiparous, * one location only
PROMISING NEW TECHNOLOGIES FOR SEMEN EVALUATION

George R. Dawson, J.N. Oyarzo, M.E. Bellin, H.M. Zhang, T.C. McCauley and R.L. Ax
Department of Animal Sciences, University of Arizona, Tucson, AZ

Introduction

Assessment of seminal plasma and sperm-membrane associated constituents has led to the isolation of four proteins that provide utility for predicting fertility potential of bulls. All four of these proteins have been isolated and characterized biochemically. They have also proved to be very valuable as a diagnostic indicator of fertility beyond the traditional breeding soundness exam (BSE) identified by Chenoweth et al. (1992), since bulls with identical physical semen characteristics still may vary greatly in fertility when used for natural mating or artificial insemination.

The specific proteins found in bovine semen that reflect fertility potential include osteopontin (OPN), a lipocalin-type prostaglandin D (PGD) synthase, fertility-associated antigen (FAA) and type-2 tissue inhibitor of metalloproteinases (TIMP-2). Since a specific gene encodes for particular protein, the potential exists to easily screen for these predictors of fertility. Single nucleotide polymorphisms (SNPs) in genes are becoming valuable tools in both proteomics and genomics. Subtle mutations in the gene from one individual to another can be detected, leading to the prediction of fertility at a day of age rather than puberty to collect semen.

Within the FAA gene, SNPs have been isolated that cause conformational changes in the carboxyl terminus of the protein. When a monoclonal antibody (M1) is used to screen for the FAA protein, the aberrant FAA form is not detected and the bull is subsequently labeled FAA-negative. The use of polymerase chain reactions (PCR) techniques will allow researchers to amplify and easily diagnose those individuals containing mutations or SNPs.

The four proteins as fertility markers in bulls and the promising new technologies for assessing semen/donors will be discussed as evaluators of fertility potential.

Semen Proteins as Predictors of Bull Fertility.

Utility of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) led to the recovery of four proteins in seminal plasma that were linked to fertility (Killian et al., 1993). This original work led to the development of a regression equation (R=0.89) to predict fertility based on the density of these proteins in Holstein bull seminal plasma (for formula see Killian et al., 1993). The proteins discovered consisted of a 55 kDa (pI 4.1) protein and a 26 kDa (pI 6.2) protein, which were correlated with above, or high fertility bulls, and two 16 kDa proteins with pI’s of 6.7 and 4.1, respectively. The latter two proteins were correlated with below average fertility bulls and are currently uncharacterized.
The 55 kDa protein was later characterized and found to be osteopontin (OPN; Cancel et al., 1997). Based on protein density, OPN was positively correlated (0.48) with fertility (Cancel et al., 1997). An OPN localization study illustrated the primary sources in seminal plasma were contributed from the ampulla and seminal vesicle secretions (Cancel et al., 1999). The functional role of OPN with respect to regulating bull fertility remain to be elucidated, however OPN has been shown to play an integral part in a number of signal transduction pathways (for review see Denhardt et al., 2001) including defense mechanisms and inflammatory conditions. The potential also exists for it to serve as a functional cell attachment protein that may assist in stabilization of the plasma membrane.

When bulls are categorized as above or below average fertility (average = 0), lipocalin type PGD synthase is 3.5-fold more prevalent in high fertility Holstein bulls (Killian et al., 1993; Gerena et al., 1998; 2000). Fluorescent microscopy revealed lipocalin-type PGD synthase to be associated with the apical ridge of the acrosome on ejaculated sperm, as well as being localized in a number of other areas (Gerena et al., 2000). Its localization in numerous tissues including the epididymis lends support to having a role in maturation of sperm and may reflect why individuals with a 3.5-fold higher concentration are more fertile.

In the early 1990’s a series of proteins were isolated using liquid chromatography, which possessed a high affinity for binding heparin. Of these heparin-binding proteins (HBPs), a 31 kDa protein was later isolated and coined fertility-associated antigen (FAA; Bellin et al., 1996). Biochemical studies indicate FAA is basic and non-glycosylated yielding a N-terminal 26 amino acid sequence which is 73% identical to human deoxyribonuclease (DNase) I-like protein (McCauley et al., 1999). Tissue extracts indicate the presence of FAA primarily to be in the seminal vesicles and prostate glands (McCauley et al., 1999), however previous work illustrated a similar molecular weight protein in all three accessory sex glands (Nass et al., 1990). Heparin-binding proteins are present on ejaculated sperm, but not on epididymal sperm (Miller et al., 1990). They augment the effects of heparin, a glycosaminoglycan, commonly used to capacitate bovine sperm in vitro (Miller et al., 1990).

Another 24 kDa HBP was also isolated and characterized as being a predictor of bull fertility. The 24 kDa protein was identified as tissue-inhibitor of metalloproteinases-type 2 (TIMP-2) as it was shown to be 90% identical to the N-terminus of a bovine aortic TIMP-2 (McCauley et al., 2001). TIMP-2 RNA has been isolated from all three bovine accessory sex glands (McCauley et al., 2001). This was the first report of TIMP-2 in bovine semen and potential roles and mechanisms are currently under investigation.

Using SDS-PAGE gels and Western blotting techniques with the monoclonal antibody designated as M1, bovine sperm extracts can be assayed and characterized as FAA or TIMP-2 positive or FAA or TIMP-2 negative. Tables 1 and 2 represent field trials that have occurred and the impact of segregating beef bulls as positive or negative with respect to either FAA or TIMP-2 content.
Table 1. Fertility of range beef bulls following a 60 d exposure period and AI beef bulls after three projected services (equivalent to 60 d exposure) when categorized as FAA-positive or FAA-negative.

<table>
<thead>
<tr>
<th>FAA Status</th>
<th>Number Of Bulls</th>
<th>Number Of Cows</th>
<th>Pregnant at 60 days or 3rd Service</th>
<th>Percent of Cows Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAA-negative</td>
<td>199</td>
<td>4,267</td>
<td>2,764</td>
<td>64.8 %</td>
</tr>
<tr>
<td>FAA-positive</td>
<td>260</td>
<td>6,081</td>
<td>4,998</td>
<td>82.1 %</td>
</tr>
<tr>
<td>Total</td>
<td>459</td>
<td>10,348</td>
<td>7,762</td>
<td>75 %</td>
</tr>
</tbody>
</table>

(Data adapted from Bellin et al., 1994; 1996; 1998; and Sprott et al., 2000)

Table 2 summarizes results from field trials recently completed to compare pregnancy outcomes of beef cows exposed to bulls after their spermatozoa had been tested for presence or absence of TIMP-2. Bulls were pastured for 60 days at a ratio of 25 cows per bull in multiple-sire pastures. Fertility was 13% higher for bulls whose sperm was qualified as TIMP-2 positive compared to their herdmates categorized as TIMP-2 negative.

Table 2. Fertility of range beef bulls after a 60 d exposure period following characterization of sperm as either TIMP-2 positive or TIMP-2 negative.

<table>
<thead>
<tr>
<th>TIMP-2 Status</th>
<th>Number Of Bulls</th>
<th>Number Of Cows</th>
<th>Number Of Cows Pregnant</th>
<th>Percent Of Cows Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIMP-2 Positive</td>
<td>180</td>
<td>3,985</td>
<td>3,431</td>
<td>86.1 %</td>
</tr>
<tr>
<td>TIMP-2 Negative</td>
<td>67</td>
<td>1,225</td>
<td>894</td>
<td>72.9 %</td>
</tr>
<tr>
<td>Total</td>
<td>247</td>
<td>5,210</td>
<td>4,325</td>
<td>83 %</td>
</tr>
</tbody>
</table>

(Data adapted from Dawson et al., 2002)

Bulls which are categorized at positive for FAA or TIMP-2 represent a 17 % or 13 % higher fertility rate (based on pregnancy) than their counterparts. This represents a
potential large increase in economic gain versus the cost of assaying for these proteins which is estimated to be approximately $1.00 per cow in a 100-head herd.

As mentioned earlier, proteins are unique and they are encoded for by a specific gene, so what other technologies may we have access to in the near future?

**Gene Frequencies and Genotypic Frequencies:**

Since a gene is unique, we can calculate its frequency if we have a measure of its distribution in a population. Using FAA as an example, a genotypic frequency is distributed as \( p^2 + 2pq + q^2 \). To date, approximately 6,000 bulls have been screened and 16% of those are FAA-negative, that means they are homozygous recessive, providing \( q^2 \). The square root of .16 is .4. One minus .4 = .6, the frequency of \( p \). From this information, we deduce that .6² = .36, the frequency of animals homozygous FAA-positive, \( 2pq = 2(.6)(.4) = .48 \), so 48% of the population is presumed heterozygous, carrying one chromosome with FAA positive form of the gene, and one chromosome carrying the FAA-negative form of the gene. They would be classified overall as FAA-positive if FAA were assayed in a semen sample. If different breeds had varying genotypic distributions of FAA, or if we culled homozygous recessive FAA-negative animals to decrease their frequency, the following table illustrates what is happening mathematically. Keep in mind, we are referring to animals. The gene frequencies should be similar in males and females because no coordinated industry-wide culling has yet occurred.

**Table 3.** Hypothetical gene and genotypic frequencies based on the proportion of homozygous recessive (FAA-negative) individuals in a population.

<table>
<thead>
<tr>
<th>Proportion of Homozygous Recessive (FAA-negative)</th>
<th>Gene Frequency</th>
<th>Genotypic Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( p )</td>
<td>Homozygous Dominant (FAA+)</td>
</tr>
<tr>
<td>25%</td>
<td>.5</td>
<td>.25</td>
</tr>
<tr>
<td>16%</td>
<td>.6</td>
<td>.36</td>
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<tr>
<td>5%</td>
<td>.78</td>
<td>.61</td>
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</tbody>
</table>

From the Table above, it is clear that if only 5% of bulls sampled were classified as FAA-negative from a semen test, there would still be 34% of cattle that would be heterozygotes carrying one copy of the undesirable form of the FAA gene. How then, do we develop a method to find animals lacking two copies of the good form of FAA (or any other protein of choice)?
Mutations

Bulls with sperm categorized as FAA-negative actually produce a protein which is almost identical to FAA. That aberrant form represents a subtle mutation within the FAA protein that causes it to fold slightly different from normal. Obviously, that fold occurs at or near the portion of the FAA which is recognized by the monoclonal antibody (M1) that was originally produced against FAA in 1992 (Bellin et al., 1996). A specific domain on a protein recognized by an antibody is referred to as an epitope. Bulls with semen classified as FAA-negative produce the protein lacking the epitope, leading to them labeled as FAA-negative.

Molecular biology techniques can now be applied to semen for defects within the FAA gene that lead to an altered form of the protein lacking the epitope. First, the FAA genes from a normal fertile bull and subfertile bull were sequenced. The portion of the gene where the mutation was detected, along with the corresponding change in amino acids encoded at that portion of the FAA protein are illustrated below.

**Figure 1.** Two DNA SNPs found between the cDNA sequences of bovine FAA gene isolated from sex glands of a normal bull and the sex glands of a subfertile bull. Both of the 550 and 553 nucleotides of the normal bull FAA gene were deoxyguanilic acids (G), whereas two deoxythymidylic acids (T) were found instead at the same positions in the subfertile bull FAA gene. The two SNPs led to two (183 and 184) amino acid substitutions of Glycine (G) in the normal bull by two Valine (V) in the subfertile bull FAA.

<table>
<thead>
<tr>
<th>Resulting cDNA Sequences:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bull # 1 (Fertile) 535 ATATTGTCAACTCTGGTGCTCTCAATCAAAC 566</td>
</tr>
<tr>
<td>Bull #2 (Subfertile) 535 ATATTGTCAACTCTGTGCTCTCAATCAAAC 566</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Corresponding Peptide Sequences:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bull # 1 (Fertile) 177 Q N I V N S G G P Q S N L 189</td>
</tr>
<tr>
<td>Bull #2 (Subfertile) 177 Q N I V N S V V P Q S N L 189</td>
</tr>
</tbody>
</table>

The illustration above is an example of SNP genotyping and clarifies what is happening genetically (for more intensive reviews and/or methods see Kwok, 2001; Mir and Southern, 2000). A bull designated as FAA-negative would have the gene and amino acid sequences shown above for a subfertile bull on both of his chromosomes. A heterozygote would have each form shown above, and a homozygous FAA positive bull would have the normal forms on each chromosome inherited from each of his parents.
DNA-Based Diagnostics

Enzymes referred to as restriction endonucleases will cut DNA at specific sequences. Thus, DNA can be fragmented chemically using these enzymes.

When a mutation occurs within a particular gene, such as the FAA mutation shown in an earlier section of this paper, that mutation may lead to appearance or disappearance of a site recognized by a restriction endonuclease enzyme. Fortunately, that is the case with FAA. In the normal genotype, there is a site between nucleotides 532 and 534 recognized by a restriction enzyme, so DNA is subsequently snipped at that specific location. The mutated form of FAA lacks that recognition site, so cleavage of DNA cannot occur. For example, if PCR is used to amplify 150 nucleotides containing the normal restriction site, and the enzyme is added, two pieces of DNA will result that can be visualized after separation in an electric current. An animal with two copies of the aberrant FAA will yield a single 150 nucleotide piece, making it simple to identify diagnostically.

A heterozygous FAA bull will have the normal and mutated DNA strands amplified. Therefore, three total bands will be visible, leading to the distinction that segregates those animals from the homozygous ones carrying the desirable or mutant genotypes.

An example is portrayed schematically below:

**Figure 3.** Example of DNA-based identification of FAA genotypes assuming a 150 nucleotide piece of DNA was amplified with the restriction site approximately two-thirds into the DNA sequence.

<table>
<thead>
<tr>
<th>Base-pair Size</th>
<th>Genotype</th>
<th>Electrical Current</th>
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</thead>
<tbody>
<tr>
<td>150</td>
<td><img src="image" alt="Normal" /></td>
<td><img src="image" alt="Mutant" /></td>
</tr>
<tr>
<td>100</td>
<td><img src="image" alt="Normal" /></td>
<td><img src="image" alt="Mutant" /></td>
</tr>
<tr>
<td>50</td>
<td><img src="image" alt="Normal" /></td>
<td><img src="image" alt="Mutant" /></td>
</tr>
</tbody>
</table>

1. The restriction site is present on both chromosomes. Therefore, the entire amplified DNA is susceptible to restriction enzyme cleavage.

2. Mutant lacks enzyme restriction site, no DNA digestion occurs.

3. Heterozygote has one normal/one mutant copy. Therefore, one-half of the DNA in the sample is digested, with one-half stable. Thus, three bands of DNA are visible.
Sexing Semen

Like many new biotechnologies, the first attempts to sort X and Y-bearing sperm was unsuccessful (Gledhill et al., 1976). In the late 1980’s and early 1990’s, rapid progress was made in an attempt to provide individuals with the ability to pre-select for gender. Met at first by controversy and concern ranging from ethical issues, to monetary costs and normality of the resulting offspring the refinement of this technology weathered the storm. Predictions have been made that sex-sorted semen will be used for artificial insemination use by the year 2005 (Amann, 1999). Sexed semen has already reached the marketing phase in the United Kingdom (Garner, 2001). So, where are we in terms of efficiency, accuracy and marketing of sorted sperm?

Since the DNA content of the Y chromosome is less than the larger X chromosome, this allows for sperm to be segregated easily following the application of a DNA dye using a high intensity light beam. Johnson (2000) reported their original flow cytometer would sort approximately 350,000 sperm per hour, whereas today’s high-output sorters now reach near six million per hour when sorting both X and Y sperm. If only the X-bearing sperm are desired, output has reached nearly 18 million per hour (Johnson, 2000). Pressures exerted for segregating at this speed range from .84 kg/cm² for standard units to 4.22 kg/cm² for high-speed cell sorters. Many hours are needed to sort multiple AI doses. As a result, low-dose insemination numbers of spermatozoa have been under investigation in multiple species. Successful attempts in cattle have been reported with sexed semen using $2 \times 10^5$ sperm per insemination of nonfrozen sperm (Seidel et al., 1997). This is significantly less than the 20 million normally used in a commercially available frozen insemination dose, even if one considers 50% of those are rendered incapable of fertilization following the rigors of freezing/thawing. Continued instrument refinement will enhance this process, but currently, multiple machines, people-power and hours will be required to produce large lots of commercially available sexed semen.

The accuracy of sorting X and Y-bearing is not 100%, but most laboratories report reaching 90% or greater (Rath et al., 1999; Seidel, 1999; and Johnson 1992). When sorting sperm, the accuracy is dependent on the amount of DNA difference between X and Y sperm. Johnson (2000) reported the highest accuracies are obtained when the differences in DNA content are greater than 3.5%. For all livestock species, the differences have been reported to be greater than 3.5% while the human resides at approximately 2.8% (Johnson, 2000). Other factors that may affect the accuracy of the sorting process include dead sperm and any morphological variation in sperm. Post-thaw motilities of sorted frozen bull sperm have been reported around 30-35% (Schnek et al., 1999; and Johnson et al., 1996) and percentage of intact acrosomes (PIA) of 40% (Johnson et al., 1996).

The United States beef industry is greater than 95% natural mating in contrast to the dairy industry where it has been reported to be approximately 65% AI (Amann, 1999). For the dairy industry, sexed semen has tremendous advantages where bull calves are undesirable and the cost of replacement heifers often a limiting factor in increasing cow numbers. The beef industry will be slower to benefit from this technology, except for purebred breeders who will more likely accept added costs to sorted sperm to capture those genetically superior male and female lines.
Cost of operating a flow cytometer for one year is estimated at $183,200, this excludes cost of raw semen, storage and distribution (Amann, 1999). Using dairy semen that has been sex sorted may have a 2-fold increase in sale price, and supply may be limited due to current sorting efficiency. Large-scale use in the dairy and beef industry may be limited to those genetically superior individual previously mentioned. However, if the current cost of equipment decreases significantly, sorted X and Y-bearing semen will be met with less hesitation.

References


FACTORS AFFECTING FERTILIZATION IN ESTROUS SYNCHRONIZED CATTLE

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Introduction
In addition to the requirements for healthy well-managed cattle and the sound application of synchronizing drugs, many other factors can also play a role in determining the success of an AI – estrous synchronization program. Considering the economic investment in semen and drugs, the success of such a program must be judged on the basis of pregnancy rate to the first artificial insemination service. Also, a good first service pregnancy rate response usually signifies that conditions are good for second service and the breeding season in general. Additional key factors to be considered as impacting pregnancy rate to first service are semen quality (primarily dependent on choice of bull), the timing of insemination and the competence of the inseminators in handling and placement of semen. In most breeding strategies, whether estrous synchronization is employed or not, the semen quality, placement, and timing of insemination are critical to a successful pregnancy. The nature of subfertility due to the male/inseminate is proving as complex as that due to the female. Current research in our laboratory utilizing accessory sperm (measure of sperm available for fertilization) and embryo quality (measure of fertilizing sperm and egg competence) have given us some insights to the problems associated with attempts to optimize pregnancy rate to AI. In this presentation I would like to address some of these insights particularly those associated with the semen/bull and the timing of insemination.

Compensable and Uncompensable Seminal Deficiencies
We now know that success or failure of an AI dose due to the male or inseminate resides in whether or not the egg was fertilized (fertilization rate) or whether or not the embryo developed normally and hatched in time to signal pregnancy to the dam (embryonic death). Both scenarios are embraced by semen quality and quantity and they must be considered together to address “pregnancy rate”. Salisbury and Vandemark (1961) were the first to suggest the nature of the relationship between sperm quality and quantity. They proposed that fertility increases with increasing numbers of viable sperm delivered to the cow up to a threshold, after which limiting factors in the female population become important and further increases in sperm are without effect on fertility. From the standpoint of semen quality, Pace et al. (1981) found this relationship to hold true for numbers of structurally intact and motile sperm in the inseminate. Sullivan and Elliott (1968) showed that the minimum number of motile sperm required for maximum fertility (threshold) differed among bulls and that bulls also differed in the maximum fertility at any dosage (Figure 1). They also observed that low fertility bulls required that more sperm be inseminated than for high fertility bulls in order to reach
their maximum fertility. They postulated that the requirement of more sperm by the subfertile bulls was due to the presence of abnormal sperm unable to negotiate barriers in the female tract precluding their access to the site of fertilization. This was shown be true in a later study (Saacke et al., 1998). From AI data in the Netherlands, den Daas et al. (1992) found that the minimum number of sperm required to reach maximum fertility for a given bull (threshold) was independent of the maximum fertility achievable by that bull. Collectively, these studies, cited above, indicate that it is now critical to recognize that seminal deficiencies fall into two major categories (compensable and uncompensable). Seminal deficiencies that are compensable would be those impacting pregnancy rates when numbers of sperm in the dosage are below threshold levels; i.e. pregnancy rate differences among bulls due to compensable seminal deficiencies could be minimized or eliminated simply by raising sperm numbers per AI dose. Such adjustments in the AI dose are made by reliable AI organizations when such deficiencies are known. However, where semen handling techniques or AI placement of semen is not adequate, impairment of pregnancy rate can be expected simply because lower than threshold numbers of viable sperm may be delivered to the cow. Seminal deficiencies that are uncompensable would be those that result in subfertility to AI regardless of sperm dosage and are represented by incompetent sperm that can fertilize, but not sustain an embryo. Such a deficiency is not compensable because the incompetent sperm have a chance of preempting fertilization by a competent sperm equal to their frequency of occurrence in the semen dose. These deficiencies are intrinsic to the bull and can therefore only be minimized by bull selection.

![Relationship of Semen Quality and Quantity to Fertility](image)

**Figure 1.** Relationship between pregnancy rate and the number of spermatozoa inseminated. The semen of different bulls varies in the maximum non-return rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (modified from Sullivan and Elliott (1968)).

**Accessory sperm and their implication to pregnancy rate**

Accessory sperm are those sperm trapped in the zona pellucida (outer covering of the egg), one of the important egg vestments sperm must penetrate in order to fertilize. Although there is only one fertilizing sperm, a range in number of sperm may be simultaneously competing for this honor. Once the fertilizing sperm enters the egg proper, a reaction occurs stopping progress of these competing sperm as well as the
binding of additional sperm to the surface of the zona pellucida. Thus, accessory sperm are thought to represent, in number and quality, those sperm competing for fertilization in the oviduct of the cow during that short window in time provided by the fertilizable egg. Through several years of experimentation in our lab we have now recovered nearly 1000 eggs/embryos from single-ovulating cows 6 days post artificial insemination (nearly 30 different bulls were represented in these studies). Figure 2 shows the distribution of accessory sperm found in the zona pellucida of embryos and eggs from these cows as being very skewed, having an average, median and mode of 12.0, 2.4 and 0 sperm per ovum/embryo, respectively. Of reproductive importance is the association of accessory sperm number per egg/embryo to the fertilization status and embryo quality. This is best described by the median (50 percentile of cows) number of accessory sperm per egg/embryo (Table 1). Clearly, unfertilized eggs are simply sperm hungry, having a median accessory sperm number of 0. These data also show that embryo quality is positively related to median accessory sperm number. Good to excellent embryos have more accessory sperm than do degenerate

Figure 2. Frequency distribution of accessory sperm per embryo or ovum in artificially inseminated single-ovulating cows. Quality and quantity of semen used varied, but was within acceptable standards for commercial artificial insemination. Similar distributions have been reported for individual experiments utilizing both frozen and fresh semen (Saacke et al., 2000).

<table>
<thead>
<tr>
<th>Fertilization status/Embryo quality</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent/good</td>
<td>449</td>
<td>24.5 ± 44.1</td>
<td>7</td>
</tr>
<tr>
<td>Fair/poor</td>
<td>213</td>
<td>17.2 ± 32.2</td>
<td>5</td>
</tr>
<tr>
<td>Degenerate</td>
<td>80</td>
<td>13.5 ± 38.1</td>
<td>1</td>
</tr>
<tr>
<td>Deg/UFO</td>
<td>12</td>
<td>2.7 ± 5.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Unfertilized</td>
<td>173</td>
<td>1.6 ± 16.5</td>
<td>0</td>
</tr>
</tbody>
</table>

or fair to poor embryos. This has been interpreted to suggest that the larger accessory sperm numbers are most likely associated with higher embryo quality because they represent greater competition among potential fertilizing sperm at the time of fertilization and that this competition favors a more competent sperm (i.e., there is sperm selection in the zona pellucida of the egg, Howard et. al., 1993). On this basis, we ascribed a score to the embryos within categories of increasing accessory sperm number to determine the approximate number of accessory sperm (competing sperm) required to maximize embryo quality in artificially inseminated cows. These data are presented in Figure 3 and were based upon 884 embryos recovered from the 927 ova/embryos represented in Figure 2 and Table 1. It is apparent from Figure 3 that approximately 11 to 20 sperm per embryo were necessary to reach the maximum embryo quality index, after which increasing accessory sperm numbers had no further relationship to embryo quality. This stresses the importance of semen handling and placement in the cow if we are to achieve threshold or above threshold numbers of sperm to the egg (i.e., greater than 11 sperm/egg) necessary to maximize both fertilization rate and embryo quality.

![Figure 3. Histogram showing the numbers of accessory sperm required to maximize embryo quality index for 6 day-old embryos (morulae) derived from artificial insemination of single-ovulating cows. Embryo grading was according to Lindner and Wright (1983) as modified by DeJarnette et al., (1992). Embryo quality index was the average embryo quality based on the numerical score listed above. As may be noted, a minimum of 11 – 20 accessory sperm per embryo was required to maximize embryo quality index. The number within each bar is the number of embryos recovered in that accessory sperm category.](image)

Important to this discussion is that one understands how embryo quality affects pregnancy rate. The best data on this point is that of Lindner and Wright (1983), who developed the embryo scoring system we used in the data presented above. They showed that embryos classified as excellent to good produce twice as many pregnancies upon transfer to recipients as those that are classified fair to poor. One would expect much of this difference in embryo performance to carry over to embryos permitted to remain in utero. Of course degenerate embryos and unfertilized eggs produce no pregnancies under any circumstance. Based upon the median number of 2.4 accessory sperm per
egg/embryo (Figure 2) and the threshold need for more than 11 sperm per ovum/embryo to optimize embryo quality (Figure 3), it is clear that breeding practices favoring sperm access to the egg be adopted where possible. The effort to raise accessory sperm number per egg/embryo using several different strategies in artificially inseminated cows has been a central focus of our research program for the past several years. The outcome of our efforts have been reviewed previously (Saacke et al., 1994 and 2000) and thus, will not be repeated here except to emphasize two of the major positive factors impacting accessory sperm numbers per egg/embryo important to estrous synchronization and timed insemination.

The effect of bulls and time of insemination on sperm access to the egg and embryo quality

When cows are bred at the conventional time following onset of heat (approximately 6 – 16 hours following onset), there is considerable variation among bulls with respect to numbers of sperm accessing the egg (Nadir et al., 1993). Data from this study comparing 4 bulls is presented in Table 2. Clearly, Bull A in this comparison has high egg access as denoted by the high accessory sperm number (median of 40 sperm per egg) compared to the other 3 bulls. It would be expected that such a bull as A would perform as well at low sperm dosages as at normal dosage and/or that this bull would be less vulnerable to inseminator error than the other bulls when maximizing fertility and embryo quality. Under the same premise, bulls B and C would also match the fertility and embryo quality of bull A, but one would expect that while sperm dosage is appropriate, there is less room for inseminator or semen handling error with these two bulls. For bulls B and C, pregnancy rates will depend more heavily on inseminator competence and timing of insemination. Based on a median of 2 sperm/egg, bull D would be expected to be inferior in optimizing fertilization rate and embryo quality under current use in AI. The seminal differences that we address across these four bulls would be considered compensable differences. Some of the semen traits involved in these differences are known and used by AI organizations in processing semen and determining sperm dosage rate. However, there are compensable differences among bulls that we still do not yet understand and can only be determined by fertility data from the artificial insemination of adequate numbers of cattle.

<table>
<thead>
<tr>
<th>Bull</th>
<th>n</th>
<th>Median</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>40</td>
<td>53 ± 61</td>
</tr>
<tr>
<td>B</td>
<td>37</td>
<td>8</td>
<td>15 ± 23</td>
</tr>
<tr>
<td>C</td>
<td>16</td>
<td>13</td>
<td>36 ± 65</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>2</td>
<td>11 ± 16</td>
</tr>
</tbody>
</table>

Nadir et al., 1993
and in particular, the health of the DNA contributed to the embryo by the male (for
review see Saacke et al. 2000). DeJarnette et al., (1992) examined the 6 day old embryos
from cows bred to semen of AI bulls having average and below average quality (within
the AI center) based upon counts of abnormal sperm. Their data are shown in Figure 4.
Clearly, the below average semen produced fewer excellent to good embryos and greater
numbers of degenerate embryos and unfertilized eggs when compared to semen of
average quality. Bulls in AI are generally screened for significant numbers of abnormal
sperm prior to acceptance into AI. In addition, in reliable AI organizations, routine
examination of semen for abnormal sperm is practiced to check for changes in a bulls
spermatogenic status. Sperm morphology evaluation is also one of the main components
of the BSE (breeding soundness exam) of bulls practiced by veterinarians in approving
breeding bulls for service. Availing oneself of a reliable semen service and/or BSE for
bulls will minimize risk of using semen with significant uncompensable deficiencies.
Posing a particular problem in uncompensable semen deficiencies are fat bulls and a
percentage of those coming off “hot rations” from test stations, where testicular
thermoregulation has been impaired.

![Effect of Average vs Below Average Semen](image)

**Figure 4.** Effect of average and below average semen (based upon content of abnormal
sperm) on fertilization status/embryo quality in single ovulating cattle. Both, fertility and
embryo quality were influenced by the semen as noted in the shift in distribution across
categories (n = 21 and 22 for the average and below average semen, respectively).
(DeJarnette et al. 1992).

More recently we have examined the effect of insemination time on numbers of
accessory sperm, fertilization status and embryo quality (Dalton et al., 2001). In this
experiment, the HeatWatch® system was used to dictate time of artificial insemination
for each cow. In this heat detection system, an electronic device is placed on the rump of
the cow and a signal is transmitted via antennas to a computer when the device is
activated for 2 seconds by the pressure of a mounting cow. On this basis, first mount,
duration of mounting and number of mounts were permanently recorded along with the
identification of the standing cow. In lactating Holsteins, ovulation occurs 27.6 ± 5.4
hours following the first mount for either natural estrous cycles or prostaglandin
synchronized cycles (Walker et al., 1996). Our experimental artificial insemination time was either 0 hour, (heat onset indicated by first mount), 12 or 24 following first mount. However, due to logistics associated with monitoring the computer every three hours followed by retrieving the cow for insemination, actual times of insemination were: 2.0 ± 0.9 hours, 12.1 ± 0.6 and 24.2 ± 0.7 hours following the first mount, respectively. Six days following insemination, the embryo was recovered non-surgically and examined for fertilization status/embryo quality and numbers of accessory sperm according to previously published methods (DeJarnette et al. 1992). Artificial insemination was to one of three bulls used at random and balanced in number of resulting eggs/embryos recovered for each time of insemination.

Accessory sperm data are presented in Table 3. Clearly, accessory sperm number per embryo/egg was favored by breeding later, rather than earlier. Fertilization rate and embryo quality are presented in Figure 5 for each insemination interval (0, 12, or 24 hours post estrus onset).

Table 3. Effect of artificial insemination time on accessory sperm per embryo or egg

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>% Fert</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour</td>
<td>39</td>
<td>9 ± 23</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td>12 hour</td>
<td>39</td>
<td>21 ± 46</td>
<td>2</td>
<td>74</td>
</tr>
<tr>
<td>24 hour</td>
<td>39</td>
<td>33 ± 53</td>
<td>4</td>
<td>82</td>
</tr>
</tbody>
</table>

*Ovulation 27.6 ± 5.4 hours

*25 x 10^6 sperm/dose

(Dalton et al., 2001)

Figure 5. Effect of time of artificial insemination following onset of standing heat (HeatWatch System®) on fertilization status and embryo quality judged 6 days following artificial insemination (n = 117). (Dalton et al. 2001).
From Figure 5, increasing fertilization rate can be observed to follow increasing accessory sperm number (Table 3), as expected. Fertilization rate is favored by breeding late (24 hours post heat onset) and poorest by breeding early, near onset of heat. However, examination of embryo quality in relation to time of insemination shows a shift from high quality embryos achieved by inseminations at/near onset of heat to low quality embryos from insemination at 24 hours following heat onset. On the basis of these data it appears that optimum reproductive efficiency (pregnancy rate) is a compromise using our current techniques and recommendations in AI. If we inseminate too early, we suffer from lower fertilization rates (but embryo quality is good) and if we breed too late, we suffer from lower embryo quality (but our fertilization rate is good). Thus, the intermediate time of 12 hours post heat onset would prove optimal when using a precise method for determining heat onset (Figure 6). This optimum was verified in field studies using “HeatWatch®” (Dransfield et al., 1998) where 6-16 hours post onset of heat provided the best pregnancy rates.

Figure 6. Calculated pregnancy rate from data presented in Figure 5 and based upon the ability of embryos classified excellent to degenerate to constitute a pregnancy (according to Lindner and Wright, 1983). AI as a compromise is based upon early inseminations being inadequate due to high levels of unfertilized ova, and late inseminations characterized by poor embryo quality, most likely due to an aging egg. However, high embryo quality appears to be associated with early insemination and high fertilization rates are associated with late insemination (Saacke et al., 2000).

The basis for pregnancy rate failure by breeding late (24 hour post onset) could reside in the fact that we would often have an aging egg waiting for sperm if we assume that ovulation occurs 27.6 ± 5.4 hours post heat onset as detected by HeatWatch®. Sustained sperm transport to the site of fertilization in the oviduct requires a minimum of 4 – 6 hours following insemination in the cow (Hunter and Wilmut, 1984). Thus, sperm arrival in the oviduct following a 24 hour insemination would be 28 to 30 hours post heat onset, after many eggs were already ovulated. This would indicate that in the current study, a rather large portion of eggs would be aging awaiting sperm arrival. This probably accounts for most of the degenerate embryos from this late insemination. On the other hand, the high embryo quality associated with early insemination suggests that
duration of sperm residence in the female tract may result in exertion of additional selection pressure favoring fertilization by a more competent sperm, particularly where there are uncompensable sperm deficiencies in the semen (Figure 6). The correct explanation is probably a combination of the two but must await further research.

**Closing Comments**

Important to the insemination strategies employed with the new burgeoning regimes of estrous synchronization is knowing the time of ovulation and the variation in time over which ovulation can be expected. Only by such information can we make the correct decision on when to inseminate in relation to injection events or behavioral clues. The data presented here would indicate that insemination must be late enough to maximize sperm access to the egg, but not so late to risk the possibility of an aging egg awaiting sperm arrival in the cow’s oviduct. Thus, if a synchronization regime were to postpone ovulation until 30 or 35 hours following heat onset, the 24 hour insemination could be the best in optimizing pregnancy rate (both fertilization rate and embryo quality). Clearly, the CL and follicular control of the estrous cycle in cattle, currently under intensive research, offers tremendous advantages in synchronizing as well as tightening the variation in the ovulatory event.

Finally, I would end this discussion by again recognizing the magnitude of bull differences that can greatly influence results to a synchronization program. Differences that we have seen among bulls in response to time of insemination for one of our studies is shown in Figure 7. Although the trends were similar, the magnitude of differences in performance of bulls at different insemination times is quite great. In a timed insemination program, Bull A would be considered to perform well over a broad time span relative to ovulation, whereas bulls B and C really required later breeding to optimize their efficiency in sperm access to the egg. Unfortunately, and as you might expect, this is difficult, time consuming and therefore expensive data to acquire and therefore not available on commercial bulls. The best protection one can have is to be aware of bull differences and to avoid a problem bull, subscribe to a reliable source of quality semen.

![Figure 7. Variation among bulls in sperm access to the egg relative to time of insemination post heat onset using the HeatWatch® system of recording mounts. Mean sperm per egg/embryo is shown by the bars and the median number in brackets. Bull A has adequate numbers of sperm accessing the egg at all breeding times while bulls B and C require insemination closer to ovulation (Dalton et al., 2001).](image-url)
Acknowledgements

I am appreciative of the support of the following agencies relative to our male research regarding accessory sperm, fertilization and embryo quality in artificially inseminated cattle: Select Sires Inc., National Association of Animal Breeders and the Virginia Ag Council.

References


BULL BREEDING SOUNDNESS EXAMS AND BEYOND

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College of Veterinary Medicine, Kansas State University

History

The development of routine on-ranch bull testing coincided with technical advances in bull electro-ejaculation (EE) which permitted safe, relatively effective, semen collection of un-handled bulls. Approximately 50 years ago, the Rocky Mountain Society for the Study of Fertility in Bulls (RMSSFB) was formed "to share and disseminate the essentials for evaluation of beef bulls for fertility and to standardize procedures". A landmark report (Carroll et al 1963) on 10,940 bulls examined showed that on-ranch bull "fertility" testing was feasible. The RMSSFB subsequently became the Society for Theriogenology (SFT), which developed a revised breeding soundness evaluation (BSE) system in 1992, as described below.

Bull Breeding Soundness

The Breeding Soundness Evaluation (BSE) is a relatively quick and economic procedure for screening bulls prior to sale or use. Its objective is to establish a baseline, above which bulls could be regarded as satisfactory potential breeders. As it is intended for wide application with a variety of breeds in different environments, it needs to be simple, repeatable and unambiguous. However, the BSE should not substitute for professional judgment or common sense. In the BSE, bulls are placed into the categories of satisfactory, unsatisfactory and classification deferred. The process is most effective in identifying bulls at the lower end of the potential fertility spectrum. It is relatively less effective in predicting individual bull performance at the upper end of the fertility spectrum. Reasons for this include:

1. Fertility is a complex trait which is influenced by both male and female traits as well as by extraneous factors (e.g. nutrition, environment, disease etc).
2. The BSE aims to identify bulls which are satisfactory (not necessarily those which are superior).
3. The BSE is a relatively quick and simple screening procedure which does not comprehensively assess all aspects of male fertility.
4. Knowledge and understanding of male fertility keep increasing and changing.

BSE Procedures

A routine BSE generally includes the following:
1. Physical examination.
2. Reproductive examination (including measurement of scrotal circumference).
3. Collection and examination of semen.
In addition, a libido/serving capacity test may be included, as may special tests for diseases (e.g. vibriosis or trichomonosis). These procedures will add predictive value to the assessment process and may be specifically indicated in some situations, but they are not part of the routine BSE.

Limitations of the BSE include:
1. Results are most valid at the time of examination only.
2. The system works best to identify infertile bulls
3. It is not designed to predict the precise fertility of individual bulls
4. It does not routinely include assessment of bull libido and mating ability
5. It does not routinely include testing for infertility diseases.

Current SFT Thresholds
Revision of the American Society for Theriogenology 1975 bull BSE procedures resulted in the system (Chenoweth et al., 1992) described below.

For bulls to be classified as Satisfactory Potential Breeders, they must pass the physical examination and equal or exceed the minimal thresholds in each of the following categories:

<table>
<thead>
<tr>
<th>Category</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Scrotal Circumference</td>
<td>30 cm at #15 mo</td>
</tr>
<tr>
<td></td>
<td>31 cm at &gt;15 #18 mo</td>
</tr>
<tr>
<td></td>
<td>32 cm at &gt;18 #21 mo</td>
</tr>
<tr>
<td></td>
<td>33 cm at &gt;21 #24 mo</td>
</tr>
<tr>
<td></td>
<td>34 cm at &gt; 24 mo</td>
</tr>
<tr>
<td>- Sperm Morphology</td>
<td>$70% normal sperm</td>
</tr>
<tr>
<td>- Sperm Motility</td>
<td>$30% individual motility &amp;/or &quot;fair&quot; gross motility</td>
</tr>
</tbody>
</table>

Bulls which do not equal or exceed these thresholds will either be classified as Unsatisfactory Potential Breeders or as Classification Deferred (as below).

Satisfactory
Bulls which equal or surpass minimum thresholds for scrotal circumference, sperm motility and sperm morphology, and which do not show genetic, infectious or other problems or faults which could compromise breeding or fertility.

Unsatisfactory
Bulls which are below one or more thresholds and which are highly unlikely to ever improve their status. Also, bulls which show genetic faults or irrevocable physical problems (including infectious disease) which would compromise breeding or fertility are included.

Classification Deferred
Any bull which does not fit into the above categories and which could benefit from a retest. Provision is provided for the scheduling of a retest. This category includes young bulls with an "immature" semen profile as well as any bulls whose semen is substandard but considered to be capable of improvement. Also bulls from which a representative ejaculate
was not obtained, as well as bulls with treatable problems. In general, if doubt exists concerning a bull's status, he should be considered as a candidate for a retest and placed into the "classification deferred" category.

**Rationale for Current Thresholds**

1. **Minimum acceptable standards for scrotal circumference, sperm motility and sperm morphology.**

   Previous SFT BSE systems placed over-reliance on numerical scores. A bull could pass the BSE even if he was very poor in one particular category as long as other scores were high enough to give an overall passing grade. Also, scores could be used to "rank" bulls in terms of potential reproductive performance. Both approaches could lead to error and/or misrepresentation, especially as the original intention of numerical scores was to help place bulls in categories or groups. In general, the threshold standards selected are not overly rigorous. The case has been made to establish more rigorous thresholds to encourage genetic progress in reproductive traits. However, this approach was not adopted for several reasons. Firstly, it was considered necessary to have bull BSEs more generally accepted by industry before higher standards might be imposed. Secondly, producers need to have flexibility to select for other traits. Thirdly, it was acknowledged that current skills and knowledge were better able to detect animals which would perform at the low end of the fertility spectrum than at the top end. However, although the present system describes minimum acceptable standards only, higher thresholds may be implemented by veterinarians in consultation with their clients. In other words, the SFT BSE standards represent the backstop for reproductive adequacy. The use of higher standards wherever possible should be encouraged particularly with seedstock breeders and their associations.

2. **Lowest scrotal circumference threshold of 30 cm.**

   Relatively low thresholds for scrotal circumference were selected for the reasons given above. **These are minimal acceptable measures for ALL bulls raised on a good plane of nutrition.** Most emphasis is placed on standards for pubertal bulls up to 2 years of age (i.e. the most common and most important test population). Even though variations occur with age, nutrition level and genotype, the use of low thresholds provides considerable latitude. However, use of a minimal threshold lower than 30 cm. will depend upon individual professional judgement and it will have to be justified accordingly. Again, these thresholds are based upon considerations of reproductive adequacy and not necessarily upon those of optimal genetic merit.

3. **70% threshold for normal sperm morphology.**

   The practice of separately classifying different sperm abnormalities based upon underlying assumptions concerning their etiology and their significance has been severely challenged. The arguments for using a single threshold for "normal" sperm in the light of present knowledge receives support from a number of sources (e.g. Barth and Oko (1989). It is reinforced by knowledge that many sperm abnormalities previously considered as discrete entities, may in fact reflect stages within a spectrum of standardized responses to stress by the spermatogenic epithelium (Larsen and Chenoweth, 1990; Vogler, 1990). This approach is not inconsistent with the pioneering conclusions of Lagerlof (1934) who argued that damage to spermatogenesis might well influence many more sperm than those with easily
discernable faults, and that a threshold level for satisfactory fertility appeared to exist. The threshold of 70% normal sperm is consistent with the observations of Lagerlof (1934) and, more recently, work by Wiltbank and Parrish (1986). The threshold of 70% normal sperm does not make any distinction between types of abnormalities involved. However, the categories of "primary" and "secondary" sperm abnormalities were retained as they are often still used to assist in the mechanics of collating totals, prognosticating and monitoring progress, despite reservations concerning their underlying assumptions. A case was made to use the categories to "major" and "minor" (Blom 1972). However, it became apparent in discussion that the lists of sperm abnormalities in both systems were identical, at least in terms of practical application. It was considered that the "compensable/non-compensable" sperm anomaly classification system, as described by Saacke et al (1991), requires further refinement and testing before widespread use is recommended. It is quite possible that progress will mandate a further change to our sperm morphology assessment procedures before too long. In the interim, employment of an overall threshold for sperm abnormalities, as recommended herein, should lessen the emphasis and debate on the significance of particular categories of sperm abnormality.

4. 30% threshold for progressive (individual, %) sperm motility.

The use of a relatively low threshold for sperm motility is challengeable. Some consider that this should be higher while others question the inclusion of any estimate of sperm motility at all. Taking into account the varied and often trying environmental conditions encountered in the field, a higher threshold might well be an obstacle to general acceptance of this scheme, or at least to its proper observance. Similar sentiments were expressed in 1975 when the motility component of the BSE was reduced. It should be realized that this relatively low threshold in no way diminishes the potential importance of motility assessment when performed under optimal conditions.

5. Use of "Classification Deferred".

Some unease occurred with the term “questionable”, despite its widespread use for many years. The difficulty arose from the realization that being placed in this category almost invariably meant that a bull was destined for a retest; certainly it was intended as a temporary or "holding" category. Bulls might be placed in this category for many reasons, both trivial and serious. Whatever the reason for its application, the term "questionable potential breeder" could be misinterpreted and it could disadvantage the subsequent sale of such animals, even if they subsequently proved to be “satisfactory”. The substitution of a "classification deferred" category, a description which has neutral connotations, does not have such disadvantages. However, its does imply that a retest will be scheduled and a slot is provided for this purpose.
Thus, the B.S.E is.....

1. A Screening Test
   The bull BSE represents a rapid, economic screening test which screens bulls for detectable problems and puts them into groups which generally behave as classified.

2. A Veterinary Procedure
   In many parts of the world, the BSE is regarded as a veterinary procedure. As such, it represents a privilege, responsibility and accountability. The responsibility includes the assumption that the procedure will be done competently and professionally, without shortcuts or compromises. As a veterinary procedure, it is also assumed that the findings and recommendations will reflect sound professional judgment.

3. A Management Tool
   The BSE should be part of management scheme to improve herd fertility, genetics and profitability. As such it can help implement decisions on bull numbers, ratios, rotation and group composition. It should also play a role in genetic selection and planning. Lastly, the BSE is an important tool in infertility investigation.

4. A Sound Investment
   A conservative estimate may be made of a 6% or greater fertility advantage for bulls passing a BSE and/or semen quality thresholds over unevaluated bulls (with larger differences possible when satisfactory bulls are compared with those which fail the BSE). In addition to increased calf crop, benefits accrue through increased weaning weights of older calves at weaning because females become pregnant earlier in the breeding season. Based upon current U.S. prices, a 6% increase in calf crop at weaning would represent an approximate return of $20-$25 for each $1 invested in the BSE. Additional benefits accrue via increased weaning weights (more females pregnant earlier) and subsequent improvements to herd fertility via male-female genetic links.

5. A Welfare Issue?
   Welfare concerns with bull BSE are mainly with the use of electro-ejaculation (EE) to obtain semen. The introduction of effective EE allowed routine testing procedures with unhandled range beef bulls. Such bulls cannot be safely collected with an artificial vagina (AV), while collection via per-rectal massage can be inconsistent. Semen collected via proper use of EE is comparable to that obtained with an AV. In addition to its advantages with range-type bulls, EE also allows semen to be collected from bulls in which physical problems rule out AV collection. However, EE does cause some discomfort to bulls, although improvement in probe design and machine circuitry have led to a marked reduction of such signs. One study ascertained that bull heart rates were less when an epidural was given prior to EE, although no comparison was made with other activities, either painful or pleasurable. Other work showed that, whereas blood cortisol levels were elevated with bull EE, these were not as high as those due to restraint alone, or per-rectal palpation. With rams, EE did not result in higher cortisol levels than either shearing or restraint. A relevant question is whether or not EE is a routine, useful managerial procedure. If so, does it cause more distress than other such procedures? Does it cause unnecessary pain or harm? This debate may differ between regions with predominantly natural breeding systems (e.g. the
Americas and Australasia) and those in which A.I. predominates (e.g. Europe). Although male fertility is important for both, it is usually assessed with unhandled bulls in the former case, compared with bulls which are used to handling and A.V. collection in the latter. For unhandled bulls, the choice is either to employ a method of semen collection which poses minimal danger to both man and animal (e.g. EE), or to avoid semen assessment altogether; an option that would lead to decreased fertility in breeding herds, add to the economic burdens of producers and increase strains on natural resources. If EE is a useful, routine managerial tool, then it is still valid to ask whether or not the pain outweighs the gain. Although objective studies are lacking, observations indicate that this procedure is no more distressful than other accepted procedures such as vaccination or lateral restraint. Thus bull EE may be considered as a valid and useful veterinary procedure which should be performed as humanely as possible.

References


HEALTH AND BIOSECURITY CONCERNS TO IMPROVE BEEF CATTLE REPRODUCTION

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Maintaining the health of animals in a beef breeding herd is critical to insure optimum herd productivity since the major contributor to overall herd performance is the reproductive rate. Because of this level of importance, it is essential to carefully monitor all aspects of herd reproduction on a production year basis in order to monitor each year’s production and make good management decisions.

Since reproduction is the basis of herd productivity, the effects of suboptimal health or disease is the primary cause of reduced performance. We should define “disease” as any deviation from normal in an individual animal. This includes non-infectious and infectious causes of disease. Physical injury, management or environmental stress, toxic agents or nutrient deficiencies alone or in combination with infectious agents can contribute to disease and, in turn, reduce reproductive performance.

The risk of introducing infectious diseases into a herd can be reduced by following good management practices. The term “biosecurity” has recently been used to address the areas related to disease prevention, containment, and control within a herd.

Components of Biosecurity

In this discussion, herd biosecurity will be covered under four parts: (1) to reduce or eliminate the source of disease; (2) to minimize disease transmission; (3) to maximize each animal’s ability to resist disease; and (4) to record animal health and productivity parameters to monitor herd health and permit early detection/control of problems.

1) Reduce the source of disease

A common source of disease in beef cow herds is through contact with other cattle. Some additional risks are involved through other livestock species, visitors, feed, water, manure and purchased semen as well as less apparent sources such as dogs, cats, rodents, birds, and other wildlife. This is especially true in regard to several primary reproductive disease agents that are primarily spread by close or direct animal contact and makes it important to determine the individual and herd of origin health status of new animals before herd entry. In all cases an appropriate quarantine, testing, and pre-entry immunization program should be mandatory for all animals prior to herd entry, even if they originated in the same herd. It is advantageous to plan and manage breeding programs and pastures to minimize exposure, including fence-line contact with neighboring livestock. Avoiding visitor and vehicle contact with livestock, being aware of potentially contaminated feed or water sources, and sound sanitation practices are an important part of breeding herd management. A specific consideration in breeding herd biosecurity is the potential of introducing disease through the use of outside semen or embryos. As a general rule, national semen suppliers conform to specific standards for certified semen but smaller bull studs may
provide collection and freezing services without a complete health protocol.

2) **Minimize disease transmission**

It is best to avoid commingling various age or management groups within herds, especially prior to breeding and calving. Maintaining separate management groups whenever possible can limit the spread of disease within a herd. The common use of livestock equipment without proper cleaning and disinfection can also be an avenue for disease exposure. Finally, the proper handling of livestock waste and carcass disposal is an important part of herd biosecurity.

3) **Maximize the animals ability to resist disease**

Individual animal disease resistance is of paramount importance in the prevention and control of certain reproductive diseases. Obviously, animals should be fed an adequate diet based upon NRC requirements and management stress should be avoided whenever possible to assure a healthy animal that can respond to disease challenges. This form of disease resistance or immunity is known as innate or natural disease resistance. Another important part of disease resistance is that from “induced or acquired” immunity. It is important to realize that even though animals may recover from the initial challenge of an infectious agent and become somewhat resistant, they may also become carriers of the disease and be a source of infection for other herd mates.

4) **Record animal health and productivity parameters to monitor herd health and permit early detection/control of problems**

Good records are invaluable for monitoring all aspects of herd reproductive outcomes and animal health. They provide a basis for comparing year to year variation and can be important documents for monitoring subclinical disease effects such as marginal nutrient deficiencies on the breeding herd. Small variation in final results are expected but significant decreases in reproductive rates should be examined and the cause corrected if efficient production is to be maintained.

An early diagnosis permits the institution of immediate control practices and minimizes the spread of disease between animals. For example, detection of repeated estrus during the breeding season is better than detection of delayed pregnancy or pregnancy failure and detection of abortion problems is preferable to waiting to find cows that fail to calve, in terms of diagnostic and control measures.

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"IMMUNIZATION OF THE BEEF COW AND ITS INFLUENCE ON FETAL AND NEONATAL CALF HEALTH"

Brief Synopsis
Immunization of the dam is critical to optimizing the health of the gestating cow and fetus and the perinatal calf. Vaccine use, by itself, is inadequate. Strategic management decisions, including types and timing of vaccination, are required. These require a knowledge of the host-pathogen relationship, including immune mechanisms, pathogenesis, and epidemiology. This article selectively reviews the immune system of the cow and fetus during gestation and explores the use of active immunization of the dam as a management tool to control certain reproductive diseases in the beef herd.

Introduction
The successful outcome of pregnancy requires the dam to have, and the fetus to develop, functional immune systems; yet both must tolerate the other. In this immunologic balancing act the dam must protect the fetus from maternal infections but not reject the fetus. The fetus must attain the ability to differentiate self from non-self but not respond to antigens of the dam. Finally, the dam must produce a high quality colostrum and the precocious calf must consume it in sufficient quantity soon after birth. Superimposed on the immunologic interactions of the cow and fetus/calf are our attempts to manipulate the immune response through management and vaccines. There are few well-designed and executed clinical trials in the scientific literature to evaluate the clinical efficacy of many vaccines. This discussion selectively reviews the immune system of the cow and fetus during gestation and the use of active immunization of the dam to control certain reproductive diseases in the beef herd.

Immune Defenses of the Reproductive Tract
The reproductive tract of cattle is one of several mucosal interfaces between the animal and the environment. At these mucosal interfaces much of the early interaction between the host and the pathogen occurs. Not surprisingly, these systems with extensive mucosal surfaces, such as the reproductive, respiratory, and gastrointestinal tracts, are also the sites of many of the significant disease of cattle.

The primary function of the immune system at mucosal surfaces is to prevent pathogens from entering the body. This function can be severely compromised by the other physiologic roles of the mucosal surface such as absorption. There are differences in the mucosal surfaces within the reproductive tract; for example, the normal vagina has a resident microflora while the healthy uterus is normally sterile. The defense systems at the reproductive mucosal surfaces include both innate and acquired immunity.

The innate immune system is usually the first line of protection at the reproductive mucosal interface. It includes physical barriers, such as the epithelium, mucus, and the sometimes closed cervix; humoral factors, such as complement, lysozyme, lactoferrin, and peroxidase; and some cellular responses mediated by macrophages, polymorphonuclear neutrophils, and natural killer (NK) cells. The mediators of innate immunity are not antigen
specific and do not require immunologic priming.

Acquired immunity is mediated by lymphocytes and is the type of immunity we attempt to manipulate with vaccines. Lymphocytes, along with some accessory cells, are responsible for recognizing foreign substances, responding to them, making soluble factors such as interleukin and interferon, killing infected and foreign cells, and producing antibodies. In contrast to innate defenses, acquired defenses are antigen specific, antigen driven, and mediated by antibodies, cytotoxic T lymphocytes, and cytokines produced during an immune response.

**Acquired Immune Response**

An acquired immune response may be divided into three phases: cognition, activation, and effect. Depending on the immunologic experience of the animal, these phases take a varying number of days to occur and the response will not be maximal for two to four weeks after exposure to antigen.

The acquired immune response is initiated by the recognition of a foreign substance called antigen. This can be a virus, bacteria, toxin, or any other non-self substance. During the cognition phase, antigen-presenting cells process and present the antigen to lymphocytes for recognition.

The activation phase is the sequence of immune events that occurs as a result of the cognition phase. Lymphocytes undergo two major changes in response to antigens: 1) they proliferate, leading to expansion of the clones of antigen-specific lymphocytes and amplification of the immune response; and 2) they differentiate to cells that function to eliminate foreign antigens.

The effector phase of immune responses is the stage in which antigen-activated lymphocytes perform functions that lead to elimination of the antigen. This includes production of antibodies by B lymphocytes and elimination of infected cells by cytotoxic T lymphocytes.

Different subtypes of lymphocytes have specific functions in the overall immune response. Some, called helper cells, are responsible for producing and releasing factors that turn on the immune system. Others, called suppressor cells, are able to turn off the immune response. The balance between the number and/or the net effects of these two cell types, or helper/suppressor ratio, is important in determining the ability of the animal to respond to a vaccine. Certain lymphocytes are able to recognize and destroy cells that have been infected by viruses or bacteria. These are known as killer or cytotoxic cells and are important in an animal's ability to fight intracellular infections. The cells mentioned above; helper, suppressor, and cytotoxic cells, are all part of the cell mediated immune system and are lumped under the general classification of T lymphocytes, since they come from the thymus.

The phrase "cell-mediated immunity" (CMI) can have several meanings, especially regarding modified-live and killed vaccines. This inconsistent usage has resulted in confusion. In its most general usage, the term CMI can include any immune phenomenon mediated by a cell. In more specific usage, it includes only effects mediated by cytotoxic T lymphocytes. Most commonly it is used to describe any effect mediated by a T lymphocyte. This includes the effects of T helper, T suppressor, and T cytotoxic cells. During the activation stage of the immune response a T helper cell response is a normal and necessary part of antibody production from B lymphocytes.

Indirect measures of immune function that assess T helper cell function, such as lymphoproliferation, are likely to show a positive response even if the effector component of CMI, cytotoxic T lymphocytes, is not stimulated. For example, following administration
of a modified-live BHV1 vaccine to a naive animal, virus replication occurs. The immune system responds to this "infection" as outlined above. T helper cells are activated, cytokines are produced, antibody titer rises, and cytotoxic T lymphocytes "see" virus infected cells and are primed. In response to a killed vaccine all of this would occur as well, except the immune system would not be exposed to virally infected cells. The practical implications of these differences would vary from pathogen to pathogen and are difficult to assess. The differences may partially account for the differences noted in the immune responses to modified live versus killed virus vaccines.

The cells responsible for the production of antibodies are called B lymphocytes. When B lymphocytes are presented with a foreign substance they recognize (cognition phase), they undergo repeated divisions and eventually mature into antibody-producing lymphocytes (activation phase). The increased number of activated lymphocytes producing antibodies results in elevation of the antibody titer of the animal to the inducing antigen (effect phase). We use this increase in antibody titer to evaluate the effectiveness of a vaccine; however, as we have briefly discussed, antibody response comprises only one part of a very complex process. In the ruminant, immunoglobulin (Ig) G is a major secretory immunoglobulin,(Butler 1982) and like secretory IgA has been shown to be capable of defending mucosal surfaces.

**Fetal Immunity**

The ruminant fetus is particularly susceptible to infectious agents for three reasons: 1) the syndesmochorial placentation does not allow passive transfer of maternal immunoglobulins during pregnancy, 2) the fetal immune and accessory systems are immature and therefore not fully functional, and 3) the fetal environment provides factors or cells which are conducive to microbial replication.(Osburn 1981)

Fetal immunocompetence develops during gestation. Lymphocytes have been observed as early as 42 days of gestation in the bovine fetal thymus, day 45 in the fetal blood, and in the spleen and bone marrow by 55 days.(Schultz 1974) Lymphocytes that contain IgM were demonstrated by day 59 and those containing IgG by day 145 of gestation. IgM is not observed in the serum until day 130 of gestation.(Osburn 1982) Lymph nodes begin to form at around 60 days and the size of all fixed lymphoid organs increases as gestation progresses.(Schultz 1974)

Bovine fetal lymphocytes demonstrated a suboptimal response to mitogens at 75-80 days of gestation. The lymphoproliferative response increases and by 120 days of gestation, the response to mitogens for many fetuses is in the range of values obtained for lymphocytes from normal adult cattle.(Jensen 1988) Lymphocytes from bovine fetuses inoculated with Mycobacterium bovis at approximately 125 days of gestation are not stimulated by purified protein derivative of M. bovis (PPD) at 20 or 50 days post-infection, whereas lymphocytes taken from adult cattle at similar intervals after M. bovis inoculations are stimulated by PPD.(MacLachlan 1984) These fetal lymphocytes did demonstrate a response to mitogens (plant glycoproteins used in vitro to stimulate lymphocytes in a nonantigen specific manner). When fetal lymphocytes obtained by cannulation of the thoracic duct after day 121 of gestation were stimulated with a mitogen they displayed patterns of secretion of the cytokine interleukin-2, a potent activator of T and B lymphocytes, indistinguishable from those of similarly treated lymphocytes from an adult animal.(Hein 1988) Newborn calves can reject skin grafts just as vigorously as adults, indicating the CMI develops in the bovine by the time of birth.(Billingham 1957)

Granulocytes appear in the fetal blood at day 130 of gestation.(Schultz 1974) The fetal
ruminant inflammatory response differs from that of the adult. Observations of inflammatory lesions occurring in a variety of infectious diseases show a fetal response composed primarily of monocytes and macrophages while the response induced in the adult is a predominantly polymorphonuclear leukocyte reaction.(Enright 1981)

**Neonatal Immunity**

Once a normal calf is born, the most important determinant of its immunocompetence is the timely consumption of colostrum.(Perino 1992) The virtually agammaglobulinemic calf receives large amounts of passive IgG1 via intestinal absorption during the first 12 to 24 hours of life.

Serum IgG1 in trapped by receptors on mammary epithelial cell of the dam, transported through these cells and secreted into the colostrum in the acinar ducts.(Banks 1989) In gestating dairy cows there is a gradual decrease in the serum levels of IgG1 during the weeks prior to parturition, then a gradual increase during the following weeks.(Kiddy 1974)

The duration of protective titers following passive transfer is a function of dose and timing. The half-life of IgG in cattle is around 20 days.(Menanteau-Horta 1985) By 100 days of age (five half-lives), 97% of the maternal antibody will be gone.(Banks 1982) However, residual passive antibody must be considered when designing calf vaccination programs because, depending on the pathogen and the vaccine, even low residual titer may interfere with immunization.(Menanteau-Horta 1985)

Colostrum also contains leukocytes that can influence the immune response of the newborn. Compared to calves fed cell-depleted colostrum, calves fed complete colostrum showed no decrease in lymphocyte numbers in the blood on the second day of life, uniform blastogenic response to a mitogen, slightly enhanced antibody formation against sheep erythrocytes, and a high spontaneous proliferation of mononuclear cells during the first week of life.(Riedel-Caspari 1991) Calves fed colostral leukocytes isolated from heifers immunized with M. bovis had increased lymphocyte blastogenesis to PPD between 3 and 21 days compared to calves fed colostral cells from control heifers.(Duhamel 1986)

The lymphoid systems of cattle and sheep contain a large number of gamma-delta (γδ) T cells, in contrast to the lymphoid systems of humans and mice. This is especially true in neonates where γδ T cells comprise 60% of the T cell pool.(Hein 1991) These cells are found in the epidermis, intestinal epithelium and lamina propria, the basal layers of the stratified squamous epithelium of the tongue and esophagus, the pseudostratified epithelium of the trachea, and the transitional epithelium of the bladder. Based on their tissue distribution and circulation patterns, the most probable function of γδ T cells is the protection of epithelial surfaces, which may be a particularly vital role in the precocious bovine neonate. Newborn calves cannot respond to all antigens with the same magnitude. Newborn calves were able to respond to soluble protein antigens, chicken RBC, and a bacteriophage at birth.(Banks 1982) However, antibody to certain bacterial, protozoal, and viral antigens was not produced or did not appear until 14 to 30 days of age. Salmonella bacterin administered to Holstein calves starting at 1 to 19 weeks of age failed to elicit antibody responses to the lipopolysaccharide (LPS) cell-wall antigen in calves less than 12 weeks old but did stimulate immunoglobulin responses to whole-cell antigen regardless of age. In contrast, modified-live S. dublin vaccine given to calves at one to three weeks of age stimulated anti-LPS immunoglobulins, although the response was not as rapid and was of lesser magnitude than that of older calves given Salmonella bacterin.(Roden 1992) The practical implication of these observations is that not only will the effects on vaccination of
the newborn or young calf be affected by their passive immune status, but also by the specific antigens in question.

**Immunization Considerations**

Vaccine induced immunity is one of several management tools available to the veterinarian to help livestock achieve optimum productivity through disease prevention, control, and eradication. Disease surveillance is a critical part of each herd program to determine need and evaluate the effectiveness of each immunization procedure. This surveillance requires accurate monitoring of clinically affected animals and should be routinely done on breeding females that do not become pregnant or fail to calve as well as herd sires. Additions to the herd should be from known sources, examined, tested, immunized, and isolated for an accepted time before being mixed with the herd. Duration of isolation is dependent on the source of the cattle and the disease(s) of concern. Other risk factors that should be considered are animals in surrounding herds, common grazing agreements, other species that may be carriers or the use of frozen semen or embryos from outside herds.

We manipulate the immune system in two ways: management decisions and vaccines. The two key components required for a successful immunization are an efficacious vaccine and an immunocompetent animal. Despite its simplicity, these, along with some environmental considerations, are the basis for all vaccination successes. Vaccine failures arise from inattention to details in these critical areas and are discussed later.

Both live and killed vaccines are in use. The advantages of one are usually the disadvantages of the other. Modified-live vaccine attributes include: strong, long lasting antibody response achieved with fewer doses; less reliance on adjuvants; virus vaccines may stimulate interferon production; stimulation of the effector component of cell mediated immunity (cytotoxic T lymphocytes); and the bacteria or virus may look and behave more like the pathogenic form of the organism. Some of the advantages of killed vaccines are that they are more stable in storage and they are unlikely to cause disease due to residual virulence or reversion. Some vaccine considerations that impact the health of the fetus and the calf are discussed below.

**Bovine Herpesvirus-1 (BHV1)**

Bovine herpesvirus-1 (BHV1) is a widespread disease primarily affecting the respiratory and reproductive systems. (Blood and Radostits, 1989-IBR) The respiratory form, BHV1 type 1, referred to as infectious bovine rhinotracheitis (IBR) may terminate pregnancy at any stage of gestation. (Chow 1964, Miller 1991) It may contribute to neonatal losses in calves from susceptible dams. (BLOOD and Radostits, 1989-IBR) A strain that may interfere with conception is BHV1 type 2, which causes the disease known as infectious pustular vulvovaginitis (IPV). The IPV form affects the genital mucosa of heifers and bulls and, if severe, may interfere with conception by reduced mating activity but does not appear to cause abortion. (Miller, 1991 VM/SAC; Miller, Whetstone, et al. 1991)

The use of intramuscular modified-live vaccine at the correct time of the production cycle provides protection against respiratory signs and abortion in cattle. (Blood/Radostits 1989-IBR, Chow 1971, Kahrs 1977, Hjerpe, Saunders 1972) It does not prevent latency induced by aerosol exposure to four ml of $10^{6.5}$ TCID50 of virulent BHV1/ml. (Narita M et al.) Animals with passive immunity from immune dams may fail to respond to vaccination before six months of age but the cellular immune function may be primed. (Brar et al. 1978;
The vaccine should be administered a minimum of one additional time approximately one month before breeding to insure stimulation of the immune system. Achieving successful immunization while avoiding complications requires proper timing of administration and handling of vaccine. Vaccination at the time of breeding with intramuscular modified-live vaccines may seriously decrease the conception rate in susceptible cattle. Intravenous administration of a five ml of cell culture medium containing from $10^{6.5}$ to $10^{7.3}$ TCID_{50}/ml of one of four vaccine strains of BHV1 on post-breeding day 14 resulted in infertility in four of eight heifers. Failure of a single injection of modified-live agent to immunize may be due to improper handling, storage, or administration. The importance of a solid immunity of long duration that minimizes the chance of a sporadic natural infection at critical stages of reproduction or production is essential for a well managed breeding herd.

Declining immunity may be stimulated by natural infection, reactivation of latent virus, or the administration of modified-live vaccine. The annual use of intramuscular modified-live IBR products is unnecessary. Immunity of long duration follows infection by virulent virus or by modified-live injection. This would include protection of the fetus from transplacental infection in most cases.

Recent work indicates that BHV1 type 2 virus administered to seronegative pregnant heifers did not cause abortion. This may indicate a possible use of BHV1 type 2 virus for an intramuscular modified-live product that could improve safety and still provide a durable immunity. Similarly, thymidine kinase-negative mutants of Cooper and Los Angeles strains of BHV1 may also be useful as vaccines as they did not cause abortion when administered to pregnant cattle. In utero inoculation of a modified-live BHV1 vaccinal strain into the fetus and the amniotic fluid via right flank laparotomy resulted in vaccine related abortion in one of nine cows, while 4,543 pregnant cows administered the same virus intramuscularly had no reported incidence of vaccine related abortion.

Since the modified-live products must replicate (cause infection) in order to stimulate immunity, caution should always be used in planning the herd vaccination program to avoid the exposure of susceptible or nonvaccinated animals. Viral shedding has been a concern as a source of infection to susceptible animals with modified-live vaccines. The use of intranasal modified-live vaccine offers a safe alternative in nonvaccinated pregnant or stressed cattle and is recommended for use in bulls which are to be used in artificial insemination programs with frozen semen. The duration of immunity has not been determined following use of intranasal immunization and it is more difficult to properly administer than intramuscular products. The use of an intramuscular modified-live vaccine at the next opportunity following intranasal immunization increases the likelihood of a durable immunity. The use of additional modified-live immunizations may be necessary under certain situations and should be carefully planned for each herd.

The use of killed IBR vaccines has increased because of safety concerns related to modified-live vaccines. Critical studies demonstrating the ability of killed BHV1 vaccines to protect the fetus are not available. Since repeated injections are necessary, it may be difficult to avoid periods of susceptibility due to low levels of immunity during some stages of production.
Bovine Virus Diarrhea Virus - BVDV

The BVDV is distributed worldwide and has a high rate of prevalence based on serology. (Radostits 1988) The main concern for the beef breeding herd is fetal infection with resulting abortion, congenital defects, or the development of persistently infected carriers that are a constant source of infective virus. (Baker, Radostits, Blood/Radostits 1989-BVD) Studies have reported a serious effect on conception if local BVDV infection occurs by experimental inoculation (Grahn, Whitmore) or following natural service with a persistently infected bull. (McClurkin) Following local infection, susceptible animals seroconverted due to systemic infection, resulting in immunity.

Confusion and controversy have surrounded the disease syndromes caused by BVDV since the first modified-live vaccine became available. (Radostits 1988) Fortunately, research during the past few years has unclouded much of the confusion related to the spread of BVDV and the cause of the severe or chronic "mucosal disease" form. (Brownlee, Bolin 1985, Moennig) Current information does not conclusively document the duration of protection following natural infection or the use of modified-live BVDV vaccine, although available information indicates that infection confers more than a single year of protection to the fetus. (Duffel, Kendrick, Kahrs 1981, Radostits, Moerman 1993)

The virus can cross the placenta in susceptible pregnant cattle and result in fetal infection either through exposure to the field virus or the improper use of intramuscular modified-live BVDV vaccines. (Trautwein 1986) If this occurs during the first six months of pregnancy, fetal losses or immune tolerance may result. Fetal infection during the last trimester of gestation usually results in the birth of an immune, seropositive, healthy calf. (Liess 1987) Seronegative cattle, vaccinated with modified-live BVDV in the last trimester of pregnancy, had calves that seroconverted as fetuses whereas over 90% of cattle that were seropositive had calves that did not, indicating that transplacental infection of previously exposed dams did not occur. (Orban 1983)

Critical studies comparing the ability of modified-live and killed BVDV vaccines to protect the fetus in field situations are not available. At the current time, it is believed that optimum protection of the beef breeding herd is dependent on active immunization with modified-live BVDV vaccine prior to breeding. (Duffel et al., Radostits 1988, Kahrs 1981, Hjerpe, Blood/Henderson) To insure a response, the vaccine should be administered to replacement heifers, two or more times between weaning (six to eight months of age) and breeding. (Kahrs 1981, Hjerpe, Blood/Radostits 1989-BVD) The final injection should be at least one month before breeding in order to avoid detrimental effects on conception. Although not documented, the use of different strains or serotypes of modified-live vaccine virus for each injection has been proposed so as to expand the range of cross protection. The genetic and antigenic instability of BVD virus may result in the emergence of isolates that have reduced antigenic cross-reactivity. (Corapi 1990, Kelling 1990) The importance of specificity of circulating antibody and effects on cellular immunity due to viral mutation are largely unanswered at this time.

A temperature-sensitive, modified-live BVDV vaccine was shown to be safe and induce seroconversion in pregnant cattle. (Lobmann M, et al.) A killed Singer-strain vaccine prevented clinical signs following intravenous challenge. (McClurkin/Coria 1980) Pregnant cows vaccinated with a polyvalent killed virus BVDV vaccine and challenged at 80 days gestation showed resistance to fetal infections compared to nonvaccinated controls. (Harkness 1987)
The long duration of immunity and the cross protection between serotypes following the use of modified-live vaccines make them preferable for use in beef breeding herds. The opportunities for a planned vaccination at noncritical stages of production and during times of minimal stress are available. This makes infection from field strain viruses during critical periods of fetal development less likely. If immunity has declined enough to permit natural infection it may stimulate an immediate immune response without severe disease consequences and this may be the basis for maintaining long term immunity. (Kahrs 1981) Depending on the circumstances of each herd, annual, biannual, or less frequent modified-live virus vaccine injections to cows between calving and breeding may be recommended.

**Insert:** Recent information from challenge studies indicates solid fetal protection to Type I BVDV and moderate protection from Type II BVDV is conferred through immunization with a pre-breeding Type I modified live virus vaccine. (Cortese et al. 1998; Brock/Cortese 2001).

**Campylobacteriosis - (Vibriosis)**

This venereal disease of beef cattle is characterized by temporary infertility and sometimes abortion. (Carroll; Ball et al.) It continues to interfere with optimum reproductive rates in a number of beef herds, in spite of the availability of effective vaccines, from a failure to develop and utilize adequate herd vaccination programs. (Grotelueschen DM, Hudson DB, personal communication, May 1993)

The immunity induced by parenteral injection is somewhat different from natural infection. Circulating antibody may not provide protection against venereally transmitted microorganisms that invade the reproductive tract directly. (Dekeyser) It is also possible to have local immunity without a rise in serum antibody. (Wilkie et al., 1972; Dekeyser) These factors may be responsible for partial immunity which in some cases of exposure results in delayed conception or early conception with low-grade infections that may result in later abortions. (Dekeyser)

Following infection of naive animals, the organism is usually eliminated from the animal within four to five months as local and systemic immunity develop. Active immunization confers adequate protection for a high reproductive rate but does not prevent local vaginal infection of the dam. (Hoerlein; Dekeyser) Effective immunization using oil adjuvanted vaccine requires a sensitizing dose, followed one month prior to breeding, by a second injection, and then annual boosters approximately one month prior to natural service for all breeding females. (Hoerlein/Carroll; Carroll/Hoerlein; Ball et al., VCNA)

Immunization of bulls has been shown to be of value in preventing the carrier state even though they may mechanically transmit the organism for a short time. (Clark 1975; Fivaz: Vasquez) The use of 2.5 times the recommended dose, twice the first year followed by annual boosters one month prior to breeding has been shown to be effective in eliminating carriers. (Vasquez, L et al.) Generally, oil adjuvanted products (Freund's incomplete adjuvant) are preferred because of more durable immunity following single annual boosters. (Hoerlein and Carroll, Ball) Products in aluminum hydroxide adjuvants generally induce less durable immunity and, to be effective, should be given ten days prior to a limited breeding season. (Berg) The oil adjuvanted product requires an annual booster, preferably one month prior to breeding. (Carroll) Modification of these recommendations for the

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1 Vibrin® - Smith Kline Beecham, Exton, PA
prevention and control of campylobacteriosis, such as immunization of only part of the cow herd, only bulls, or failure to utilize booster injections at the correct time, may result in decreased effectiveness.

**Leptospirosis**

Leptospira interrogans serovars hardjo and pomona have been reported to be the most frequent cause of abortion in cattle. (Blood/Radostits 1989-LEPTO p759) The most common isolate in the United States is serovar hardjo genotype hardjo-bovis. (Miller DA 1991) This genotype of hardjo is antigenically different from the hardjo-prajitno genotype identified in Europe and currently used in the multivalent vaccines. (LeFebvre 1987) Although the serovar in the multivalent vaccine produced circulating antibody following one or two doses it was not protective against experimental conjunctival challenge. (Bolin/Theirman et al. 1989) Further studies using an experimental vaccine derived from a hardjo-bovis isolate also failed to prevent infection and urine shedding when challenged in a similar manner. (Bolin/Zuermen 1989 and Bolin 1991)

In endemic areas frequent immunization with multivalent antigens containing the specific serovar is recommended. (Ellis 1986) In the majority of beef herds not in endemic areas, less frequent immunization of animals is usually practiced. Previous information indicated that annual vaccination in closed herds and every six months in endemic areas was protective. (Hansen 1977) Recent studies revealed fetal infection, stillbirths, weak calves, and apparently healthy calves shedding the organisms in urine following challenge of immunized pregnant cattle at four to six months of gestation. (Bolin 1989) Based on this information it may be beneficial to administer booster injections of vaccine again during midterm pregnancy in an attempt to reduce fetal losses in later gestation and the perinatal period.

Immunization of bulls with booster injections immediately prior to breeding season may be considered due to the reported incidence in bulls, possible venereal transmission, and the potential of reducing urine shedding following natural infection. (Miller DA 1991; Ellis WA; Bolin 1991)

It is difficult to fully justify immunization of the majority of beef cattle herds based solely on the reported incidence and currently available information on vaccine efficacy. It is possible that local immunity could permit improved reproductive rates even though infection is present and the dam sheds the organism in urine. (Hansen, 1977) Further study regarding the benefit of immunization may answer these questions.

**Trichomoniasis**

Reproductive losses due to infection by Tritrichomonas fetus result primarily in delayed fertility but are also associated with abortion, pyometra, and reduced calving rates in limited breeding seasons. (Kimsey 1986) The disease is generally insidious in onset due to a single or limited number of infected animals initiating the disease in a susceptible herd. The disease is widespread in the range areas of the western United States and has been diagnosed as a significant cause of infertility in some beef herds for more than 50 years. (Johnson, Kvasnika)

Resistance and immunity to natural tritrichomonas infection are similar to other pathogenic organisms causing local infection of the reproductive tract such as campylobacteriosis. (Skirrow-a) Infected animals gradually develop enough immunity to remain pregnant and eventually eliminate the infection in four to seven months. (Abbit,
Skirrow-b) This is important for control of the disease in herds with limited breeding seasons since infected pregnant females rarely remain infected until the next breeding year.(Kimsey et al. 1980) Bulls are the primary source of disease and with the possible exception of artificial insemination, are the only method of spread. Once exposed, older bulls are more likely to remain infected than young bulls.(Clark 1974) Clinicians should not be lulled into a complacent attitude towards testing young bulls because of this characteristic. Tritrichomonas fetus has been cultured from essentially any age bull.(Grotelueschen DM, personal communication, August 1993)

Controlling the disease by immunization has been studied and a commercial vaccine\(^2\) is currently available. Immunization of bulls appears to have limited application under most situations.(Clark 1983, 1984) Immunizing breeding females has resulted in more rapid elimination of infection and a reduction in early abortion when compared to controls.(Kvasnicka; Schnackel) Further studies are needed to provide additional information on efficacy and evaluation from an economic standpoint. Vaccination is currently recommended for controlling the disease in infected or high risk herds.(Kvasniska; Hjerpe; Schnackel)

Management is critical to control trichomoniasis, regardless if vaccine is used. Due to the relative ease, accuracy and cost of diagnostic surveillance of herd bulls and open females for trichomoniasis, it should be a routine practice in beef herds.(Abbitt, Ball, Berry 1985, Mickelsen) Prevention and control of the disease require management decisions based on epidemiologic characteristics of the disease and have been reviewed.(Ball)

**Haemophilus Somnus**

Haemophilus somnus can innocuously colonize the healthy genital mucosa of the cow.(Kwiecien 1992) It has also been associated with genital inflammatory disease (Hoblet 1989) and abortion (Firehammer 1959) in cows. H. somnus associated reproductive diseases have been reviewed.(Kwiecien 1991)(Miller 1983)

Corbeil, et al. were able to experimentally induce abortion using an intravenous challenge of large numbers of organisms.(Corbeil 1986) Commercial (Williams 1978) and experimental (Stephens 1984) H. somnus vaccines have been shown to attenuate the effects of intravenous challenge. While intrauterine infusion of H. somnus resulted in increased serum anti-H. somnus antibody titer and transient genital inflammatory lesions, it provided no protection against challenge five months later.(Kaneene 1987) Similarly, vaccination with an anionic antigen of H. somnus induced an increase in serum antibodies but did not increase antibodies at the vaginal mucosa or provide protection to challenge.(Patterson 1986)

There are no reports documenting reduction of H. somnus induced infertility or abortion in vaccinated cattle in the refereed literature. Despite anecdotal reports of efficacy, gaps in our understanding of the epidemiology of H. somnus induced reproductive disease and lack of demonstration of vaccine efficacy make it difficult to justify recommendation of vaccination.

**Additional Vaccines**

Several additional vaccines are available that may influence the outcome of a successful breeding herd health program. Nearly all diseases may indirectly affect reproduction by interfering with the normal physiologic processes observed with many diseases. Brucellosis,

\(^2\) Fort Dodge Laboratories Inc, Fort Dodge, IA
caused by Brucella abortus is currently of limited distribution in the United States due to eradication efforts. Vaccination of replacement heifers is recommended under most circumstances due to requirements for interstate shipment and sale of replacement heifers. Although the vaccine has been shown to be efficacious in the past there is less information regarding the reduced dosage now recommended. (Hjerpe) Federal guidelines will dictate future use of this vaccine.

Optimizing Immunization

As stated at the outset, injection of a vaccine only ensures that the animal has been exposed to the antigens contained in that vaccine, not that a protective immune response will ensue. The two key components required for a successful immunization are an efficacious vaccine and an immunocompetent animal. We will briefly discuss why one of these components may be missing, resulting in an apparent vaccine failure.

Achieving a protective immune response to every pathogen in every animal in a population is probably impossible for several reasons. Even if it were possible, it would likely be cost prohibitive. Based on their pathogenesis, some disease agents require each individual in a population to be immune for the vaccine to be efficacious. An example would be an infectious but noncommunicable disease like tetanus. For other pathogens, especially those that are highly contagious, reducing the number of susceptible animals below a critical threshold may be sufficient for the vaccine to be efficacious by preventing a disease outbreak. This is the concept of herd immunity.

Our goal in herd immunization is to raise the level of immunity in a sufficient number of animals to prevent epidemics and the catastrophic monetary losses associated with them. This means that individual animals may still become ill, especially if other factors are present that reduce their level of disease resistance. In a population of immune animals, disease transmission is reduced as disease resistance increases. This reduces, but does not eliminate, the chances of a disease with high morbidity or mortality. Paradoxically, individual animals can still become ill when the vaccine is successfully stimulating an effective level of herd immunity.

There are pathogens that can influence fetal and/or neonatal calf health for which no vaccines are available, such as Neospora-like protozoa and Ureaplasma. There are situations where antigenic differences between strains and species of pathogens or changes in the antigens the organism displays may compromise vaccine efficacy. A previously mentioned example of this is the genetic and antigenic instability of BVD virus. (Corapi 1990) This instability was thought to be the cause of the failure of repeated annual doses of inactivated virus vaccine to protect animals from infection. (Kelling 1990); however, for many infectious agents of cattle, the immunologically important antigens are relatively stable.

A more likely cause of vaccine ineffectiveness is improper handling, as was mentioned in the discussion of IBR. Vaccines must be stored and administered as recommended or their efficacy will be reduced. Special care must be taken with any live vaccine, either viral or bacterial, to prevent inactivation of the vaccine by exposure to extreme temperature, ultraviolet radiation, disinfectants, etc.

Sanitation is an important component of any vaccination plan and helps minimize injection site reactions and abscesses. Contamination of a multidose container can result in vaccine inactivation and injection site problems. Some disinfectants will destroy vaccines, so care must be taken to properly clean all equipment that comes in contact with the vaccine.

Once we have done everything to make certain that the vaccine and the equipment are
properly cared for, we should carefully administer the vaccine. Ensuring our personnel are knowledgeable about the proper locations for vaccine administration, changing needles at intervals or whenever they become barbed or bent, and having good handling facilities help minimize injection site reactions.

Timing of vaccine administration can also influence our perception of vaccine effectiveness. If an animal is incubating a disease, or is exposed to the disease-causing agent soon following vaccination, sickness may result and the vaccine will appear ineffective. It takes several days for an animal's immune system to respond to a vaccine and for the animal to be protected, especially if the calf is immunologically naive.

Experimentally, if we give enough of the disease causing organism we can cause disease even in animals that have immunity. When cattle are assembled in close quarters, the amount of disease agent that they are exposed to may be quite large, resulting in disease even in immune animals.

Individual animal responsiveness can affect vaccination success or failure. Not all animals are able to respond to vaccines for a variety of reasons including age, nutrition, genetics, stress, and previous vaccination/disease history. As previously mentioned, the immature immune system found in a calf is not able to respond to vaccines as well as the immune system in adult cattle.\(^{(Banks \ 1982)}\)\(^{(Roden \ 1992)}\) Even though the bovine fetus is capable of recognizing and responding to antigens before birth, the immune system does not reach its peak function until around puberty. Much later immunocompetence wanes with old age.

The previous nutritional status and parasite burden of a calf or cow can affect their overall physiology and their immune responsiveness. Parasites have been shown to produce immunosuppressive substances as they progress through their larval molts.\(^{(Gasbarre \ 1985)}\) Since the immune system is a part of the larger organism, the cow or calf, nutritional deficiencies in energy and protein are likely to compromise both overall physiology and immune function. Trace minerals and vitamins are thought to play an important role in maintaining an optimally functioning immune system, although this is incompletely understood and the practical implications are even more obscure. Genetics contribute to an animal's ability to respond to a vaccine, although markers in cattle that would indicate good or poor responders have not yet been found. Genetic predisposition to disease has been described in other species and speculated on in cattle.

Stress is an important factor in determining the ability of the animal to respond to vaccines and comes from a variety of sources including transport, nutritional changes, weaning, handling, etc. The relationships between stressors and disease resistance have been speculated on for centuries. In the nineteenth century Pasteur noted that placing a chicken's legs in cold water increased its susceptibility to anthrax. Similar relationships have been described in cattle. Weaning reduces antibody responses in calves.\(^{(Gwazdauskas \ 1978)}\)\(^{(Pollock \ 1992)}\) Lymphocyte function is suppressed in transported calves.\(^{(Filion \ 1984)}\)\(^{(Blecha \ 1984)}\) Efforts should be made to minimize as many different stressors as possible to increase the chances that an animal can respond to the vaccine.

The concept of additive stressors is especially relevant when discussing the immunologic sequelae of distress. Usually it is not a single stressor that debilitates the immune system. More often, the cumulative effects of a series of mild and moderate stressors experienced over a period of hours to days depress immune function below a threshold that prevents an effective immune response from occurring. Not only does each animal have a unique immunologic history but each animal varies in its response to these stressors resulting in the
spectrum of morbidity and response to vaccine that we frequently see in cattle.

Once we appreciate the importance of the additive stressor concept, along with some of the interactions of distress and immune function, it becomes apparent that a positive intervention point for health managers is identifying and minimizing preventable stressors. Many distresses that cattle encounter are the results of the marketing and management systems inherent in the cattle industry of the United States. Often we can have little impact on such stresses. However, an objective examination of our management strategies will reveal that many controllable stressors are tolerated in the interest of economics or convenience.

**Conclusion**

Specific vaccine recommendations should be made by you, the veterinarian familiar with the operation, the type of cattle handled, and the disease problems cattle typically experienced. There are few cookbook solutions. Fine tuning the program by including or excluding certain vaccines requires working to identify the specific disease entities that are present in an operation. This requires good records, complete postmortems, and a good diagnostic support system. Effective management to optimize the immunocompetence of the cow and the timing of administration of the vaccine is as important as selecting the correct antigens and type of vaccines to be used.

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PROCEDURES THAT SUPPORT REPRODUCTIVE MANAGEMENT OF REPLACEMENT BEEF HEIFERS

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Abstract
Selection and management of replacement beef heifers involve decisions that affect future productivity of an entire cowherd. The decision to breed heifers as yearlings involves careful consideration of the economics of production and the reproductive status, breed type, or genetic make-up of the heifers involved. Reproductive competence is established as a consequence of a specific program of developmental events leading to organization of functionally competent reproductive tissues and organs. The timing of puberty is critical in determining whether a heifer remains in the herd and the extent to which lifetime productivity is achieved. Because most components of fertility that influence calving and subsequent reproductive performance are not highly heritable, it is logical to assume that the majority of factors related to reproductive performance in cattle are influenced almost entirely by management. Utilization of various prebreeding management technologies enables producers to improve breeding performance of heifers during the first breeding season and during the subsequent calving and rebreeding period as 2-yr-olds. These practices help to ensure that heifers entering the herd as raised or purchased replacements will contribute to the general performance and productivity of an entire cowherd immediately, and cumulatively long-term. This review examines the relative merits of these various practices and provides an assessment of the adoption rate of specific reproductive management procedures for replacement beef heifers.

Key Words: Beef Cattle, Heifer, Reproductive Management

Introduction
Selection and management of replacement beef heifers involve decisions that affect future productivity of an entire cowherd. Programs to develop replacement heifers are focussed on the physiological processes that influence puberty. Age at puberty is most important as a production trait when heifers are bred to calve as 2-yr-olds and in systems that impose restricted breeding periods (Ferrell, 1982). The decision to breed heifers as yearlings involves careful consideration of the economics of production and the reproductive status, breed type, or genetic make-up of the heifers involved (Wiltbank, 1978; Morris, 1980; DeRouen and Franke, 1989; Kinder et al., 1990; Marshall et al., 1990, Short et al., 1990). Geographical-region differences in the age at which heifers are first exposed for breeding depend on management systems, forage quality and

* Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 13012.
Available at: http://www.asas.org/jas/symposia/proceedings/0902.pdf.
availability, and adaptation of respective breed types to specific environmental conditions (Short et al., 1990). In some cases, the economic advantage of early breeding and calving is now offset by biological limitations of the animal and management constraints of the environment (Short et al., 1990).

Reproductive performance is the single most important economic trait in a beef cow herd (Trenkle and Willham, 1977; Melton, 1995). Most reproductive loss occurs because cows fail to become pregnant or losses at or near birth are high (Wiltbank, 1990; Bellows and Short, 1990). Reproductive management requires a broad appreciation of technical material and knowledge to minimize reproductive loss, and make decisions that ultimately result in profit (Dziuk and Bellows, 1983). This review is focused on reproductive management practices for developing replacement beef heifers and the current state of the industry concerning utilization of various management procedures.

The Reproduction Cycle of the Cow

The reproductive phase of the beef production to consumption process is characterized by the breeding, conception, birth, and early nurturing of an animal (Melton, 1995). Increased weaning rate represents the greatest time-adjusted economic value to commercial cow-calf producers, simply because without a calf to sell no other characteristic has much meaning (Melton, 1995). Reproductive failure and (or) loss within a herd occurs primarily as a result of cows failing to become pregnant or the loss of calves at or near birth (Wiltbank, 1990; Bellows and Short, 1990). Puberty in the heifer and resumption of estrous cyclicity following calving in the postpartum cow are the critical reproductive events that determine if and when pregnancy will occur.

Puberty in the bovine female is determined by an array of identifiable genetic and environmental variables. Ultimate reproductive competence is established as a consequence of a specific program of developmental events leading to organization of functionally competent reproductive tissues and organs (Bartol et al., 1995). Studies that were designed to determine the sequence of events that occur at puberty gave way to research focused on basic factors that influence the onset of puberty and the interplay of reproduction, growth and metabolism. Reviews of the literature provide answers to questions concerning control of puberty in the heifer and factors influencing its onset. These perspectives include genetics (Martin et al., 1992), nutrition and season (Schillo et al., 1992), reproductive endocrinology (Day and Anderson, 1998), and management (Kinder et al., 1990; Patterson et al., 1992a; Larson, 1998).

Production of forage and the reproductive process in beef cattle are cyclical events (Figure 1; Bellows, 1987). The broad general categories that describe this cycle include: 1) developing the replacement heifer and 2) rebreeding the lactating dam. Growth and weight gains are integral to both reproductive events and attainment of profitable production (Bellows, 1987). Collectively, this suggests that life-cycle feeding approaches are needed, in which higher levels of supplemental feeding are used during key periods of growth and development.
Heifers bred to calve as 2-yr-olds should be exposed for breeding before mature herdmates and early calving periods can be used as a means of increasing production efficiency (Wiltbank, 1970). This practice often results in heifers being bred on their pubertal estrus. Fertility of heifers bred at the pubertal estrus was 21 percent lower than for those bred on their third estrus (Byerley et al., 1987; Perry et al., 1991). This means that heifers should reach puberty 1 to 3 mo before the average age at which they are to be bred (Short et al., 1990). Earlier age at puberty in relation to breeding ensures that a high percentage of heifers are estrous cycling and the effects of lowered potential fertility at the pubertal estrus are minimized (Short et al., 1990).

The timing of puberty is critical in determining whether a heifer remains in the herd and the extent to which lifetime productivity is achieved. Because most components of fertility that influence calving and subsequent reproductive performance are not highly heritable, it is logical to assume that the majority of factors related to reproductive performance in cattle are influenced almost entirely by management. Patterson et al. (1992a) provided a sequential review of the consequences associated with use of various management practices that may be imposed during each phase of the development process; beginning with the suckling phase of the heifer calf and progressing through the first postpartum period.

A number of factors influence the ability of a cow to calve in a given year and successively over a number of years. Management of replacement heifers during the postweaning to prebreeding period influences to a large extent when puberty, pregnancy, and parturition will occur. Heifers that calve early during their first calving season have higher lifetime calf production than those that calve late (Lesmeister et al., 1973). Because most calves are weaned at a particular time rather than on a weight-constant or age-constant basis, calves born late in the normal calving season are usually lighter than those born early, decreasing lifetime productivity of their dams (Lesmeister et al., 1973).

Reproductive Management Procedures for Replacement Beef Heifers

Long-term survival and prosperity of the U.S. beef cattle industry depends on its economic viability, which is best served by its competitiveness, profitability and economic efficiency (Melton, 1995). Managing an enterprise requires the fundamental ability to make decisions based on information that exists rather than something one imagines. A range of procedures are available to cow/calf producers to aid in reproductive management of replacement beef heifers and determine the outcome of a development program. These procedures, when collectively viewed as a “program”,
assist producers in more effectively managing reproduction in their herds. Producers that utilize these procedures are able to use data generated on their own farms and with their own heifers to plan, execute, and accomplish reproductive and genetic goals for their herds. These procedures facilitate improvements in breeding performance of replacement beef heifers during the first breeding season and during the subsequent calving and rebreeding period as 2-yr-olds. Adoption of specific procedures for an operation depends on factors including current level of performance, availability of facilities and labor, and economic return.

Table 1 provides a summary from USDA’s National Animal Health Monitoring System (NAHMS, 1994a) which reviews the percent of beef cattle operations in the U.S. using selected management procedures on replacement beef heifers. These procedures gained only marginal acceptance, despite their potential impact and resulting contribution to the reproductive integrity of an entire herd, both short and long-term. Collectively, these practices help to ensure that heifers entering a herd as raised or purchased replacements will contribute immediately, and cumulatively long-term, to the general performance and productivity of that herd. These procedures provide an objective assessment of the postweaning to prebreeding development phase and a useful means of selecting or culling potential replacements. A sequential review of these practices is required to establish the relative merit of each practice singly, and more importantly, the cumulative contribution of these practices to an improvement in total reproductive management of an entire cowherd.

<table>
<thead>
<tr>
<th>Management practice</th>
<th>Percent of operations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed separately</td>
<td>31.8</td>
</tr>
<tr>
<td>Pelvic measurements</td>
<td>3.0</td>
</tr>
<tr>
<td>Reproductive tract scores</td>
<td>1.2</td>
</tr>
<tr>
<td>Breed prior to the mature herd</td>
<td>12.7</td>
</tr>
<tr>
<td>Synchronize estrus</td>
<td>3.0</td>
</tr>
<tr>
<td>Artificial insemination</td>
<td>3.3</td>
</tr>
<tr>
<td>Body condition score</td>
<td>4.6</td>
</tr>
<tr>
<td>Weigh</td>
<td>7.9</td>
</tr>
<tr>
<td>Pregnancy diagnosis/palpation</td>
<td>15.9</td>
</tr>
</tbody>
</table>

*Adapted from NAHMS, 1994a.

**Target Weight.** The target weight principle calls for feeding heifers to a prebreeding target weight that represents 65 percent of the heifer’s projected mature weight. Puberty can be expected to occur at a genetically predetermined size among individual animals (Lamond, 1970; Taylor and Fitzhugh, 1971), and only when heifers reach genetically predetermined target weights can high pregnancy rates be obtained. Genotype of the heifer must be considered in the development program (Laster et al., 1976; Brinks et al., 1978; Toelle and Robison, 1985; Cundiff, 1986). Effects of postweaning nutritional development manifest themselves at different points within the reproductive cycle. Furthermore, vulnerability of specific breeds or breed crosses to these effects differs at specific points within this cycle (Patterson et al., 1991, 1992b). Heifers with the genetic potential to reach a heavier mature weight must attain a heavier
prebreeding weight before the first breeding season. Using the standard set by the Beef Improvement Federation (BIF, 1990) for nine frame-size classifications for U.S. breeding cattle, producers can estimate body composition and energy requirements per kg of gain at various weights during the feeding period (Fox et al., 1988). Optimum growth rates for replacement females of various body types are also available. These growth rates represent optimums for heifers that vary in mature size and were developed to maximize female lifetime productivity (Table 2; Fox et al., 1988).

<table>
<thead>
<tr>
<th>Table 2. Optimum growth rate for breeding herd replacement heifersa</th>
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<tbody>
<tr>
<td>Frame size</td>
</tr>
<tr>
<td>Optimum weight at first estrus, kg</td>
</tr>
<tr>
<td>Mature weight, kg</td>
</tr>
</tbody>
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aFrom Fox et al., 1988.

Although rate of gain is important for heifers to reach puberty at an early age, rapid growth during the prepubertal period can decrease subsequent milk production (Mangus and Brinks, 1971; Kress and Burfening, 1972; Holloway and Totusek, 1973; Beltran, 1978; Martin et al., 1981; Sejrsen et al., 1982; Harrison et al., 1983; Johnsson and Obst, 1984; Laflamme, 1993; Sejrsen, 1994; Sejrsen and Purup, 1997). Stair-step nutritional management regimens were used to limit growth during critical periods of mammary development and to subsequently allow periods of rapid growth to permit heifers to reach puberty at an early age (Park et al., 1989, 1998; Barash et al., 1994; Choi et al., 1997; Lynch et al., 1997). Grings et al. (1998, 1999) reported little direct effect of either trace mineral supplementation or altering rates of gain from weaning through the beginning of the breeding season on reproductive performance and subsequent milk yield for beef heifers gaining over .6 kg/d. These authors, therefore, suggested some flexibility in gain strategy and diet formulation with subsequent alterations in feed costs (Grings et al., 1999).

Patterson et al. (1992b) reported a significant negative relationship between age at puberty (AAP) and subsequent length of the postpartum interval (PPI) to estrus after parturition. The increase in PPI among heifers that reached puberty at younger ages was associated with weight of the heifer at weaning. Heifers that weighed more at the time they were weaned as calves reached puberty at younger ages and heavier weights. These same heifers, however, experienced longer PPI after calving, and weaned heavier calves at the end of their first year in production as 2-yr-olds. Heifers experienced longer PPI when both weight and condition at calving declined. Ferrell (1982) showed that large heifers were younger and heavier at puberty, produced more milk, and had lower body condition scores than did small heifers. Large cows that produce more milk are expected to have higher feed requirements than small cows that produce less milk. Lower condition scores suggest that large heifers are less able to meet their feed requirements during lactation than are small heifers (Ferrell, 1982; Buttram and Willham, 1987). These
data are supported by more recent studies from Brink and Kniffen (1996), and Frazier et al. (1999). Collectively, these data characterize a common problem in the industry associated with nutritional management of the 2-yr-old cow and demonstrate that early management regimens have a significant effect on subsequent reproduction.

Until a better rule of thumb is established, the target weight principle of developing heifers to an optimum prebreeding weight seems to be the most feasible method of ensuring that a relatively high percentage of yearling heifers reach puberty by the breeding season. However, the NAHMS (1994a) data indicate that few operations either weigh (7.9%), body condition score (4.6%), or feed heifers separately from the mature cowherd (31.8%), suggesting that in many cases heifers are not being fed adequately in order to meet their unique nutritional needs (Table 1).

Prebreeding Exams: Reproductive Tract Scores (RTS) and Pelvic Measurements.
Reproductive Tract Scores. A practice developed recently (Anderson et al., 1991) can be used to assist beef producers with selection of potential herd replacements and support timing of estrus synchronization programs. A reproductive tract scoring (RTS) system was developed to estimate pubertal status (Table 3). Scores are subjective estimates of sexual maturity, based on ovarian follicular development and palpable size of the uterus. A RTS of 1 is assigned to heifers with infantile tracts, as indicated by small, toneless uterine horns and small ovaries devoid of significant structures. Heifers scored with a RTS of 1 are likely the furthest from puberty at the time of examination. Heifers assigned a RTS of 2 are thought to be closer to puberty than those scoring 1, due primarily to larger uterine horns and ovaries. Those heifers assigned a RTS of 3 are thought to be on the verge of estrous cyclicity based on uterine tone and palpable follicles. Heifers assigned a score of 4 are considered to be estrous cycling as indicated by uterine tone and size, coiling of the uterine horns, as well as presence of a preovulatory size follicle. Heifers assigned a score of 4 do not have an easily distinguished corpus luteum. Heifers with RTS of 5 are similar to those scoring 4, except for the presence of a palpable corpus luteum (Table 3). Prebreeding examinations that include RTS furnish the opportunity to assess reproductive development, but further provide an appraisal of possible aberrant situations that may detract from a heifer’s subsequent reproductive potential.

Table 3. Reproductive tract scores\textsuperscript{a}

<table>
<thead>
<tr>
<th>RTS</th>
<th>Uterine horns</th>
<th>Ovarian length (mm)</th>
<th>Ovarian height (mm)</th>
<th>Ovarian width (mm)</th>
<th>Ovarian structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immature, &lt; 20 mm diameter, no tone</td>
<td>15</td>
<td>10</td>
<td>8</td>
<td>No palpable follicles</td>
</tr>
<tr>
<td>2</td>
<td>20-25 mm diameter, no tone</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>8 mm follicles</td>
</tr>
<tr>
<td>3</td>
<td>20-25 mm diameter, slight tone</td>
<td>22</td>
<td>15</td>
<td>10</td>
<td>8-10 mm follicles</td>
</tr>
<tr>
<td>4</td>
<td>30 mm diameter, good tone</td>
<td>30</td>
<td>16</td>
<td>12</td>
<td>10 mm follicles, CL possible</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 30 mm diameter</td>
<td>&gt; 32</td>
<td>20</td>
<td>15</td>
<td>CL present</td>
</tr>
</tbody>
</table>

\textsuperscript{a}From Anderson et al., 1991.
Figure 2 represents a modified interpretation of the conceptual model for puberty onset in the heifer presented by Day and Anderson (1998). This model combines the associated endocrine and ovarian changes that occur as heifers approach puberty, in addition to the corresponding RTS that would be assigned at respective points in development. A RTS of 1 corresponds to the point in time at which the pattern of LH release is characterized by low-frequency pulses. This is due to the fact that the hypothalamic-pituitary axis is highly responsive to estrogen negative feedback. Reproductive tract scores of 2 and 3 are associated with the peripubertal phase, at which responsiveness to estradiol negative feedback decreases, causing increases in LH pulse frequency, follicle growth, and estradiol secretion. The decline in estradiol negative feedback and increase in LH secretion result in significant increases in follicular growth, and elevated concentrations of estradiol sufficient to induce estrus and the preovulatory LH surge. Reproductive tract scores of 4 and 5 are assigned to heifers that have reached puberty, but differ in stage of the estrous cycle at the time of the prebreeding exam (follicular phase = 4; luteal phase = 5).

Growth-promoting implants are used extensively in the nursing, growing, and finishing phases of the beef cattle production cycle (Hargrove, 1990; Simpson and Moore, 1990; Deutscher, 1991). Growth promoting or anabolic agents are compounds containing estrogen and (or) progesterone, nonsteroidal compounds that have estrogenic activity (zeranol), or potent synthetic androgens (trenbolone acetate). Bartol et al. (1995) designed a study to determine: 1) if exposure of neonatal heifer calves to progesterone or estradiol, delivered from a commercial growth-promoting implant (Synovex-C®) would affect adult uterine structure or function evidenced by changes in gross morphology, histoarchitecture, or uterine luminal protein content; and 2) whether such effects would be related to the neonatal age at which steroid exposure first occurred. The results from Bartol’s study are shown in Table 4. Results from this study (Bartol et al., 1995) clearly indicate that chronic exposure of heifer calves to progesterone or estradiol, beginning on or before postnatal d 45, reduced uterocervical wet weights and altered uterine wall histology. It is especially important to note that these effects were observed in heifers 15
mo after the first steroid exposure. Regardless of the neonatal age at which treatment began, chronic administration of progesterone and estrogen was ultimately reflected in the adult uterine wall by significant reductions in cross-sectional areas for both myometrium and endometrium and by reduced uterine gland density. In some cases, developmental loss of adult endometrial parenchyma was reflected by reductions in both endometrial area and glandularity, in some cases approaching 75 percent. Although this study was not designed to evaluate implant effects on bovine fertility, the changes that occurred cannot be considered desirable effects, because both maternal uterine tissues and uterine secretions are recognized to play critical roles in support of conceptus development (Bartol et al., 1995).

Table 4. Effects of neonatal exposure to progesterone and estradiol on reproductive tract development of adult beef heifers

<table>
<thead>
<tr>
<th>Response</th>
<th>Neonatal age at treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Birth</td>
</tr>
<tr>
<td>Uterocervical weight (g)</td>
<td></td>
</tr>
<tr>
<td>113.7 e</td>
<td>123.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myometrial area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td>123.7&lt;sup&gt;h&lt;/sup&gt;</td>
<td>141.8&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Endometrial area (mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td>29.9&lt;sup&gt;j&lt;/sup&gt;</td>
<td>32.4&lt;sup&gt;j&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gland density (hits/mm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td>172.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>380.3&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uterine luminal protein content (mg/flush)</td>
<td></td>
</tr>
<tr>
<td>2.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adapted from Bartol et al., (1995).
<sup>b</sup>Data were collected from cyclic adult heifers on d 12 of an induced estrous cycle.
<sup>c</sup>Treated heifers received a single Synovex-C<sup>®</sup> implant containing progesterone (100 mg) and estradiol benzoate (10 mg). Implants were placed (sc) on the designated day of neonatal life. Control heifers were untreated.
<sup>d,e,f,g,h,i,j</sup>Means within a row with different superscripts differ (P < .05).

The significance of these findings as they relate to RTS pertain to situations involving heifers in which the management history of the heifer is unknown at the time the prebreeding exam is performed. The changes that occur in uterine morphology as a result of implant administration are in many cases palpable per rectum at the time the RTS is performed. These observations are made in heifers that are examined up to 15 mo after the first steroid exposure, as noted by the 75 percent reduction in endometrial area and glandularity (Bartol et al., 1995). The reproductive tract scoring system can be used to select heifers that are “reproductively ready” for the breeding season and thus minimize carrying costs of heifers that will very likely fail to cycle and conceive. Reproductive tract scores, when timed appropriately, serve as a useful indicator in determining whether heifers are ready to be placed on an estrus synchronization treatment and are useful too, in determining the most appropriate method of estrus synchronization to use. However, just over 1 percent of producers use this relatively new management tool (Table 1).

Pelvic Measurements. Pelvic measurements should be used in addition to, not in place of, selection for size, weight, and above all fertility (Bellows and Staigmiller, 1990). Producers should be aware that selection for pelvic area will not likely result in increased pelvic dimensions alone, but will result in increased size of the entire skeleton and animal (Morrison et al., 1986). Increased skeletal size of the dam will be reflected in higher
birth weights and dimensions of the calf. Pelvic measurements, on the other hand, can be used successfully to identify abnormally small or abnormally shaped pelvises. These situations, left unidentified, often are associated with extreme dystocia, resulting in Cesarean delivery and even death of the calf or dam (Patterson et al., 1992a).

Recent estimates indicate that nearly 20 percent of beef heifers require some degree of calving assistance (NAHMS, 1994b). The NAHMS (1994b) survey indicates that over half of producers (57.2 percent) only check their heifers one to two times per 24-hr period during the calving season. Furthermore, recent statistics indicate that calf losses due to dystocia may run as high as 20 percent. Selection of sires with low BW-EPD mated to heifers that are screened for pelvic area could contribute to a decrease in the incidence and (or) severity of calving problems and minimize calf losses from dystocia.

Bullock and Patterson (1995) reported that puberty exerts a positive influence on pelvic width and resulting pelvic area in yearling heifers, however, differences that were seen among heifers as yearlings did not carry through to calving as 2-yr-olds. Therefore selection (culling) decisions based on pelvic measurements and contemporary grouping for genetic analysis of pelvic measurements should include consideration of pubertal status at the time of the examination. The data suggest that puberty plays a role in pelvic size as yearlings, but once heifers reach puberty the effects may no longer be present. An independent culling level for pelvic size on heifers that are at different stages in their reproductive development appears to be more restrictive for those heifers that are peripubertal at the time of the exam. Despite the fact that pelvic measurements can be a useful management tool to eliminate heifers with a higher potential for calving difficulty, only 3 percent of producers reported using this technique in their herds (Table 1).

**Estrus Synchronization and Artificial Insemination.** The percentage of beef cattle inseminated artificially is predicted to increase substantially with the advent of sexed semen (Seidel, 1998). Currently, however, only 3.3 percent of the beef cattle operations in the U.S. practice AI on their heifers and only 3 percent of total operations use estrus synchronization to facilitate their AI programs (Table 1).

Although hormonal treatment of heifers and cows to group estrous periods has been a commercial reality now for years, producers have been slow to adopt this management practice. Perhaps this is because of past failures, which resulted when females that were placed on estrus synchronization treatments failed to reach puberty or to resume normal estrous cycles following calving. Estrus synchronization and artificial insemination remain however, the most important and widely applicable reproductive biotechnologies available (Seidel, 1995).

Estrus synchronization and artificial insemination contribute to a total heifer development program in several ways. Estrus synchronization improves time management for producers that use AI by concentrating the breeding and resulting calving periods. Managers are able to spend more time observing heifers as they calve because calving occurs over a shorter time period. Calf losses in many cases are reduced because of improved management during the calving period. Artificial insemination provides the opportunity to breed heifers to bulls selected for low BW-EPD with high accuracy. This practice minimizes the incidence and severity of calving difficulty and decreases calf loss that results from dystocia. In addition, heifers that conceive during a
synchronized period typically wean calves that are older and heavier at weaning time (Schafer et al., 1990). Finally, heifer calves that result from AI can be an excellent source of future replacements facilitating more rapid improvement in the genetic makeup of an entire herd.

Methods of synchronizing estrus were reviewed by Patterson et al. (1989), Odde (1990), Larson and Ball (1992), Beal (1998), and Patterson (1999). The development of methods to control the estrous cycle of the cow has occurred in five distinct phases (Patterson, 1999). Phase I included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in Phase II; whereas Phase III involved prostaglandin F$_{2\alpha}$ (PG) and its analogs as luteolytic agents. Treatments that combined progestational agents with PG characterized Phase IV.

Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the changes that occur during a follicular wave. Growth of follicles in cattle occurs in distinct wave-like patterns, with new follicular waves occurring approximately every 10 d (range 6-15 d). We now know (Phase V) that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan. A single injection of gonadotropin-releasing hormone (GnRH) to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronized ovulation or luteinization of most large dominant follicles. Consequently, a new follicular wave is initiated in most cows within 2 to 3 d of GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 d later (Twagiramungu et al., 1995). The GnRH-PG protocol increased estrus synchronization rate in beef (Twagiramungu et al., 1992a,b) and dairy cattle (Thatcher et al., 1993). However, a drawback of this method is that approximately 5 to 15 percent of the cows are detected in estrus on or before the day of PG injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period. Furthermore, this system has not been effective when administered to heifers.

In recent years, the 14-17 d melengestrol acetate (MGA)-PG treatment has become a widely used method of synchronizing estrus in replacement beef heifers (Brown et al., 1988). Melengestrol acetate is an orally active progestational steroid (Zimbelman and Smith, 1966) capable of inhibiting estrus and ovulation in heifers when consumed (0.5 mg) on a daily basis. Melengestrol acetate is fed for 14 d in a supplement carrier. Heifers exhibit estrus beginning 48 h after MGA withdrawal from the feed, but should not be inseminated or exposed for natural service at this time. Prostaglandin F$_{2\alpha}$ should be administered 17 d after MGA withdrawal with insemination based on detected estrus. This treatment avoids problems with reduced conception and offers advantages compared with untreated controls (Brown et al., 1988; Patterson and Corah, 1992). The advantages of MGA for synchronization of estrus are ease of administration and cost. Furthermore, MGA recently received clearance (Federal Register, 1997) for use in reproductive classes of beef and dairy females. Although, this treatment works effectively to synchronize estrus, growth in the use of estrus synchronization and artificial insemination depends upon the development of methods that shorten the time period required to detect heat and (or) facilitate use of fixed-time insemination.
Wood et al. (1999) evaluated a modified MGA-PG protocol for inducing and synchronizing a fertile estrus in yearling beef heifers. The first modification changed the day of PG injection from d 31 to d 33 of treatment. The second modification was the addition of a GnRH injection on d 26 of treatment (MGA feeding d 1 to 14, GnRH on d 26, and PG on d 33; MGA-GnRH-PG). Injection of GnRH on d 26 of this protocol successfully induced luteal tissue formation and initiated a new follicular wave on approximately d 28. This modification resulted in a significant increase in the proportion of animals with synchronized follicular waves on d 33 and an associated improvement in the degree of synchrony after PG. This sequential approach to estrous cycle control (progestin-GnRH-PG) appears to offer significant potential to more effectively synchronize estrus with resulting high fertility (Wood et al., 1999; Kojima et al., 2000; Patterson et al., 1999).

Potential for Induced Estrous Cyclicity with Progestins. Progestins were used to induce estrus in peripubertal heifers (Gonzalez-Padilla et al., 1975) and are often combined with estrogen to mimic changes that occur in concentrations of blood hormones around the time of puberty. Increased progesterone is thought to be a prerequisite for the development of normal estrous cycles. Progesterone increases during the initiation of puberty in the heifer (Berardinelli et al., 1979), and before resumption of normal ovarian cyclicity in postpartum suckled beef cows (Prybil and Butler, 1978; Rawlings et al., 1980). Progestins stimulate an increase in follicular growth that results subsequently in increased production of estrogen by ovarian follicles (Henricks et al., 1973; Wetteman and Hafs, 1973; Sheffel et al., 1982; Garcia-Winder et al., 1986). Melengestrol acetate initiates estrous cyclicity in peripubertal beef heifers (Patterson et al., 1990) and is associated with increased LH pulse frequency during the treatment period (Smith and Day, 1990; Imwalle et al., 1998). Recent studies suggest that the stimulatory effects of progestins on LH secretion are greatest after removal of the steroid (Hall et al., 1997; Imwalle et al., 1998). Furthermore, improvements in observed pubertal induction response following treatment with a progestin occur with an increase in age (Hall et al., 1997). The increase in pulsatile release of LH that occurs in response to progestin treatment in peripubertal heifers results in a decrease in estrogen receptors within neuronal systems that mediate negative feedback actions of estradiol on GnRH secretion (Anderson et al., 1996).

Burfening (1979) suggested that because puberty is a heritable trait, induced puberty in replacement heifers over several generations might result in situations in which attainment of puberty would be difficult without hormone treatment. This consideration cannot be overlooked. However, there is a need to explore treatments to induce puberty in breeds of cattle that are late-maturing but of sufficient age and weight at the time of treatment to permit successful application (Patterson et al., 1990). The decision to utilize this practice within a herd perhaps differs with various types of beef operations. For instance, the common goal of most managers of commercial cow-calf herds is to maximize weaning rate. In other words, the investment in time and resources in a heifer from weaning to breeding requires that management efforts be made to facilitate puberty onset and maximize the likelihood of early pregnancy. In this scenario, a method to induce puberty in heifers could serve as a valuable tool to improve reproductive performance of heifers retained for breeding purposes. On the other hand,
seedstock managers should weigh the economic importance of puberty onset in their herds, as well as their customers’, and the associated potential and resulting implication of masking its true genetic expression.

Early Pregnancy Diagnosis. Determining pregnancy rates and accurately evaluating their distribution by period within a breeding season requires that pregnancy diagnosis be performed at a fixed time. To accurately determine conception date and resulting calving date, this time point should represent a maximum number of days from when breeding began. This information can then be used to determine the success of an estrus synchronization and AI program, project subsequent calving dates and cull late-bred or non-pregnant replacements.

Diagnostic ultrasonography provides a non-invasive form of visual access to the cervix, uterus and ovaries for evaluating normal, morphologic changes in cattle (Pierson and Ginther, 1988; Kastelic et al., 1988; Griffin and Ginther, 1992). The potential advantages of using ultrasonography for pregnancy diagnosis are that the presence of an embryo can be detected earlier than by palpation per rectum. Use of ultrasonography rather than manual palpation of the reproductive tract may improve consistency of early (< d 45) pregnancy diagnosis by reducing variation in accuracy among technicians (Beal et al., 1992). In addition, fetal sexing using ultrasonography may be an effective management and marketing tool (Muller and Wittkowski, 1986). Knowing the sex of the developing fetus can provide valuable information to the breeder and (or) purchaser of bred replacement heifers. Pregnancy diagnosis is one of the more widely used reproductive procedures, however, only 15.9 percent of the beef cattle operations in the U.S. routinely determine pregnancy status of their heifers (NAHMS, 1994a).

Interpreting Data Obtained from Various Reproductive Procedures to Make Management Decisions

Collectively, prebreeding weight, reproductive tract score, pelvic height, pelvic width, and total pelvic area can be used to evaluate success of a development program. Timing these procedures is critical in determining whether heifers are ready to be placed on an estrus synchronization treatment, the type of treatment to be used, and the anticipated outcome of a particular treatment regarding estrus response and subsequent pregnancy. Table 5 summarizes prebreeding data that were collected on 2,664 heifers (Patterson and Bullock, 1995). Measurements were obtained within 2 wk prior to administration of a 14-17 d MGA-PG treatment. Reproductive tract score was correlated with prebreeding weight \((r=.39)\), pelvic height \((r=.30)\) pelvic width \((r=.34)\) and total pelvic area \((r=.39)\). Poor reproductive performance of heifers with RTS of 1 points to the importance of identifying and culling these heifers before the breeding season begins (Table5).
Table 5. Prebreeding weights, measurements, and subsequent estrus response after synchronization of estrus with MGA-PG

<table>
<thead>
<tr>
<th>RTS</th>
<th>N</th>
<th>Weight (kg)</th>
<th>Pelvic height (cm)</th>
<th>Pelvic width (cm)</th>
<th>Pelvic area (cm²)</th>
<th>Estrus response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>270b</td>
<td>13.9b</td>
<td>10.9b</td>
<td>152b</td>
<td>54b</td>
</tr>
<tr>
<td>2</td>
<td>278</td>
<td>282c</td>
<td>14.1b</td>
<td>11.2b</td>
<td>158b</td>
<td>66c</td>
</tr>
<tr>
<td>3</td>
<td>1103</td>
<td>317d</td>
<td>14.5c</td>
<td>11.4c</td>
<td>166c</td>
<td>76d</td>
</tr>
<tr>
<td>4</td>
<td>494</td>
<td>333c</td>
<td>14.7c</td>
<td>11.7c</td>
<td>172c</td>
<td>83c</td>
</tr>
<tr>
<td>5</td>
<td>728</td>
<td>343c</td>
<td>14.7c</td>
<td>11.7d</td>
<td>172d</td>
<td>86c</td>
</tr>
</tbody>
</table>

Adapted from Patterson and Bullock, 1995. Weights and measurements were taken within 2 wk prior to the first day of MGA. Estrus response is the percentage of heifers that exhibited estrus and were inseminated within 144 h after PG.

In situations where heifers are scheduled to begin an estrus synchronization treatment with MGA, we recommend that RTS be performed within 2 wk prior to the initiation of treatment. We further recommend that heifers are ready to begin treatment with MGA if 50 percent of the heifers within a group are assigned RTS of 4 or 5. This indicates that these heifers have reached puberty and are estrous cycling. Based on the age and weight of prepubertal or peripubertal contemporaries, up to 70 percent of these heifers can be expected to exhibit estrus and ovulate after MGA withdrawal, so the potential estrus response during the synchronized period is up to 80 percent (Table 5). Estrus response among heifers that were assigned scores of 2 or 3 was lower than for those assigned scores of 4 or 5. However, as RTS increased, estrus response improved.

Inadequacies in nutritional development programs often are associated with situations in which the desired degree of estrous cyclicity has not been achieved. This necessitates reevaluation of the nutritional development program and in many cases a postponement of the breeding season. The results obtained from a prebreeding exam provide an objective assessment of the success or failure of a development program and are useful in determining the appropriate timing of estrus synchronization treatments (Anderson et al., 1991; Patterson and Bullock, 1995; Randle, 1999).

Reasons for Failure to Utilize Reproductive Procedures

Producers are often restricted in their operations from implementing production-enhancing technologies. Figure 3 provides a summary of the most common reasons for not using specific procedures (NAHMS, 1998). The reason cited most for not utilizing these practices is “lack of time and labor”. Some “other” reason was the next most common explanation followed by “too complicated” or “costly”. In some cases, respondents believed that benefits of incorporating these improved technologies into their management schemes outweigh the costs. Not only can these practices ameliorate profitability by improving production, some can also decrease costs (NAHMS, 1998).

Modern-day production agriculture is an increasingly competitive arena. In many cases technology can help increase production while maintaining or decreasing costs. However, low adoption rates of these and other management practices leads one to question the future competitive position of the U.S. beef cattle industry, when compared
with change in technology adoption that is occurring in other parts of the world. For instance, the United States and Brazil are world leaders in total numbers of beef cows in production. Table 6 summarizes the change in use of AI that occurred over a 5-yr period in these two countries. Growth in the use of artificial insemination in Brazil outpaced that of the U.S. by 93 percent (ASBIA, 1998; NAAB, 1998). Beef producers in Brazil are inseminating 3.5 times more cows annually compared with producers in the U.S., based on the sale of import and domestic beef semen. Furthermore, nearly one half of the semen used in Brazil is imported, a large portion of which comes from the U.S. Given this scenario, it is likely to assume that in the years ahead, elite seedstock herds in the U.S. will provide a sizeable percentage of the germ plasm used worldwide. However, unless owners of commercial cowherds in the U.S. begin to aggressively approach reproductive and genetic improvement within their herds, one could argue that this country would lose its competitive advantage in the production of high quality beef. International players that are more technically astute and competitively advantaged will position themselves to dominate the production and sale of beef worldwide.

Figure 3. Reasons for not using reproductive procedures (adapted from NAHMS, 1998).

Table 6. Import and domestic beef semen sales in Brazil and the U.S. over a 5-year period

<table>
<thead>
<tr>
<th>Country</th>
<th>1993</th>
<th>1998</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,874,996</td>
<td>3,256,259</td>
<td>+74</td>
</tr>
<tr>
<td>United States&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,117,798</td>
<td>906,923</td>
<td>-19</td>
</tr>
</tbody>
</table>

Export sales in the U.S. rose from 393,365 units in 1993 to 848,677 units in 1998 (+ 46 percent, NAAB, 1998).

<sup>a</sup>ASBIA, 1998.

<sup>b</sup>NAAB, 1998.
Replacement Heifer Programs that Utilize Reproductive Procedures in Development and Marketing

The advent of coordinated on-farm heifer development and marketing programs (e.g., the Bourbon County Kentucky Elite Heifer Program and the Missouri Show-Me-Select Replacement Heifer Program and Sales), and commercial heifer development facilities that focus on the procedures presented here, remove much of the risk of developing replacement beef heifers compared with situations in which replacements are raised or purchased without these criteria being taken into consideration (Patterson, 1998; Randle, 1999).

Marketing heifers that are developed according to established guidelines has been shown to be a viable means of rural economic development in specific regions of the U.S. (Patterson and Bullock, 1995). Programs in Kentucky and Missouri were designed to: 1) improve existing efforts through a total quality management approach to heifer development; 2) increase marketing opportunities for and add value to the heifer portion of the calf crop; and 3) provide reliable sources of quality replacement females concerning genetics and management.

These programs require compliance with specific guidelines, and provisions for various management and reproductive practices and (or) procedures. These guidelines include provisions for ownership; health and vaccination schedules; parasite control; implant use; weight, pelvic measurement and reproductive tract score; estrus synchronization and artificial insemination; service-sire requirements for BW-EPD; early pregnancy diagnosis, and body condition score (Patterson, 1998).

Statistics that Warrant Change. Table 7 provides a summary of the distribution of the over 900,000 beef operations in the U.S. with regard to herd size (NAHMS, 1998). These statistics indicate that 91.7 percent of beef operations in the U.S. are involved with herds of < 100 cows. However, the cumulative number of cows on these operations accounts for 50.3 percent of the total number of cows in production nationwide.

Table 7. Number of beef cow operations and herd size (NAHMS, 1997)a

<table>
<thead>
<tr>
<th>Number of head</th>
<th>1-49</th>
<th>50-99</th>
<th>100-499</th>
<th>≥ 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of U.S. beef operations by herd size</td>
<td>79.8</td>
<td>11.9</td>
<td>7.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Percent of U.S. beef cow inventory by herd size</td>
<td>30.8</td>
<td>19.5</td>
<td>35.7</td>
<td>14</td>
</tr>
</tbody>
</table>

aPercentages represent beef operations in the U.S. for 1996.

Larger size herds make use of more of the technologies currently available (NAHMS, 1997a). There is also indication of regional differences in use of reproductive technologies in cow-calf herds. In general, operations in the Southeast and Southcentral regions are less likely to use any of the reproductive procedures listed. Only 35.4 and 58.3 percent of operations in the Southeast and Southcentral regions, respectively, used any of the reproductive procedures currently available (i.e., estrus synchronization, artificial insemination, pregnancy diagnosis, pelvic measurement, body condition scoring,
sperm evaluation). This compares with 77.7 percent of operations in the West, 77.3 percent in the Northcentral, and 67.1 percent in the Central regions.

According to the NAHMS (1997b) survey, only 46.4 percent of beef operations in the U.S. maintain restricted breeding and calving seasons. Furthermore, up to 40 percent of heifers nationwide that become pregnant as yearlings fail to conceive in their second breeding season, or lose calves by the end of their second calving period (Bellows and Short, 1990; Wiltbank, 1990). The demographics of U.S. beef production that include large numbers of operations with small numbers of cows in production, low adoption rate of technology, and failure to adopt technology because of limited time and labor, point to an industry destined to concede its competitive position worldwide.

Sources of Information and Implementing Change

Veterinarians serve as a key information source for U.S. beef producers and will be essential in facilitating the adoption of various reproductive procedures (NAHMS, 1997c). Nearly two-thirds (60.8%) of cow-calf producers cited their veterinarian as a “very important” source of information for their cow-calf operation including health, nutrition, or questions pertaining to production or management. Differences in importance of various information sources based on size of the cowherd are illustrated in Figure 4.

![Figure 4. Sources of information (adapted from NAHMS, 1997c).](image)

On-farm development programs that involve local veterinarians, state, regional, or county livestock specialists, and individual farm operators provide the structure from which change can occur. Organized on-farm programs such as Kentucky’s Bourbon County Elite Heifer Program and Missouri’s Show-Me-Select Replacement Heifer Program are examples that draw on the fundamental basis upon which extension and the
Land Grant System were founded: the use and application of what we know to create knowledge (Patterson, 1998). In these programs evaluation has an impact in itself, because meaningful assessment of these programs builds in evaluation as part of the design. Data collection is part of the delivery process and reinforces the development of sound management practices on individual farms regardless of their size (Randle, 1999). Farmers use data generated on their own farms. The focus of these programs centers on action alternatives based on data generated. Methods flow from issues with a negotiated participatory process that involves veterinarians, livestock specialists, and farmers.

Implications

During the years 1993-1997 roughly 6 million beef replacement heifers entered the U. S. cowherd annually, and of these approximately 12 percent (720,000) were purchased as bred replacements on an annual basis (NAHMS, 1998). It is safe to assume that a very small percentage of these heifers were “programmed” per se in terms of reproductive procedures currently available. The expertise to develop and market programmed heifers exists, but requires a team approach to managing heifers in terms of nutrition, reproduction, genetics, health and emerging management practices. Effecting change in reproductive management of the U.S. cowherd will require a fundamental change in the approach to management procedures and development practices being used on heifers retained for breeding purposes. We have reached a point concerning reproductive management of our nation’s beef cowherd at which the tasks of transfer and development of technology must be equally emphasized and must progress together for the U.S. to maintain a strong beef cattle sector in our economy. Unless efforts are taken to implement change in the U.S. beef cattle industry, the products of our research and technology may be exported to more competitive international markets.

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PRACTICAL APPLICATIONS OF ULTRASOUND FOR REPRODUCTIVE MANAGEMENT OF BEEF AND DAIRY CATTLE

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Introduction

O. J. Ginther stated: “gray-scale diagnostic ultrasonography is the most profound technological advance in the field of large animal research and clinical reproduction since the introduction of transrectal palpation and radioimmunoassay of circulating hormones.” (Ginther, 1986). It is hard to imagine that many discoveries and procedures related to ovarian, uterine and fetal function that we use today would have been considered without the development of real-time ultrasound. The research and commercial applications of ultrasound developed for reproduction over the last 15 years would support the statement by O. J. Ginther.

The area that has arguably benefited more from the development of ultrasound technology than any other area is reproduction in large animals. In many cases, rectal palpation has been replaced by transrectal ultrasonography for pregnancy determination, and diagnoses associated with uterine and ovarian infections. In addition, ultrasonography has added benefits such as fetal sexing, early embryonic detection and is less invasive than rectal palpation. From a research standpoint, ultrasound has given us the ability to visually characterize the uterus, fetus, ovary, corpus luteum, and follicles. More accurate measurements of the reproductive organs has opened doors to new areas of research and validated or refuted data from past reports.

Practical applications of ultrasound by bovine practitioners for routine reproductive examinations of beef and dairy cattle is the next contribution this technology is positioned to make to the livestock industry. Most veterinary students continue to be taught that ultrasound is a secondary technology for bovine reproductive work; however, the information-gathering capabilities of ultrasonic imaging far exceed those of rectal palpation (Ginther, 1995). This paper will discuss the impact and practical applications of ultrasound for conducting routine reproductive examinations in dairy and beef cattle.

Veterinary Ultrasound Equipment

In general, linear-array, real-time, B-mode ultrasound scanners are best suited for veterinary applications involving cattle reproduction. Most ultrasound machines consist of a console unit that contains the electronics, controls, and a screen upon which the ultrasound image is visualized by the operator, and a transducer, which emits and receives high-frequency ultrasound waves. Linear-array transducers consist of a series of piezo electric crystals arranged in a row. These crystals emit high frequency sound waves upon being energized. The configuration of a linear-array transducer results in a rectangular image on the field of scan (as opposed to a pie shaped image produced by a sector transducer).
Bovine reproductive organs are most commonly scanned per rectum using a linear-array transducer specifically manufactured for transrectal use. However, specialized applications including ovum pickup and follicle ablation involve a transvaginal approach using a sector transducer. Linear-array transducers of 5.0 and 7.5 MHz frequency ranges are most commonly used in cattle, and most veterinary ultrasound scanners are compatible with probes of different frequencies. Depth of tissue penetration of sound waves and image resolution is dependent upon and inversely related to the frequency of the transducer. Thus, a 5.0 MHz transducer results in greater tissue penetration and lesser image detail, whereas a 7.5 MHz transducer results in lesser tissue penetration and greater image detail. An ultrasound scanner equipped with a 5.0 MHz transducer is most useful for bovine practitioners conducting routine reproductive examinations, however, small ovarian structures such as developing follicles are best imaged with a 7.5 MHz transducer.

It is clear that ultrasound has made a tremendous impact as a scientific tool; however, ultrasound holds much promise as a tool to improve reproductive management in beef and dairy operations. There are several reasons that transrectal ultrasound is not widely used among bovine practitioners at present. First, research-grade ultrasound machines are relatively expensive, costing from $10,000 to $20,000. Second, most ultrasound machines require a cart and an external power source, thereby making them cumbersome to use under field conditions. Recently, several ultrasound manufacturers have developed and marketed ultrasound machines that are cheaper, smaller, and battery operated. At present, these portable ultrasound machines lack the image quality of the larger console based units but may be easier to use on a routine basis. Continuation of the trend toward portability will foster future use of this technology by bovine practitioners for routine reproductive management.

Imaging the Bovine Ovary

Ovarian Structures as Diagnostic Aids

The use of ultrasound technology to evaluate ovarian activity has been reviewed in great detail (Pierson and Ginther, 1988; Beal et al., 1992). Ovarian stroma, ovarian vessels, follicles, cysts, corpora haemorrhagica (CH), and corpora lutea (CL) are all structures that have been previously identified by real-time ultrasonography (Pierson and Ginther, 1988; Kastelic et al., 1990a,b; Beal et al., 1992; Singh et al., 1997). The most distinguishable ovarian structures are antral follicles. Because follicles are fluid-filled structures they absorb ultrasound waves and are displayed as black on the screen (i.e., anechoic or non-echogenic). In contrast, the ovarian stroma, CH, and CL all contain varying degrees of dense cells, which reflect the ultrasound waves and result in a gray image on the screen.

Routine reproductive examinations should include visualization of the major structures (or the lack thereof) on both ovaries. Although rectal palpation can be an accurate method for diagnosing pregnancy, rectal palpation is a poor method for resolving ovarian follicles (Pieterse et al., 1990). By contrast, ultrasonic imaging is a highly accurate and rapid method for assessing ovarian structures (Griffin and Ginther, 1992). Too often, bovine practitioners proceed directly to scanning the uterus during reproductive examinations and neglect the ovaries all together. This is unfortunate because the ovaries contain a wealth of information that can be used to aid in diagnosing the reproductive status of the cow and for selecting appropriate therapies or reproductive interventions. For example, presence or absence of a corpus luteum aids in diagnosing pregnancy status, especially when conducting pregnancy exams early post-AI. When present, the size and location (i.e., left vs. right ovary) of the corpus luteum indicates the location
of the conceptus within the uterus if the cow is pregnant. Because most twinning in cattle is dizygous (Wiltbank et al., 2000), the presence of multiple corpora lutea is a diagnostic indicator of the presence of twin fetuses. Ovarian pathologies such as “static ovaries” and follicular and luteinized cysts can easily be distinguished. Use of ovarian structures as diagnostic aids during reproductive examinations, however, requires a thorough understanding of ovarian and reproductive anatomy and physiology. In addition, there are limitations to the conclusions that can be made from a single (as opposed to serial) ultrasound examination.

**Ovarian Follicles**

Folliculogenesis is the process of forming mature follicles capable of ovulation from the pool of nongrowing, primordial follicles in the ovary (Spicer and Echternkamp, 1986). Ovarian follicles are fluid-filled structures surrounded by an inner layer of granulosa cells and an outer layer of thecal cells. The oocyte is suspended within the antrum by a specialized pedicle of granulosa cells called the cumulus oophorous. Because fluid absorbs rather than reflects ultrasound waves, fluid-filled structures such as follicles appear as black circular structures surrounded by echogenic ovarian tissue. Most veterinary grade ultrasound scanners can resolve ovarian follicles with a diameter of 2 to 3 mm or greater, and larger follicles can easily be tracked during serial scanning sessions (Pierson and Ginther, 1988). The ability to noninvasively track follicular growth during the estrous cycle using ultrasound has revolutionized our understanding of reproductive physiology.

**Follicular Waves**

Scientific studies using transrectal ultrasonography have led to clarification of the nature of antral follicular development in cattle (For a review, see Ginther et al., 1996). The first studies using ultrasound revealed that follicular growth occurs in waves, each wave culminating with formation of a large follicle (Figure 1).

A follicular wave begins with emergence of a group or cohort of small antral follicles just before the day of ovulation. During the next several days, one of the follicles in this cohort continues to grow and becomes dominant, thereby suppressing subordinate follicles within the wave from which it originated as well as emergence of follicles in an ensuing follicular wave. As the dominant follicle continues to grow, growth of the remaining follicles in the cohort ceases or slows, and these subordinate follicles eventually

![Figure 1. Schematic diagram depicting two-wave (top panel) and three-wave (bottom panel) patterns of follicular growth during the bovine estrous cycle. Growing follicles before selection of the dominant follicle are depicted as black circles, the dominant follicles of each wave are depicted as gray circles, and atretic follicles are depicted as open circles.](image-url)
undergo atresia. A second wave of growth emerges on approximately Day 10 after ovulation and, for three-wave cycles, an additional wave emerges at Day 16 after ovulation. For both two and three-wave cycles, the ovulatory follicle arises from the final wave (Ginther et al., 1996).

Manual palpation or ultrasonographic examination of the cow's genital tract are currently used by veterinarians involved in reproductive management. A recent report (Aslan et al., 2000) evaluating the difference in detection of follicles by ultrasound or rectal palpation concluded that ultrasound was more effective at identifying follicles greater than 10 mm in diameter than rectal palpation. Follicles 10 to 15 mm in diameter were detected in 90% of cases using ultrasonography versus 62% of the cases using rectal palpation. Follicles greater than 15 mm were detected in 100% of the cases for both ultrasonography and rectal palpation. In a similar review (Hanzen et al., 2000) manual diagnosis of follicles <10 mm was inaccurate, but ultrasound offered the possibility to diagnose follicles <5 mm and to measure the diameter of those follicles. Figure 2 demonstrates the appearance of the ovary at various stages of follicular development prior to emergence of a follicular wave, during proestrus, and after development of a follicular cyst.

**Figure 2.** Ultrasound image of bovine ovaries prior to emergence of a follicular wave (note two small follicles [< 5 mm]; Panel A), during proestrus (note pre-ovulatory follicle [13 mm]; Panel B), and after development of a follicular cyst (note delamination of granulose layer into the antrum; Panel C). Images were taken using a 7.5 Mhz transducer (Lamb, 2001).
**Corpora Lutea**

The CL is a transient endocrine gland that forms after ovulation from the tissues that previously composed the ovarian follicle. Thus, the CL can be viewed as the terminal stage of follicular development. Corpora lutea appear as distinctly echogenic areas within the ovarian stroma. Many corpora lutea appear as a solid tissue masses but may also contain fluid-filled cavities. Based on ultrasonographic examinations in dairy heifers, 79% of otherwise normal CL contain cavities ranging from less than 2 to greater than 10 mm in diameter at some time during the estrous cycle and early pregnancy (Kastelic et al., 1990b; Singh et al., 1997). The appearance of the CL may be used to estimate the stage of the bovine estrous cycle (Kastelic and Ginther, 1989; Kastelic et al., 1990a,b; Singh et al., 1997), yet differences in CL development decrease the accuracy of estimates. A higher percentage of corpora lutea in early diestrus tend to have a fluid filled lumen versus the corpora lutea during late diestrus and advanced stages of pregnancy. We (Spell et al., 2001) determined that luteal diameter was not associated with concentrations of progesterone on day 7 of the estrous cycle, but area and volume were correlated to concentrations of progesterone.

Ultrasonographic attributes of CL including cross-sectional diameter, luteal area, and echogenicity have been correlated to luteal structure and function (Battocchio et al., 1999; Kastelic et al., 1990a; Singh et al., 1997). Use of luteal characteristics to improve accuracy of pregnancy diagnosis has been reported in dairy heifers (Kastelic et al., 1991), but similar data does not exist for beef or lactating dairy cattle. Luteal size and echogenic characteristics assessed at specific times post breeding may prove useful as a method to improve accuracy of early pregnancy diagnosis in dairy cattle. Although ultrasound is more accurate than rectal palpation for assessing ovarian cysts, it is difficult to distinguish between developing corpora lutea and older regressing corpora lutea using either technique (Pieterse et al., 1990).

**Ovarian Cysts**

For beef cattle the diagnosis of cysts is of little practical importance, whereas, diagnosis of cysts in dairy cattle most often occurs during routine postpartum rectal examinations conducted by a bovine practitioner. Palpation per rectum of a large, fluid-filled structure is commonly used as a clinical indication of a follicular cyst. Differentiation between follicular and luteal cysts via rectal palpation is difficult, even for experienced practitioners (Dawson, 1975; Farin et al., 1992). Accuracy of diagnosis increases when using transrectal ultrasonography, with correct identification of greater than 90% of luteal and nearly 75% of follicular cysts (Farin et al., 1990, 1992). Follicular and luteal cysts also can be classified based on serum progesterone concentrations (Farin et al., 1990). Diagnosis of a cyst in conjunction with low serum progesterone is indicative of a follicular cyst, whereas a cyst in conjunction with high serum progesterone is indicative of a luteal cyst. Using these criteria, a benign follicular cyst would fall into either category depending on the stage of the estrous cycle when they were detected.

Treatment for ovarian cysts depends on the classification of the cyst. Follicular cysts are most commonly treated by administration of synthetic GnRH analogs approved for use in lactating dairy cows (Bierschwal et al., 1975; Seguin et al., 1976; Whitmore et al., 1979). Manual rupture of cysts via rectal palpation is not recommended because adverse side effects including adhesions around the ovary and adnexa may impair fertility (Archibald and Thatcher, 1992). Interestingly, approximately 20% of untreated cows with follicular cysts recover spontaneously (Bierschwal et al., 1975), supporting the notion that many of these cysts may be benign. Treatment with GnRH induces luteinization rather than ovulation of the follicular cyst, and
ultimately results in formation of a luteal cyst (Garverick, 1997). Once formed, regression of a luteal cyst can be induced by administration of PGF$_{2\alpha}$ (Nanda et al., 1988). Administration of GnRH to cows with benign follicular cysts often induces ovulation of a normally growing dominant follicle rather than the cyst itself (Fricke and Wiltbank, 1999, Table 1), and other researchers have reported similar observations (Archibald and Thatcher, 1992; Garverick, 1997).

Table 1. Effect of ovarian cysts on synchronization rate and conception rate in lactating dairy cows after synchronization of ovulation using Ovsynch (Adapted from Fricke and Wiltbank, 1999).

<table>
<thead>
<tr>
<th>Item</th>
<th>Yes</th>
<th>No</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>11.0%</td>
<td>89.0%</td>
<td></td>
</tr>
<tr>
<td>Synchronization rate$^b$</td>
<td>73.1%</td>
<td>85.3%</td>
<td>84.0%</td>
</tr>
<tr>
<td>Conception rate$^c$</td>
<td>36.8%</td>
<td>48.8%</td>
<td>47.6%</td>
</tr>
</tbody>
</table>

$^a$A fluid-filled ovarian cyst $\geq$ 25 mm in diameter present at the time of the second GnRH injection of the Ovsynch protocol.

$^b$Ovulation of a normal dominant follicle after the second GnRH injection of the Ovsynch protocol.

$^c$Ultrasoundographic determination conducted at 28 d post AI.

Ovsynch, a protocol for synchronizing ovulation in lactating dairy cows, uses injections of GnRH and PGF$_{2\alpha}$ (Pursley et al., 1995, 1997) and is an effective treatment for ovarian cysts. A recent field trial using Ovsynch and ultrasonographic monitoring of ovarian structures (Table 1) revealed that 11% of lactating cows exhibited a large ovarian structure that would have been diagnosed as a cyst using rectal palpation. Treatment with Ovsynch induced ovulation of a follicle other than the cyst that was present at the time of the second GnRH injection in 73% of cows, and nearly 37% of these synchronized cystic cows conceived after a timed AI. Thus, Ovsynch the treatment of choice for establishing pregnancy in lactating dairy cows exhibiting ovarian cysts. Data from a separate study has recently corroborated these findings (Bartolome et al., 2000).

Diagnostic Limitations of Ultrasonic Imaging

Under most circumstances, practical application of ultrasound for routine reproductive management consists of a single ultrasound examination at a given point in time. It is important to understand that the physiological status of a follicle (e.g., dominant, subordinate, growing, regressing) or corpus luteum cannot be determined during a single ultrasound exam. In addition, ultrasonic imaging aids in distinguishing anatomical attributes of a structure but confers little information regarding physiological or endocrine status. For example, ovarian cysts can be categorized by anatomical attributes such as diameter and presence or absence of luteal tissue; however, no information regarding functionality such as plasma hormone concentrations can be conferred. One exception would be the visualization of a fetal heartbeat as a diagnostic indicator of a viable fetus. The diagnostic limitation of ultrasonic imaging becomes important especially when the limitation is exceeded and an incorrect therapy or reproductive intervention is recommended. A thorough understanding of ovarian physiology and the mechanisms by which
hormonal programs succeed or fail is imperative for correct interpretation of ultrasonic imaging information.

**Imaging the Bovine Uterus and Conceptus**

Of all the ultrasound applications utilized by technicians in the industry, scanning of the uterus for infection and pregnancy are the most commonly practiced commercial applications that we have seen in the cattle industry. In a nonpregnant, cycling cow the uterine tissue appears as a somewhat echogenic structure on the screen. Because the uterus is comprised of soft tissue it absorbs a portion of the ultrasound waves and reflects a portion of the waves. In this way we can identify the uterus as a gray structure on the screen. A cross-sectional view of the uterus is displayed as a “rosette” and is easily distinguished from other peripheral tissues, whereas the longitudinal section is less recognizable, yet a trained technician can differentiate between the elongated view of the uterus and other tissues that may appear similar (Figure 3). Physiological changes during the estrous cycle leads to physical changes (such as tone) in the uterus, which alters the echogenic properties of the uterus (Pierson and Ginther, 1987a). Even though a scoring system has been developed to describe changes in uterine echogenic ability during different stages of the estrous cycle (Pierson and Ginther, 1987a), predicting the stage of the estrous cycle remains inconsistent.

**Figure 3.** Ultrasound image depicting an elongated (Panel A) and cross-sectional (Panel B) view of the non-pregnant uterus. Images were taken using a 5.0 Mhz transducer (Lamb, 2002=1).
Pathological applications for ultrasound technology have extended to identifying endometritis, pyometra, mucometra, and hydrometra (Perry et al., 1990). With the aid of ultrasound, researchers have determined that uterine infections were related to delayed postpartum folliculogenesis (Peter and Bosu, 1988), to the occurrence of short luteal phases after the first postpartum ovulation (Peter and Bosu, 1987), and to the development of follicular cysts on the ovaries (Peter and Bosu, 1987).

Detection of the embryo proper as well embryonic and fetal developmental characteristics during early fetal development are shown in Table 2 and 3. The bovine fetus can be visualized beginning at 20 d post breeding and continuing throughout gestation, however, because of its size in relation to the image field of view, the fetus cannot be imaged \textit{in toto} after about 90 days using a 5.0 MHz linear-array transducer.

\begin{table}[h]
\centering
\caption{Day of first detection of ultrasonographically identifiable characteristics of the bovine conceptus (Adapted from Curran et al., 1986).}
\begin{tabular}{lcc}
\hline
Characteristic & Mean & Range \\
\hline
Embryo proper & 20.3 & 19 to 24 \\
Heartbeat & 20.9 & 19 to 24 \\
Allantois & 23.2 & 22 to 25 \\
Spinal cord & 29.1 & 26 to 33 \\
Forelimb buds & 29.1 & 28 to 31 \\
Anmion & 29.5 & 28 to 33 \\
Eye orbit & 30.2 & 29 to 33 \\
Hindlimb buds & 31.2 & 30 to 33 \\
Placentomes & 35.2 & 33 to 38 \\
Split hooves & 44.6 & 42 to 49 \\
Fetal movement & 44.8 & 42 to 50 \\
Ribs & 52.8 & 51 to 55 \\
\hline
\end{tabular}
\end{table}

\textbf{Early Pregnancy Diagnosis}

Reports have indicated the detection of an embryonic vesicle in cattle as early as 9 (Boyd et al., 1988), 10 (Curran et al., 1986a), or 12 days (Pierson and Ginther, 1984) of gestation. In these situations the exact date of insemination was known and ultrasonography simply was used as a confirmation of pregnancy or to validate that detection of an embryo was possible within the first two weeks of pregnancy. In contrast, Kastelic et al. (1989) monitored pregnancy in pregnant and nonpregnant yearling heifers that were all inseminated. Diagnosis of pregnancy in heifers on day 10 through day 16 of gestation resulted in a positive diagnosis for pregnant or nonpregnant of less than 50%. On days 18, 20, and 22 of gestation accuracy of pregnancy diagnosis improved to 85%, 100%, and 100%, respectively. Although evidence of a pregnancy via ultrasound during days 18 to 22 of gestation yields excellent results, a technician needs to ensure that confusion between fluid accumulation in the chorioallantois during early pregnancy (Kastelic et al., 1989)
and uterine fluid within the uterus during proestrus and estrus are not confused when making the diagnosis.

Several further reports (Taverne et al., 1985; Hanzen and Delsaux, 1987; Pieterse et al., 1990, Badtram et al., 1991) also indicate the presence of an embryonic vesicle as early as day 25 of gestation. Although Hanzen and Delsaux (1987) utilized a 3.0 MHz transducer for pregnancy diagnosis, they concluded that by day 40 of gestation a positive diagnosis of pregnancy was 100% accurate, whereas overall diagnosis of pregnancy and absence of pregnancy from day 25 of gestation proved to be correct in 94% and 90% of cases, respectively. In 148 dairy cows, pregnancy diagnosis from day 21 to day 25 was 65% accurate, whereas diagnosis of pregnancy from day 26 to day 33 was 93% accurate (Pieterse et al., 1990). In their conclusions, the authors state that probable causes of misdiagnosis from day 21 to day 26 were either an accumulation of proestrus or estrus uterine fluid, or the accumulation of pathological fluid in the uterus, or were diagnosed pregnant but experienced early embryonic loss.

Although we have indicated that an embryonic vesicle is detectable by ultrasound as early as 9 days of gestation, accuracy of detection approaches 100% after day 25 of gestation. For practical purposes, the efficiency (i.e., speed and accuracy) of a correct diagnosis of pregnancy should be performed in females expected to have embryos that are at least 26 days of age (Figure 4). This information can be used to determine the age of bovine fetuses with a high degree of accuracy (Pierson and Ginther, 1984; Boyd et al., 1988; Ginther, 1995). Crown-Rump length measurements were summarized by Hughes and Davies (1989; Table 3). There was a significant correlation (r = 0.98) between embryo age and crown-rump length.

Figure 4. Ultrasound images of the bovine fetus at various stages of development.
Table 3. Fetal crown-rump length in relation to age in weeks (Hughes and Davies, 1989).

<table>
<thead>
<tr>
<th>Fetal age, weeks</th>
<th>No. of observations</th>
<th>Crown-rump length, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>16</td>
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<tr>
<td>7</td>
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<td>10</td>
<td>43</td>
<td>61</td>
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<tr>
<td>11</td>
<td>39</td>
<td>95</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>107</td>
</tr>
</tbody>
</table>

Ultrasound is a rapid method for pregnancy diagnosis, and experienced palpators adapt to ultrasound quickly. The time required to assess pregnancy in beef heifers at the end of a 108-day breeding season averaged 11.3 seconds using palpation per rectum versus 16.1 seconds required to assess pregnancy and fetal age using ultrasound (Galland et al., 1994). Fetal age also affected time required for diagnosis with older fetuses requiring less total time for diagnosis (Galland et al., 1994). Although ultrasound at ≥45 d of gestation did not increase accuracy of pregnancy diagnosis for an experienced palpator, it may improve diagnostic accuracy of a less experienced one (Galland et al., 1994).

Two caveats must be considered when using ultrasound for routine early pregnancy diagnosis in a cow herd. First, when using ultrasound for early pregnancy diagnosis, emphasis must be given to identifying nonpregnant rather than pregnant cows. Second, a management strategy must be implemented to return the nonpregnant cows to service as quickly as possible after pregnancy diagnosis. Such strategies include administration of PGF$_{2\alpha}$ to cows with a responsive CL, use of estrus detection aids, or a combination of both methods.

Early Embryonic Loss

Prior to the development of ultrasound for pregnancy diagnosis in cattle, technicians were unable to accurately determine the viability or number of embryos or fetuses. Because the heartbeat of a fetus can be detected at approximately 22 days of age, we can accurately assess whether or not the pregnancy is viable. Studies in beef (Diskin, M.G. and J.M. Sreenan. 1980; Beal et al., 1992; Lamb et al., 1997) and dairy (Smith and Stevenson, 1995; Vasconcelos et al., 1997; Fricke et al., 1998; Szenci et al., 1998) cattle have used ultrasound to assess the incidence of embryonic loss. The number of fetuses can most accurately be assessed at between 49 and 55 days of gestation (Davis and Haibel, 1993).
Table 4 summarizes the incidence of embryonic loss by study in beef and dairy females. The fertilization rate after artificial insemination in beef cows is 90%, whereas embryonic survival rate is 93% by day 8 and only 56% by day 12 post artificial insemination (Diskin and Sreenan, 1980). The incidence of embryonic loss in beef cattle appears to be significantly less than in dairy cattle. Beal et al. (1992) reports a 6.5% incidence of embryonic loss in beef cows from day 25 of gestation to day 45. Similarly, Lamb et al. (1997) noted a 4.2% incidence of embryonic loss in beef heifers initially ultrasounded at day 30 of gestation and subsequently palpated rectally at between day 60 and 90 after insemination. In dairy cattle, pregnancy loss from 28 to 56 days after artificial insemination was 13.5%, or 0.5% per day (Fricke et al., 1998). This rate of pregnancy loss is similar to the 12.4% reported by Smith and Stevenson (1995) and the 19.1% reported by Vasconcelos et al. (1997) during a comparable stage of pregnancy in lactating dairy cows. The greatest occurrence of pregnancy loss was between day 28 and 42 of gestation (10.5%) and between day 42 and 56 of gestation (6.3%). After day 56 of pregnancy, embryonic losses were reduced to 3.4% from 56 to 98 days of pregnancy and 5.5% from 98 days to calving (Vasconcelos et al., 1997). Specific physiologic mechanisms responsible for pregnancy loss in dairy cattle may include lactational stress associated with increased milk production (Nebel and McGilliard, 1993), negative energy balance (Butler and Smith, 1989), toxic effects of urea and nitrogen (Butler et al. 1995) or reduced ability to respond to increased environmental temperature (Stevenson et al., 1984; Hansen et al., 1992). These studies indicate the usefulness of ultrasonography as a tool to monitor the success of a breeding program, by determining pregnancy rates and embryonic death.

Additional investigators have reported a range of embryonic mortality from day 21 to 60 to be 8% (Boyd et al., 1969) to 35% (Beghelli et al., 1986). In those reports, embryo mortality was determined by the presence of high blood concentrations of progesterone at day 21 to 23 after breeding (presuming a high concentration of progesterone was caused by the embryonic signal to prevent luteal regression) but the absence of an embryo or fetus by rectal palpation at 40 to 60 days after insemination. The authors rationale assumed that the embryo was lost between day 21 of gestation and the time of palpation; however, there was no positive identification of a viable embryo at day 21 to 23 of gestation. Therefore, ultrasonography provides a tool to accurately differentiate between the failure of a female to conceive or the incidence of embryonic mortality because a heartbeat is detectable at 22 days of gestation.

At present, there is no practical way to reduce early embryonic loss in cattle. However, recognizing the occurrence and magnitude of early embryonic loss may actually present management opportunities by taking advantage of new reproductive technologies that increase AI service rate. If used routinely, transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by reducing the period from AI to pregnancy diagnosis to 26 to 28 days with a high degree of diagnostic accuracy.
**Table 4.** Incidence of embryonic/fetal loss in cows after an initial diagnosis of pregnancy by ultrasound, followed by a second diagnosis prior to or at calving

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. pregnant, days of gestation</th>
<th>No. pregnant, days of gestation</th>
<th>No. of embryos lost</th>
<th>Embryonic mortality, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beef Cattle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beal et al., 1992 (Cows)</td>
<td>138 25 days</td>
<td>129 45 days</td>
<td>9</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>129 45 days</td>
<td>127 65 days</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>138 65 days</td>
<td>127 65 days</td>
<td>11</td>
<td>8.0</td>
</tr>
<tr>
<td>Lamb et al., 1997 (Heifers)</td>
<td>149 30 days</td>
<td>143 60 days</td>
<td>6</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>271 35 days</td>
<td>260 75 days</td>
<td>11</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>105 30 days</td>
<td>100 90 days</td>
<td>5</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Dairy Cattle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith and Stevenson, 1995 (Cows and Heifers)</td>
<td>129 28 to 30 days</td>
<td>113 40 to 54 days</td>
<td>16</td>
<td>12.4</td>
</tr>
<tr>
<td>Vasconcelos et al., 1997 (Cows)</td>
<td>488 28 days</td>
<td>437 42 days</td>
<td>51</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>42 days</td>
<td>437 42 days</td>
<td>56 days</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>409 42 days</td>
<td>402 56 days</td>
<td>7</td>
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<td>56 days</td>
<td>70 days</td>
<td>7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>402 56 days</td>
<td>395 70 days</td>
<td>7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>488 56 days</td>
<td>395 98 days</td>
<td>93</td>
<td>19.1</td>
</tr>
<tr>
<td>Fricke et al., 1998 (Cows)</td>
<td>89 28 days</td>
<td>77 56 days</td>
<td>12</td>
<td>13.5</td>
</tr>
<tr>
<td>Szenci et al., 1998 (Cows)</td>
<td>64 26 to 58 days</td>
<td>52 Full term</td>
<td>12</td>
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</table>
Fetal Sexing

Many cattle operations are developing strategies to use fetal sexing as either a marketing or purchasing tool. At approximately day 50 of gestation, male and female fetuses can be differentiated by the relative location of the genital tubercle and development of the genital swellings into the scrotum in male fetuses (Jost, 1971). Fetuses at 48 to 119 days of age have been successfully sexed (Müller and Wittkowski, 1986; Curran et al., 1989; Wideman et al., 1989; Beal et al., 1992). The procedure is reliable and accuracy has ranged from 92 to 100% (Müller and Wittkowski, 1986; Wideman et al., 1989; Beal et al., 1992). Beal et al. (1992) noted that of 85 fetuses predicted to be male 84 were confirmed correct, resulting in 99% accuracy. In addition, of 101 fetuses predicted to be female 98 were confirmed correct, resulting in 97% accuracy. Recently, we (Lamb, 2001) determined the sex of 112 fetuses in Angus heifers with 100% accuracy.

For optimal results the ultrasound transducer should be manipulated to produce a frontal, cross-sectional, or sagittal image of the ventral body surface of the fetus. In larger framed cows (i.e. Holsteins and Continental beef breeds) or older cows the optimum window for fetal sexing usually is between day 55 and 70 of gestation, whereas for smaller framed cows (Jerseys and English beef breeds) the ideal window usually is between day 55 and 80 of gestation. There are two limitations that could inhibit the ability of a technician to determine the sex of a fetus: 1) as the fetus increases in size it becomes more difficult to move the transducer relative to the fetus to obtain the desired image; and, 2) the gravid horn is more likely to descend ventrally into the abdominal cavity in larger or older cows, making fetal sexing virtually impossible without retracting the gravid horn.

Figure 5 illustrates the cross-sectional image of female fetus (65 days of gestation; Panel A) and a sagittal view of a male fetus (65 days of gestation; Panel B; Lamb, 2001). The umbilicus can be used as an excellent landmark when determining the location of the genital tubercle or presence of a scrotum in males. In the male, the genital tubercle is located adjacent to and caudal to the umbilicus, whereas the genital tubercle in the female is located just ventral to the tail. The scrotum is detectable between the hind legs of the male fetus. The genital tubercle and scrotum are echogenic and are easily detected on an ultrasound screen as echogenic images. To ensure an accurate diagnosis of sex, for each patient, a technician should view an image at three locations: 1) adjacent to the umbilicus, where the umbilicus enters the abdomen (possible male genital tubercle); 2) the area between the back legs (possible scrotum); and, 3) ventral to the tail (possible female genital tubercle).

In beef cattle operations, fetal sexing remains limited to purebred operations especially in conjunction with an embryo transfer program. Determination of sex especially after the successful transfer of embryos to recipients allows marketing of male and female embryos before the pregnancy is carried to term. This strategy can be used effectively in dairy operations trying to produce bull calves of a particular mating for sale to bull studs. From a commercial cattle operation standpoint, heifer development operations are utilizing fetal sexing as a marketing tool to provide potential buyers with females that are pregnant with fetuses of a specific sex. As more technicians become proficient at fetal sexing, commercial operations will utilize this technology to enhance the marketability and efficiency of their cattle operations.
Conclusion

The impact of real-time ultrasound on the study of reproduction has been dramatic and the further development of portable ultrasound machines has given clinicians an added tool for diagnostic reproductive management. Ultrasound is commonly used to monitor uterine anatomy, involution, and pathology. In addition, it has been used to detect pregnancy, study embryonic mortality, monitor fetal development, and determine fetal sex. The applications of ultrasound used by scientists include the ability to monitor follicular characteristics, ovarian function, and aid in follicular aspirations and oocyte retrieval. In the future, as technology improves technicians will have an opportunity to use the internet or video conferencing for ultrasound image analyses. With every new technological development, scientists, veterinarians, and producers discover new possibilities for the use of reproductive ultrasound to enhance the scientific merit of research or improve reproductive efficiency in cattle operations.

Figure 5.
Ultrasound image of a female bovine fetus (65 days of gestation; Panel A) and a sagittal view of a male fetus (65 days of gestation; Panel B). Images were taken using a 5.0 Mhz transducer.
REFERENCES


Curran S, Pierson RA, Ginther OJ. 1986. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. JAVMA 189:1295-1302.


Semen can be collected from bulls by a variety of means including per-rectal massage, the use of an artificial vagina and by electro-ejaculation. The latter method is the one most commonly employed with range-type bulls. Method of collection should be noted as it may affect qualitative aspects of the ejaculate.

Electroejaculators and Probes.
Commercially available electroejaculators are available with power being provided by AC current, by internal rechargeable batteries, or by 12v automobile batteries. Electroejaculation requires the stimulation of pelvic nerves controlling not only the emission of semen into the penile urethra but also those controlling erection and ejaculation. Newer probe designs have full-length longitudinal electrodes which stimulate all functions simultaneously.

Preparation and Stimulation.
The bull's rectum should be emptied of feces before the probe is inserted. The lubricated probe is inserted so that the anal sphincter closes behind the main body of the unit. It is helpful to determine the lowest current level at which the animal first shows an obvious physical response. This initial response may be subtle e.g. a twitch of the tail, a tightening of the anal sphincter, or a tensing of the gluteal muscles. This stimulus "threshold" provides the starting point for subsequent stimulations which should be conducted with a smooth routine to which the bull can easily adapt.

With Bos taurus breeds, a typical approach is to deliver a smooth increase in probe current from zero to the desired level over a duration of 1 to 2 seconds, followed by a more rapid reduction to zero current and a rest period of approximately one second before the next stimulation. Once the bull is settled into the routine, 5 to 7 stimulations are given at each succeeding voltage step until erection and ejaculation occur. For machines which do not have separate voltage and current controls, the same stimulation pattern is employed except that the single control is used to generate incremental increases in probe voltage and current until ejaculation occurs. During the early stages of stimulation and erection, clear seminal plasma is often passed which is not generally collected. When the ejaculate turns cloudy, the subsequent jets of semen are collected. It is important to continue stimulation until the ejaculate starts to become clear again. Failure to proceed to this point can lead to errors of interpretation in the spermiogram as the initial portion of the ejaculate may contain large numbers of degenerating spermatozoa, especially in bulls which have been sexually quiescent for some time. For the same reason, if an ejaculate shows substandard motility in the absence of an obvious physical cause, the collection of a second sample within a short
period of time (e.g. 5 to 10 minutes) can often result in improvement.

Variations on these stimuli patterns occur with different machines and operators. With difficult bulls some experimentation might well be necessary. In all situations, however, the welfare of the animal is paramount and stimulation should be discontinued if either undue stress is being caused or physical injury to the bull might occur.

**Collection Devices.**

Semen is collected into a prewarmed insulated or jacketed tube through a funnel or cone. All surfaces coming into contact with semen should be clean, warm, dry and free of spermatoxic agents. Because "cold shock" causes irreversible damage to spermatozoa, efforts to maintain semen at 30-35°C until the "on-site" evaluation procedures are complete is an important consideration for successful semen assessment.

**Semen Evaluation**

**Initial Impressions.**

Volume, density, and gross characteristics of the ejaculate are not "front-line" BSE assessments because they have not been shown to be related to fertility. Space on the score sheet is, however, provided for the recording of such information. Likewise, the assessment of spermatozoal concentration is not a routine part of the BSE; the measurement of scrotal circumference provides a better estimate of sperm production in range-type bulls which are subject to infrequent examinations. However, informal recording of such information may help to monitor the success of semen collection and to interpret gross motility estimation.

Other gross characteristics which may be noted include evidence of contamination, hemorrhage or inflammatory material. If the ejaculate contains sufficient purulent material for this to be obvious to the naked eye, then that bull should not be classified as satisfactory at least until a benign cause is found. Debris or contamination from the sheath may be regarded as being less serious unless it represents active infection.

**Motility** should be assessed microscopically. Two methods of assessing sperm motility are traditionally employed; gross motility (or mass activity) and individual motility (or percent progressive motility). It is good procedure to use both methods as can they differ somewhat in interpretation and precision. With all motility estimations it is important to protect semen against adverse effects (e.g cold shock) and to do the estimation as soon as possible after semen collection.

Gross motility, or the amount of swirling (or wave motion) present in an undiluted semen sample, is a function of both sperm concentration and individual motility. Under field conditions, gross motility is typically assessed by placing a drop of raw semen on a warmed slide and observing it at 100 magnifications (10X eyepiece and 10X objective). With the condenser properly adjusted, mass action or "swirl" can be observed in samples which have adequate numbers of motile spermatozoa. The rankings for this estimate are as follows:
Mass Activity (Gross Motility) Rating

<table>
<thead>
<tr>
<th>Activity</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Swirling</td>
<td>Very Good (VG)</td>
</tr>
<tr>
<td>Slower Swirling</td>
<td>Good (G)</td>
</tr>
<tr>
<td>Generalized Oscillation</td>
<td>Fair (F)</td>
</tr>
<tr>
<td>Sporadic Oscillation</td>
<td>Poor (P)</td>
</tr>
</tbody>
</table>

Individual progressive motility of spermatozoa is assessed under a brightfield or phase-contrast microscope preferably equipped with a warm stage or other means of preventing cold shock of spermatozoa. Coverslipped specimens are usually examined at a total magnification of 400x. In dense samples (milky or creamy) the sample should be diluted for proper observation of individual spermatozoa. Sodium citrate or skim milk based semen extenders are serviceable diluents; physiological sterile saline (PSS) may be used although readings should not be delayed when it is used. The percentage of active, progressively motile cells is estimated. This procedure takes more practice than does the gross motility estimation, but is probably more accurate in experienced hands. Individual motility ratings are as follows:

Percent Progressive Motility Rating

<table>
<thead>
<tr>
<th>Percent Progressive Motility</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>Very Good (VG)</td>
</tr>
<tr>
<td>50 - 69%</td>
<td>Good (G)</td>
</tr>
<tr>
<td>30 - 49%</td>
<td>Fair (F)</td>
</tr>
<tr>
<td>≤ 30%</td>
<td>Poor (P)</td>
</tr>
</tbody>
</table>

Observation of the semen sample at 400x can also help to identify the presence of abnormal numbers of other cells (e.g. squamous epithelial cells, inflammatory cells or spheroids) within the sample. The identification of aberrant cellular material can benefit from the staining of a semen smear with Dif-Quik, New Methylene Blue, or other differential blood cell stains, while bacteria are best categorized using a Gram stain.

Morphology of spermatozoa (differential counts of normal and abnormal cells) is assessed either by phase microscopy (using preparations "fixed" in e.g. formol-buffered-saline or PBS-gluteraldehyde) or by using brightfield microscopy of stained smears. Common stains used for this purpose include nigrosin-eosin, William's stain, modified Giemsa and even India-ink. The Society for Theriogenology (SFT) recommends the nigrosin-eosin stain for its combination of ease and utility. Although this stain is a "supra-vital" stain (i.e. sperm which are "alive" at staining will not absorb stain while those that are "dead" will partially or completely absorb the red eosin color), here it is used for its ability to depict sperm morphology only.
With nigrosin-eosin staining of spermatozoa, the most common method is to mix a fraction of a drop of semen with a drop of background or "negative" stain and spread the mixture over the surface of a glass slide which is allowed to air-dry. Care should be taken during the smearing process to avoid trauma to sperm. It is also helpful to vary the thickness of the smear to provide a variety of background densities to the stain from which an area can be picked for best microscopic examination.

Brightfield microscopy of stained smears is best done at 1000X with an oil immersion lens. At least 100 spermatozoa should be observed in different fields and classified for normality or abnormality. Normal sperm should be at least 70% of the ejaculate for the bull to pass the BSE (see below).

In the older SFT BSE system, sperm abnormalities were classified as being either "primary" or "secondary" with the underlying assumptions being that primary abnormals (considered to be caused during spermatogenesis) were more serious than secondaries (caused subsequent to sperm release into the extragonadal system). More recent knowledge has cast doubt upon these assumptions. In the meantime a system using "major" and "minor" abnormalities was created to more accurately reflect sperm abnormalities for which fertility data was available. It was apparent that the lists of sperm abnormalities in routine use for both systems were essentially indistinguishable (except perhaps for proximal cytoplasmic droplets). Thus, as the primary/secondary scheme is currently widely used, this was retained as the reference point in the present system (see appendix for categories of abnormalities). Although total abnormalities only are employed as the threshold in the new BSE system, "primary" and "secondary" abnormalities can be collated to arrive at this number. The recording of specific abnormalities (or their category as primary or secondary) can also be useful for the monitoring of bulls and their progress.

It should be noted that a system describing compensable and uncompensable sperm defects, has been proposed (Saacke et al 2000), which shows much promise in helping to categorize sperm defects in manner which reflects their function and significance. This system is, however, still evolving.

**Advances in Semen Assessment**

To be able to accurately predict fertility by using a rapid, economic assessment of a single semen sample remains an elusive goal for researchers and clinicians alike. This is because fertility is a multifactorial trait, and also because the semen assay attempts to predict future performance from past events. Difficulty exists in defining which semen or sperm traits are most associated with fertility. For example, although motility undoubtedly plays a role in sperm transport and penetration of the zona pellucida of the oocyte, results with ICSI (intracytoplasmic sperm injection) show that it is not an essential prerequisite for actual fertilization to occur. In addition, although significant morphological abnormalities can prevent sperm from reaching the site of fertilization, this does not appear to be true for more
subtle abnormalities. However, there is some evidence that the latter group might contribute to lowered embryonic viability. In general, the best approach for semen assessment is to use a combination of several seminal quality attributes (Garner 1997), choosing those which reflect different aspects of sperm function (eg motility and morphology).

**Sperm Movement**

Visual assessment of sperm movement using a bright-field microscope is still the most common method employed. Although rapid and relatively inexpensive, this method is susceptible to subjectivity and environmental insult, resulting in a relatively low allowable threshold for this parameter in the current SFT bovine BSE system (30%). Accuracy improves with the use of phase microscopy in controlled environments and appropriate dilution of concentrated samples. More objective methods have included the use of time-lapse micro-photography, photo-electric systems (e.g. Optibreed) and computer assisted semen analysis (CASA). Some of these show considerable promise, as well as providing a wealth of additional information concerning sperm movement attributes. Cost is a constraint for routine field use of some of these systems. For such use, reasonable repeatable results may be obtained by a trained observer using a good microscope in a controlled environment.

**Sperm Morphology**

Sperm morphology represents one of the more important aspects of semen assessment (Garner 1997), as it directly affects fertility. Difficulties arise in determining which abnormalities are most problematic, and what are their acceptable levels of tolerance. Sperm morphology classification systems have attempted to categorize abnormalities in terms of their assumed origin (primary/secondary), significance (major/minor) and presumed functional contribution to infertility (compensable/uncompensable). Another approach is to assess the number of viable sperm which are free of abnormalities in relation to a estimated fertility “threshold” - the route followed by the Society for Theriogenology with its breeding soundness guidelines for bulls (Chenoweth et al 1991).

Traditionally, sperm morphology assessments have been conducted by observing stained semen samples via a bright-field microscope. Commonly used stains have included nigrosin-eosin, eosin-congo red, Williams stain and Giemsa. If these are not available, Dif-Quik or even India ink can provide adequate staining. Sperm morphology assessment is best done at a magnification of 1000 plus X, which usually implies use of an oil-immersion objective. When done with care, using a good microscope, the evaluation of 100 sperm in random fields has been shown to be sufficient for routine assessment purposes.

Improvements in sperm morphology assessment have been attributed to the use of different forms of phase-contrast microscopy, with differential-interference contrast (DIC) microscopy being regarded as the “gold standard” for certain types of abnormalities, particularly those involving the acrosome (Garner 1997), and for depicting subtle abnormalities of the sperm head or midpiece (Chenoweth et al 1994). With subtle sperm head abnormalities, DIC has allowed identification of a stereotyped spectrum of sperm abnormalities following insult, which is first observable as the classic sperm “diadem defect”. Phase microscopy has an advantage that it may be used with “fixed” semen samples, thus avoiding possible structural damage to sperm which may occur with traditional stained smears. However, it represents increased expense and sophistication, both of which reduce its attractiveness for routine use. The electron microscope (EM) represents the ultimate...
method for depiction of individual sperm abnormalities. However, this option is not regarded as practical for routine use as the process is complex, expensive and difficult to quantify.

Automated high resolution image analyzers can identify subtle differences in head shape and size, with the added advantage that they can process sperm at high speed, and thus provide quantifiable results (Gravance et al 1998). Difficulties encountered in their ability to differentiate between sperm and non-sperm particulate matter can be circumvented with the use of fluorochromes. Despite this, their applicability for routine use in domestic animals has yet to be established.

Fluorescence and Flow Cytometry

Fluorochromes can be used to assess specific functional aspects of sperm (such as live/dead) or sperm organelles (such as the acrosome or mitochondria). A number of stains have been employed to assess factors such as sperm DNA/nucleic acids (eg. propidium iodide, acridine orange, DAPI, Hoechst 33258), sperm membrane integrity (eg. CFDA, BCECF), intact acrosomes (PSA-FITC), functional mitochondria (rhodamine 123, JC-1, MITO) and sperm capacitation (CTC). The combination of fluorochrome staining of sperm with flow cytometry allows large numbers of sperm to be accurately categorized in a short period of time. Combinations of stains have also proven to be useful, with SYBR-14/PI being used in our laboratory to differentiate between living and dead sperm. Such methods have shown good relationships with other bovine sperm viability measures in our laboratory (Figure 1), as well as with other species elsewhere (Garner and Johnson 1994; Ferrara et al 1997; Pena et al 1998).

Although the use of fluorochromes in conjunction with flow cytometry provides rapid and accurate results, it does have several disadvantages including the extra expense and logistics involved. However, flow cytometry in association with cell sorting has the added advantage of being able to not only identify sperm with certain characteristics, but also to separate them. This method is being used to successfully separate X and Y spermatozoa based on differences in DNA content between the two populations (Garner 1997), although limitations are imposed by both cost and by the relatively low numbers of separated sperm which become available. However, indications are this technique will be commercially available before long.

K-State Andrology Laboratory

An Andrology Laboratory within the College of Veterinary Medicine at K-State provides male evaluation capabilities and services, including an evaluation service for semen samples sent in by practitioners. This is available for all species and for semen which is fresh as well as stored (frozen, chilled, extended). The tests available are shown after the references section as an example of andrology services available.

Selected References

Bellin ME, Hawkins HE and Ax RL (1994). Fertility of range bulls grouped according to the presence or absence of heparin-binding proteins in sperm membranes and seminal fluids.


**KANSAS STATE UNIVERSITY**  
**COLLEGE OF VETERINARY MEDICINE**  
**ANDROLOGY LABORATORY**

<table>
<thead>
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<td>Address</td>
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<td>Ph</td>
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**History/Exam Details:**
(Attach copy of BSE form if available)

**Ejaculate Details:**  
Method of Collection - Massage, AV, EEJ, Other.
Vol:  
Color:  
Density: DDDD, DDD, DD, D  
Comments:

**Tests requested:**

- **Fresh** - stain, extender, “fixed”
  - Live/dead % □
  - Morphology (stain) □
  - Morphology (phase/DIC) □
  - Sperm Concentration □
  - PIA □
  - Gram stain □
  - Other (including fluorochromes, flow cytometry) - by request.

- **Frozen**
  - Post-thaw motility (15 min & 2h) □
  - Live/dead % □
  - Morphology (phase/DIC) □
  - Sperm Concentration □
  - PIA □
  - Gram stain □

**Results**

- % Live
- **Morphology**
  - Stain  
  - Phase  
  - DIC
  - Fluoro/Other
- Normal
- Head
- Midpiece
- Tail  
  - $1^0$
  - $2^0$
- Prox. Drop
- Dist. Drop
- Acrosome  
  - $1^0$
  - $2^0$
- Detached Hd
- Other

- **Concentration:**
  - RBC
  - WBC
  - Other
  - $1^0$ Sperm Defects
  - $2^0$ Sperm Defects

- **Motility**
  - 15min  
  - 2h
- **Concentration:**
  - Fluoro/Flow Cytometry
  - “live/dead”

- **Chromatin**
  - Other

- **Other Tests**
  - HOS
  - CASA (IVOS):

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USE OF THE EAZI-BREED CIDR INSERT

Richard Pursley
Michigan State University

**Handling**

For best results, follow these simple steps:

1. Wear protective gloves whenever handling the EAZI-BREED CIDR Insert.

2. Prepare a container of clean water with disinfectant solution. Wash the applicator with the water between uses.

3. Fit the body of the insert into the applicator with the tail along the slot. The two wings will be pushed together; protruding about one inch above the top of the applicator.

4. Apply a generous amount of lubricant to the tip of the insert.

5. Shift the animal’s tail to one side, and clean the vulva.

6. Make sure the tail of the EAZI-BREED CIDR Insert is on the underside of the applicator; curling down, to ensure that the tail will be hidden from curious pen mates.

7. Open the lips of the vulva and insert the applicator at a slightly upward angle, moving forward over the pelvic bone until it meets resistance.

8. Dispense the insert from the applicator by depressing the plunger; then slowly withdraw the applicator.

9. To prevent removal by curious pen mates, you may want to clip the tail of the insert so that 2-1/2" protrude from the vulva.

10. To withdraw the insert seven days later; simply give the tail of the CIDR a gentle, but firm, pull to release the insert.

11. Dispose of used inserts in a sealed, plastic container in accordance with applicable local, state and federal regulations.
Current Recommended Protocol

Basic Program with Observed Estrus

<table>
<thead>
<tr>
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<th>Day 6</th>
<th>Day 7</th>
<th>Day 8 - 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administer the EAZI-BREED™ CIDR® Cattle Insert</td>
<td>Inject 5 mL LUTALYSE® Sterile Solution (dinoprost tromethamine)</td>
<td>Remove the EAZI-BREED™ CIDR® Cattle Insert</td>
<td>Heat detect and breed on detected estrus</td>
</tr>
</tbody>
</table>

References

NOTES

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TIPS FOR A SUCCESSFUL SYNCHRONIZATION PROGRAM

Sandy Johnson and Jeff Stevenson
K-State Research & Extension

Do you know if your herd is a good candidate for synchronization of estrus? Can you identify potential problems if AI pregnancy rates were lower than expected in an existing program? The guidelines below are designed to address these issues.

Normal Reproductive Response

- Pregnancy rates (number pregnant / number exposed) after a 60-day breeding season should be 85-90% before considering an intensive synchronization and AI program. Lower fertility may indicate that some other aspect of management such as nutrition or health is less than optimal and would reduce the success of an AI program as well.

Calving Distribution ⇐ Calving season length ⇐ Breeding season length

- The greater the proportion of cows calving in the first 21 days of the calving season, the better the response expected from a synchronization and AI program (Figure 1).

- Although some synchronization protocols can induce estrus and ovulation in some non-cycling cows, cows that calved during the 30 days just before the start of the breeding season are unlikely to respond.

- Using a synchronization protocol every year, gradually increases the proportion of cows that calve in the first 30 days of the calving season, subsequently increasing the pregnancy rates to AI in a parallel fashion (Figure 2).

- With longer breeding seasons (>70 days) and less than 60% of herd calving in the first 42 days of the calving season, expect much lower AI pregnancy rates. Timed AI of the entire herd would not be recommended.

Cow Age

- Duration of postpartum anestrus averages 20 days longer for first-calf heifers than mature cows. Even in herds that calve heifers ahead of cows, the proportion of primiparous cows cycling at the start of the breeding season was 9% less than multiparous cows (Figure 3. Stevenson, 2001).
**Body Condition**

- Body condition influences the length of postpartum anestrus and thus the proportion of cows cycling at the start of the breeding season. Cows need to be in a positive energy balance to resume normal estrous cycles. Over a range of body condition scores (BCS) of 4 to 5.5 (1=thin to 9=fat), proportion of cows cycling increased 18% for each unit increase in body condition score (Figure 4. Stevenson, 2001). This response would likely level out for cows with BCS over 6.5. The cow’s ability to conceive early in the breeding season also increases over this range of BCS.

**Mature Cows**
- BCS 5 – Good candidates for synchronization and AI.
- BCS 4 – 4.5 – AI pregnancy rates will be lower, risk of poor response may be lessened if plane of nutrition has been increasing 3-4 weeks prior to the onset of the breeding season. Timed AI is not recommended.
- BCS < 4 – Poor candidates for synchronization. Timed AI is not recommended.

**First Calf Heifers**
- BCS 5.5 – Good candidates if calved 3 weeks ahead of mature cows.
- BCS 4.0 – 4.5 – Higher risk.
  - Response to induction of ovulation with GnRH is about half of that in mature cows at similar BCS (Figure 5. Stevenson, 2001).
  - Consider using multiple methods to induce anestrus cows to cycle (e.g., calf removal and a progestin).

**Semen**
- A thorough breeding soundness exam (BSE) should be performed on bulls prior to freezing semen, including assessment of concentration, motility and morphology of sperm. This may not be done as a routine part of the semen freezing process by smaller independent collection operations.
- Semen should be processed at a CSS certified lab.
- Be aware that sire to sire variation in pregnancy rates exist for bulls even when they have passed a BSE (Figure 6. Yelich 2001).
  - Keep accurate records to check individual sire conception rates. Bull studs consider at least 250 inseminations before evaluating fertility.
at this level. However, suspected problems observed after fewer inseminations warrant further examination.

- Ask semen salesperson to identify high fertility sires before making final genetic decisions, especially for timed breeding.

**Technicians**

- Experienced technicians generally produce more pregnancies than technicians in their first season.
- Variation in conception rates between experienced technicians can range up to 20% or more. Evaluation of accurate records will allow problems to be recognized.
- When inseminating large numbers of females during timed AI, ensure you have enough technicians to complete the job.
  - Rotating jobs between loading guns and inseminating every 15-25 head is recommended to minimize effects of fatigue.
  - Size of inseminator’s forearm, particularly for heifers, can be negatively related to ability to inseminate large numbers. The pressure of the rectal sphincter on a large forearm speeds fatigue.

**Administration of treatments**

**Injections**

- Use appropriate sizes of syringes and needles, follow label directions and Beef Quality Assurance guidelines. Accuracy is the goal, not speed.
- Injections given immediately after insemination may be best administered with smaller gauge needles and syringes than normally on hand (20 or 21 gauge needles, 3-cc syringes). Do not inject in the top butt. Make sure you have the proper equipment in sufficient supplies (at least 1 needle per 10-15 cows).
- Have a specific place to discard old needles. An old milk jug will work well.

**CIDRs**

- Follow package directions, cleanliness is important during insertion.
- In confined situations and/or for heifers, you may wish to cut all except 2.5” off the tail of the CIDR so pen mates do not play with the tail and remove the CIDR early.
- Do not re-use CIDR’s.

**MGA**

- Uniform, consistent daily consumption is increased when adequate bunk space is available (18 – 24” for heifers and cows, respectively).
- Make sure all animals are up to the bunk or gathered before feeding.
- Feed MGA mixed with a small amount of grain that can be cleaned up in a relatively short time yet allows for everyone to get their share.
- When feeding MGA in a high volume total mixed ration, deliver half or less of the daily ration at first feeding with the entire MGA dose, delivering the
remaining ration later in the day. Split feeding increases the odds that those females with lower intakes will consume the entire daily dosage.

- Cows that are just getting new-growth grass in the spring at the time MGA feeding begins may ignore completely the MGA feed. To improve consumption, remove free-choice salt from the pasture prior to MGA feeding and include ½ oz of salt per head per day in the MGA supplement.

**Timing**

- Do not combine administration of synchronization drugs with routine vaccination, especially with modified live vaccines. Check with your veterinarian for appropriate timing. Most vaccinations should be completed several weeks in advance of the breeding season.

- Make sure to give the appropriate treatment on the appropriate day. Changes by even a day may seriously harm results. The Iowa Beef Center Synchronization Planner will print out a calendar of treatment days for the system of your choice. [http://www.ibc.iastate.edu/content/synch_planer.htm](http://www.ibc.iastate.edu/content/synch_planer.htm)

**Buyout System - Cow Program Only**

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*Turn in Bull Power*

**If you intend to identify AI vs. natural service calves, wait at least 10 days after the synchronized period to turn out bulls and employ early pregnancy detection. Pregnancy detection at 30 to 50 days after AI will minimize errors in proper identification of AI pregnancies.**

**Heat Detection**

*During synchronized estrus*

- Detection for 2 hours morning and evening and 1 hour at noon identified 40% more cows in estrus than checking twice daily for 30 minutes (Figure 7. Geary, 1999).
Many successful operations have someone watching cows during all daylight hours of the recommended synchronized observation period.

- During days of peak estrus, females that are identified in heat should be sorted off several times during the day. This allows animals that are just coming into heat to be more easily identified.
- Attempting to watch cows in large pastures is nearly impossible. Gathering cattle into a smaller pasture, moving cattle into a corner of the pasture or large pen always facilitates better heat detection. Moving and sorting stimulates heat activity.
- Animals need legible, clean ear tags or other forms of identification so they can be identified at a reasonable distance and accurately recorded for later sorting.
- Recommend at least one person observing heat per hundred head during peak hours. Heat detection in very large herds may be more effective if subdivided into groups of 200 or less.
- Heat detection aids may be useful, but not as effective as a visual observation.

**During naturally occurring estrus**

- Detecting for 30 minutes, twice a day is considered a minimum.
- Gomer animals, tail chalking, or heat-mount patches may be useful heat detection aids but their effectiveness depends on examining each animal twice daily for signs of standing activity.

**For first experience with synchronization**

- Make sure animals are in adequate BCS
- Start simple with a smaller group; heifers or early calving cows.
- Consider synchronizing and using bulls natural service the first year.
- Consult an expert when selecting a synchronization system.
- Trade help with an operation that has experience with AI and synchronization to learn how they do things and to have expertise on hand when it’s your turn.

**Facilities**

- Well-designed facilities in good repair minimize stress on animals and people to optimize results.
- If breeding on heat, areas for easy sorting and holding animals are needed.
- Cows generally stand quietly in a breeding box without heads caught.
- Have a plan for rainy weather.
- Semen handling and thawing should be done out of direct sunlight.
Characteristics of successful estrous synchronization programs:

- Good year-round nutrition program
- Cows are in a minimum BCS of 5 at calving time
- Total breeding season is 60 days or less
- Functional facilities for sorting, administration of treatments, and AI
- Skilled help
- Good record keeping
- Effective vaccination and health program
- Attention paid to details

References


NOTES
APPLIED BIOSECURITY

Larry C. Hollis, DVM, MAg, Kansas State University

Many beef cattle producers fail to utilize basic biosecurity measures and thus put their operation at risk. Biosecurity is a series of good management practices that can help keep a livestock producer in business, while the lack of biosecurity may cost him/her the business.

Basic biosecurity starts with protection of the existing herd. Vaccination of the existing herd against such commonly-encountered diseases as blackleg, vibrio, lepto, IBR, BVD, BRSV, PI3, etc., is the cornerstone of any biosecurity program. Depending upon your location, and diseases that are common to your area, other diseases may need to be included in your vaccination program. Check with your veterinarian for his/her recommendations.

Protection of the existing herd includes purchasing replacements from herds with known histories, which include the lack of key disease problems and an active disease prevention/management program. Don’t hesitate to ask if the purchase source herd has experienced such problems as Trichomoniasis, Vibriosis, Leptospirosis, BVD, Johnes disease, or bovine leukosis. You could easily purchase healthy-looking carriers of any of these diseases and contaminate your existing herd. Don’t let the purchase source become the exposure source.

Once “clean” animals are purchased, make sure that the trucks or trailers used to transport these animals to your location are clean. Has the truck/trailer been used to haul high-risk feeder calves from an auction market to a stocker operator or feedlot immediately prior to loading your cattle? Has it been used to haul chronic poor-doing animals from a farm/ranch/feedyard to an auction market? Has it been used to haul a sick calf to the veterinarian for treatment, or a dead calf for a necropsy? Trucks or trailers should be cleaned and disinfected if they have been used to haul anything other than normal, healthy cattle prior to hauling “clean” animals. Don’t let the transportation source become the exposure source.

Quarantine new herd additions for 45 days. Quarantine facilities should not allow fence line contact with the existing herd. This 45-day period will allow many incubating illnesses time to manifest themselves before new cattle are mixed with existing cattle. It will allow time to test for key diseases such as BVD, Johnes and bovine leukosis, and get the results back from the lab. It will also allow time to vaccinate the new animals in the same manner that the existing herd is vaccinated, and give vaccinated animals time to respond to the vaccine before being exposed to the existing herd.

Control access of people into your operation. If they have visited another cattle operation prior to coming to yours, they may bring disease from that operation to yours. Asking where they have been prior to coming onto your operation is a starting point. Ask your veterinarian what he has done earlier in the day, and don’t hesitate to ask him to scrub his boots with disinfectant or put on clean coveralls prior to working with your animals; likewise with any other visitors. Don’t let visitors become the exposure source.
Control vehicle access into your operation. Good gates and padlocks are a cheap form of biosecurity! Rendering trucks, feed trucks, and any other vehicles that travel from farm-to-farm may bring disease onto your operation. Develop a “traffic flow pattern” to limit access of outside vehicles to restricted areas on your operation. Designate a “visitors” parking area. Rather than allowing a rendering truck to drive to a dead animal, pull any dead animal to a pre-determined spot that is out of the normal traffic flow pattern. Don’t let outside vehicles become the exposure source.

Control across the fence contact. We’ve all heard the old saying, “good fences make good neighbors.” If your neighbor is constantly bringing in new cattle from a variety of sources or backgrounds, or if your property butts up against a well-traveled road, establishing a “buffer zone” between your operation and the neighbor or road will reduce the likelihood that your herd will be exposed to something from across the fence. Building a secondary fence 25-100 feet inside the perimeter fence in areas of potential exposure will provide a buffer that many pathogens will not be able to cross. Don’t let your single 5-wire fence become an exposure source.

Maintain a separate sick pen area for sick animals from your herd. This will reduce exposure within your own herd. Sick pens should not allow fence line contact with the existing herd. Always treat sick cattle after you have completed working with healthy cattle – not vice versa. Don’t you become the exposure source.

Biosecurity is not paranoia, but it does require a new way of thinking. It is thinking defensively. It is thinking preventatively. It is becoming more proactive in keeping disease out of your operation. It is a good habit to develop that could save your economic future!
Questions Submitted to Speakers

1. How do you get LH pulses? What stimulates their production?

Answer (CL): LH pulses always occur during the cycle, but the LH surge (which causes ovulation) is a result of an increase in estrogen and a decrease in progesterone. As estrogen continues to increase, a positive feedback occurs with LH resulting in a spike or surge of LH.

Answer (SJ): LH pulse frequency and amplitude vary with the amount of negative feedback from progesterone. At the end of the cycle when progesterone is decreasing as a result of luteolysis, LH pulse amplitude and frequency increases stimulating estrogen production from the preovulatory follicle and the LH surge. During the luteal phase, LH pulses are less frequent and at lower amplitude. This pattern of LH pulse frequency allows follicular wave patterns to continue normally. In the case where there is no CL and an exogenous source of progesterone is provided such as with MGA, the MGA is sufficient to prevent an LH surge and the rather low levels of progestin allow LH pulses to increase similar to what happens at the end of the cycle. This creates the situation of the persistent follicle, where there is sufficient LH to stimulate the follicle to grow to preovulatory size and beyond with high estrogen production, but enough progesterone to block the LH surge.

2. Is there any data to suggest or physiologic reason to suggest that dairy cows would have a decreased pregnancy rate on the second or third lactation that the OVSYNCH protocol was used to synchronize estrus?

Answer (CL): The data is conflicting - in some experiments first lactation cows have a better fertility response than multi-lactation cows, but in general the feeling is that there does not seem to be a net difference between the two groups of females. We need more data. In addition, much of the data is somewhat confounding because many females are presynchronized with PGF at various stages, so it is difficult to verify whether Ovsynch is the influencing factor.

3. What determines the female’s follicular wave pattern; whether it is a two-wave or three-wave follicular pattern?

Answer (CL): It generally tends to be nutritionally and age related, but there may be other factors involved too. Cows in better condition tend to have more follicular waves than cows in poorer condition. Therefore, beef and dairy heifers usually tend to be 3 or 4 wave animals, whereas beef cows tend to be 3 wave and dairy cows are predominantly 2 wave cows. Experiments manipulating diets have altered the number of follicular waves.

4. What can we do with the superovulated donor (GnRH?) to maximize the number of follicles to ovulate? And also, to decrease the number of hours over which ovulation occurs?
5. Is there any evidence that GnRH at the start of estrus on a super ovulated [cow] will increase ovulations? (↑ovums released)

Answer (CL): Timing ovulation in superovulated cattle is an issue; however, one way to ensure the maximum number of follicles ovulates is to inject GnRH at the time of first insemination (onset of estrus). Although results from studies using GnRH in a superovulation protocol are mixed, GnRH may be an insurance policy to ensure an adequate LH surge is induced.

6. Your position is that small follicles yield small CLs and therefore lower P₄ compared to larger follicles yielding larger CLs. Is this in contrast to ET recipient studies in the 80’s wherein CL size did not significantly influence P₄ levels? And if so, what is YOUR position on the influence of CL size on ET recipient pregnancy rate?

Answer (CL): In large studies where we have taken blood samples (to determine concentrations of progesterone) at the same time as embryo transfer and determined the amount of CL tissue (with ultrasound) we have seen a correlation between luteal tissue volume and circulating progesterone. However, regardless of the size or volume of the CL as long as there is luteal tissue present, pregnancy rates appear to be similar. In other words, CL size does not seem to affect pregnancy rates.

7. What pelvis growth rate do you use?

Answer (RR): I typically use 0.25 cm/day for pelvic growth rate from yearling age to calving. Producers wishing to adjust pelvic areas to a standard can use this growth factor.

8. When you pelvic measure - palpate heifers & find an elevated symphysis but the pelvic score is greater than 150 - 160 cm², do you discriminate vs. that heifer? What elevation in inches or cm do you find acceptable?

Answer (RR): I do discriminate on both size and shape of the pelvis. I know of no work to show how much elevation is acceptable, but on pelvises where it is severe and gives a “keyhole effect” shape, I specifically make note of that and inform the client.

9. Is there a correlation between birth weight (calving ease bulls) and pelvic measurement? Are calving ease bulls giving us small pelvic measurement daughters?

Answer (CL): Yes, smaller birth weight bulls tend to have smaller pelvic areas. Pelvic area is directly related to frame and selecting for smaller or lighter calves you generally select for smaller frame animals resulting in a selection for smaller pelvic areas.

Answer (SJ): Research has shown a moderate negative correlation (-.22 to -.25) between pelvic area and calving ease. So as pelvic area in females goes up the problems calving are reduced. Remember that calf birth weight is an indicator trait for calving ease, there is not a 1:1 correlation. Birth weight does have a strong positive correlation with mature weight, but there are individuals with low birth weights and good growth traits after birth. If a producer used calving ease bulls with good weaning and yearling weights and culled yearling heifers with small
pelvic sizes there shouldn't be a problem. However, selecting calving ease bulls without regard to other growth traits could result in smaller framed, smaller mature weight daughters that in turn had smaller pelvic areas as a result of the smaller frame. The moral of the story: Single trait selection usually creates problems in other areas.

10. Have you suggested marketing RTS 1 & 2's as feeders? With the number of animals that get pregnant @ 3 + cycle could there be a nutritional affect? (i.e. micro-minerals?? Or endophyte)

Answer (CL): Depending on the operation we can often move RTS 2 or 3 heifers to 4 and 5 given sufficient time. In many cases we will eliminate heifers with a RTS of 1 and feed them as feeders. Many factors play a role as to why those females do not have developed reproductive tracts. The primary factor is definitely nutrition related.

Answer (RR): Typically, heifers with an RTS of 1 are considered non-breeders and are culled. Heifers with an RTS of 2 could be a result of age and/or nutrition. Heifers with an RTS of 2 may simply be too young to fit in the current breeding season. The producer has the option of culling RTS 2’s or possibly moving them to a fall breeding herd if he has both as many of our producers in Missouri do. If it is a result of nutrition, we can often change this in a relatively short period of time (2 weeks to a month). That is why we suggest doing the pre-breeding exams 30 to 60 days before the planned breeding season so there would be time to correct nutritional problems.

11. How long after breeding should you wait to give a killed vaccine?

Answer (RR): No work has been done to specifically answer this question, but as a rule of thumb, most veterinarians would recommend waiting a minimum of 30 days after breeding before giving any vaccines.

12. When do you vaccinate cows - late term for calf immunity to avoid breeding implications?

Answer (RR): Vaccinating any time in the 3rd trimester (last 90 days) is probably effective at providing good colostral antibody levels. Certain vaccines such as the scours vaccines need to be given closer to calving (within 30 days) to get effective antibody levels in colostrum. Producers should consult with their veterinarians regarding their herd health program to determine the most appropriate vaccines and timing based on the health history of their herd.

13. Is there an economic basis to suggest not giving vaccines during synch? (i.e. why give vaccines 30 days prior to synch - is there research?)

Answer (SJ): If the vaccination reduces the synchronized pregnancy rate that would certainly have an economic impact (money invested in synchronization wasted). There are published reports that indicate conception rates are lower in heifers vaccinated just prior to AI than controls and other indications of that happening in the field. The problem is likely to be greatest in animals that have not developed immunity from previous vaccinations.
Answer (RR): Placing an exact economic value to any vaccination given at a specific time is difficult to access. Remember that vaccinations are more like an insurance policy. If a heifer is staying in a herd as a replacement, then vaccinations given are both providing protection to that heifer and also establishing the basis for long term immunity as that heifer enters the herd and reaches adulthood. If adequately immunized as a heifer, then annual booster vaccinations will provide better protection for that animal and help to maintain adequate herd immunity.

14. Any risk/concern for increased incidence of twins when giving GnRH at timed breeding? Is there higher incidence of double ovulation when giving GnRH at that time?

Answer (CL): In beef cattle – if anything twinning rates seem to have declined slightly to the average. Because GnRH only ovulates follicles after deviation (greater than 10mm) it should not alter ovulation rates.

15. Can pregnancy % be increased by giving GnRH to an animal that is heat detected & AI’d?

Answer (CL): Yes, potentially GnRH can alter pregnancy rates, but only marginally (at best). Therefore, the potential benefit does not warrant the expense giving GnRH at AI for a marginal pregnancy rate increase.

16. How far out can you go in hours before giving GnRH and TAI on a hybrid synch program? 96 hours?

Answer (CL): Certainly the further out from the PGF injection the more cows you will detect in estrus and reduce the number of cows you inseminate at a fixed-time. However, if you plan on waiting for 96 hours before timed AI you are probably better off not inseminating those cows. A rule of thumb would be to wait a maximum of 80 hours after PGF to use timed AI in Hybrid Synch.

17. What is optimum time for timed AI in OVSYNCH, CO Synch and Hybrid Synch programs? Values (??) from 48-80 hours

Answer (CL): For Ovsynch the GnRH should occur 48 hours after PGF and AI 10 to 16 hours later. For CO-Synch the research has been inconclusive to this point, but the ideal timing may be for timed AI and GnRH between 54 and 60 hours after PGF. For Hybrid Synch the amount of time between PGF and Timed AI/GnRH can vary depending on the management system and time (days) that a producer wants to detect estrus – Use Time AI before 80 hours after PGF or do not use time AI, it probably will be less effective.

Answer (SJ): We have compared CO-Synch at 48 and 60 hours after PGF and found no difference in pregnancy rates to the single timed AI. There are several indications in the literature that cows categorized as non-cycling prior to the start of synchronization come in heat sooner after PGF than cycling cows. This implies that the best timing for a timed AI would depend on the proportion of cycling and non-cycling cows. In the study mentioned previously, there was no interaction of time of insemination with cycling status.
18. Is there any difference in effectiveness between different “brands” of PGF$_2\alpha$ or GnRH?

19. Which is better - Lutalyse or Estrumate?

20. Lutalyse - Prostamate - Estrumate B which product will cause the most recipients to cycle and in the tightest, closest time frame?

21. I understand a prostaglandin analog is more effective, and the analog prostaglandin has a much longer half-life. So, is there any practical or economic advantage in using an analog product like Estrumate?

22. Is there a difference in efficacy between Lutalyse and generic? I.E. Cystorelin vs. generic.

Answer (CL): Side-by-side studies are limited. For GnRH, the few comparisons have been inconclusive or shown no difference in synchronization efficacy. Only one study has indicated an increased LH surge, but practically neither product appears to have an advantage for estrus synchronization. For PGF, side-by-side studies indicate no difference between Lutalyse and Estrumate for synchronizing cows. Other PGF products have not been compared, but at the current doses little difference would be expected to occur.

23. Is there any harm with reshooting heifers with either of these products - Lutalyse or Estrumate - after 10 days when you either missed the cycle or no heat was seen? (Using multiple doses.)

Answer (CL): No, there is not harm - a common practice is to reinject females with PGF (any product works just as well) between 11 and 14 days after the previous PGF injection if they were not previously detected in estrus.

24. Is it necessary to give the full dose (2 ml) of GnRH in synchronization protocols that utilize GnRH in any way? If not, which protocols can one get by on, say 2 dose? Or, more importantly, will the money saved in decreased drug costs be lost in decreased reproductive performance?

25. Any work with 1/2 ds GnRH in a Co-Synch program?

Answer (CL): In dairy cows decreasing the dose from 2cc to 1cc has shown little change in pregnancy rates. In beef cattle, data appears to be inconclusive. However, reducing the dose is a risk and the cost of a single GnRH injection should not be a reason to reduce pregnancy rates. I recommend a full dose.
26. Does the dose of PGF need to change with cow body weight?

Answer (SJ): A higher dose in dairy cows did not improve response, so I would use the same dose on all cows.

27. Why do cows come in heat before PGF<sub>2α</sub> with Select Synch programs?

Answer (CL): For two reasons: 1) some cows were anestrus prior to the GnRH and are having their first postpartum estrus, and 2) at the time of GnRH, many cows are at day 15, 16, 17 of the estrous cycle and either the dominant follicle is not large enough to respond to GnRH or did not respond to GnRH, allowing the cow to come into estrus spontaneously.

28. What level of progesterone needs to be reached to suppress estrus? How long will a CIDR maintain that level?

Answer (CL): Generally if circulating progesterone is above 0.5ng/mL then we are fairly sure there is luteal tissue present. This should be sufficient to suppress estrus in most cases. Data presented by Dr. Kesler shows P<sub>4</sub> above 2.5 ng/mL after the CIDR has been in place for 7 days. I’m not aware of data showing the P<sub>4</sub> levels beyond that time frame with the 1.38 g CIDR, however the expectation would be that there would be sufficient P4 to suppress estrus for 10 to 16 days.

29. The New Zealand protocol was to use an estrogen “capsule” @ CIDR insertion. Why not use in the U.S.?

Answer (SJ): The capsule was not included in the US product for two reasons. 1) There has been some question about how effective the capsule was as a delivery system for estrogen and 2) no one believed they could get approval through FDA for a combination treatment of progesterone, prostaglandin and estradiol all at one time.

30. How many hours, post CIDR removal, will you find the highest percent of estrous response?

Answer (SJ): The peak estrous response in a CIDR/PGF system is usually at 48 hours after CIDR removal. If PGF is given at the same time as CIDR removal, the peak will be flatter and later.

31. What happens if CIDR is left in longer than 7 days due to scheduling changes?

Answer (SJ): The result would depend on how much longer it is left in place. If the CIDR was in place for one additional day there would be little concern. Longer than 8 days I would not want to attempt a timed AI but rather just AI after detected estrus. In general, if the CIDR is left in place longer than 7 to 8 days, synchrony of follicular growth would likely be diminished.

32. Is there significant similarity between CIDR technology and human birth control skin patch that the “skin” delivery system should/could or is being pursued in cattle?
Answer (CL): I am unsure of the exact progestin used in the skin patch delivery system, but there is no effort to find an additional method of supplying progesterone to cattle. Several systems have been tried in the past, but retention rates were poor – trying to keep the product from being rubbed off.

33. Do you have any data on pregnancy rates on E. T. recipient cows synchronized by using CIDRs?

Answer (CL): Pregnancy rates after timed E.T. using the CIDR in the CO-Synch system have been excellent as long as a CL has been present we have achieved about a 60% pregnancy in direct transferred frozen embryos.

34. What is your recommendation for a cow that loses the CIDR? Drop her from group? How long to wait before re-starting?

Answer (SJ): If the fact that a cow has lost a CIDR is first discovered at the time of CIDR removal, I would continue as planned despite the loss. The cow may have a CL anyway, respond to PGF and come in heat with the rest of the herd. There is no way of knowing when she lost the CIDR and the loss may have occurred a few hours ago. If the cow does not show heat within 5 d after PGF you could “re-start” after that if desired.

35. When feeding MGA, how will it affect young bull calves still on the cow?

Answer (SJ): MGA will not hurt young bull calves eating with their mothers at the time of synchronization or as the bulls obtain reproductive maturity. If these calves are large enough that they consume a significant amount of feed, the total amount fed may need to be increased to account for their consumption.

36. What would be the effect of excess MGA on synchronization results? Miscalculation of dose?

Answer (SJ): Excess consumption of MGA can have negative affects. A single day’s consumption of 2 mg/hd/day of MGA can leave enough MGA in fatty tissues that the animal stops cycling for a month after MGA withdrawal. Some producers are currently using .7 mg/hd/day to prevent breakthroughs during MGA feeding with similar results as those using .5 mg. As you get up to 1 mg/hd/day, the interval to estrus after withdrawal would increase. So depending on where and how you were using MGA in a synchronization system it could change the timing of things especially if timed AI were involved immediately after MGA feeding stopped. Problems with over consumption are most likely to occur when MGA is delivered in a small amount of feed (< 2 lbs per head). Increasing the delivery system to 4-5 lbs will increase the chances for uniform consumption. As intake of MGA goes below .4 mg/hd/day, the number of animals that will break through and come into heat increases. Errors can occur in mixing and diluting MGA so having someone double check your math is a good idea. Having a record of how the dilution/mixing was done can be helpful if a question comes up later about animal response.
37. MGA Select program - with or without calf removal? Movement after AI to pasture - how long after AI should cattle be given before being moved?

Answer (SJ): In a study conducted this spring, calf removal resulted in a 7% improvement in pregnancy rates to timed AI with a modified MGA-CO-Synch system. The additional advantages to the calf removal were only sorting calves off once and not much work to gather cows for AI (cows were in a 600 + acre pasture).

The limited data that are available on the impact of transportation after AI would indicate animals should be moved sooner rather than later. When heifers were hauled about 6 hours or 300 miles, synchronized pregnancy rate was higher for those moved within 4 days after AI compared to those moved 8 to 33 days after AI. Information is lacking on variability due to distance of haul and other time periods after AI.

38. Does change in diet from TMR to grass affect conception? Effects of inclement weather at time of AI.

Answer (SJ): Depending on the energy in the TMR diet and the amount and growth of pasture, the change in diet may be detrimental if there is an extreme change. This question is often raised when heifers are AI’ed one round and then taken to grass. Keep in mind that diet change is just one of the things going on at this time. In addition to diet change, some heifers have just reached puberty, moving is a stress and can reduce conception rates and heifers may be mixed with unfamiliar cattle.

The impact of inclement weather at AI probably depends on a variety of factors. I have had heifers show strong heat in some pretty nasty weather that I really didn’t want to be out heat checking in. If a front comes through and several days of weather change are involved, a problem is more likely. Good nutrition, good drainage and windbreaks would lessen any effects. If the weather is less than pleasant it could be the heat detection is not as effective as it should be (ie, watching from a pickup vs in the pen; less time spent because of cold or wet) and could explain fewer animals observed in heat.

39. Have you seen any other reasons for cystic ovaries in heifers than prolonged MGA use?

Answer (CL): Outside of MGA induced cystic ovaries we have identified very few cases of cysts. Because the nutritional, lactational, and nursing stress tend to be reasons for causing cysts you generally will not find a high incidence of cystic ovaries in heifers.

40. Could you please discuss the practical and economic advantages of Re-Synch? (i.e. labor, costs, drugs)

Answer (SJ): The advantage to a Re-Synch system would be to pick up additional AI calves without an extended period of heat detection. For most commercial operations using an $8 CIDR that would be placed in all cows (pregnant or open) in a resynch program as just part of the cost to produce perhaps 20% more pregnancies would not be economically justified. But if
you have a purebred herd where the market value of bull calves may change by several hundred dollars per AI pregnancy, then the systems shown may be a good tool to get those additional pregnancies. Comparatively speaking, very little work has been done in this area so hopefully over time we can improve these systems by decreasing costs and improving responses.

41. Best time for body condition scoring to have a positive effect on conception date. Pelvic measurement relationship to frame size and weight.

Answer (SJ): We really need to be monitoring body condition score (BCS) year round. It is certainly useful to record BCS at preg checking in the fall to estimate adjustments needed to ensure a BCS of 5 at calving. Adequate BCS at calving is the most important target we should try to hit and then to ensure cows have enough nutrition to be in a positive energy balance prior to breeding. If we miss our target of 5 for mature cows and 6 for first calf heifers at calving, we can try to catch up after calving but usually cows will produce more milk rather than increase condition. So trying to score cows at so many days prior to breeding is generally too late to really change cow condition if that is needed. A better approach would be to score cows 30-60 days prior to calving which would allow time for cows to gain weight if needed.

Pelvic area has a high positive correlation with frame size and weight. Any selection for pelvic area should be done within a frame size. A good way to use pelvic area information is cull those with the smallest pelvic areas, rather than selecting for large pelvic area. Combined with selection of bulls with low birth weights, any calving difficulties should be minimal.

42. Economic thoughts on estrus detection vs. TAI in a large dairy herd?

Answer (CL): Estrus detection in dairy cows is very poor and is probably the single biggest reason for failing to get a cow pregnant by about 90 days after calving. If you consider that the average dairy cow is mounted about 6 times every estrus for about 2 to 3 seconds per mount the chance of a producer detecting an estrus is not likely. Therefore, TAI may in fact be more economical in the long run. In addition, the cost of labor for heat detection can be eliminated and cows present for TAI can be set up to be inseminated on the same day every week or two weeks. These are advantages of TAI that seem to be over-looked in a dairy setting. Understandably, less initial drug costs are seen with heat detection, but ultimately a pregnant female is worth more.

Answer (SJ): One of the problems that large dairies have with TAI is that all the injections must be given at the appropriate time and sometimes the labor situation is such that either the injections don’t all get given or perhaps not at the correct time. In that case the cows don’t become pregnant. So as with any AI program, overall management impacts the ability to implement a successful program.

43. We have used several estrus synchronization schemes and have experience delayed conception with clean-up bulls after AI - usually 60 days. What are some reasons for this delay? BCS of these females is 5 & 6. Cows were ≥50 days post-partum & calf removal was used.
Answer (CL): Good question, in many cases the majority of delayed conception could be that you established a pregnancy initially, the pregnancy was lost during the time the clean-up bulls were present and the cow was bull-bred late during the breeding season. Generally producers who use estrus synchronization now never used it in the past and have become more aware of their calving intervals and are better at recording breeding information. The last point also is a factor. Cows that are cycling have an excellent chance of becoming pregnant to the synchronization, but if you consider that even in well conditioned herds less than 60% of the females are cycling at the initiation of the breeding season. These could easily be the cattle that only initiate their estrous cycles later in the breeding season.

44. BCS - how high is too high - do BCS 8's or 9 settle as well as BCS 5.5 - 7.0?
Comment on the importance of proper equipment and facilities - and how the cattle are handled. Ex. ‘Cowboying’ vs Temple Grandin’s philosophy of livestock husbandry. Effect of cortisol levels and body temperature in relation to conception, etc.

Answer (CL): Anything greater than a BCS 7 is too high. Pregnancy rates begin to drop off after reaching a peak when females are at a BCS of 5.5 to 6. No question handling facilities and methods of handling make a difference to fertility. We generally find cattle that have been handled poorly in poor facilities tend to have poorer pregnancy rates. However, this could be confounded because of poorer total management, so this is tough to say from herd to herd. Nonetheless, cattle under stress release endorphins, cortisol, etc. that may play a role in decreased fertility (i.e., activating the “fight or flight” mechanism will decrease pregnancy rates). We do, however, know that transporting cattle is more effective during the first seven days of gestation than during days 7 to 14 of gestation.

45. What is the preferred heat detection methods preferred for synchronization systems? Most efficient? Heat Watch? Most economical?

Answer (SJ): When detecting heat in a synchronized group of animals, visual detection is my preferred method. There is no question if the animal is in heat or not. Most other aids end up requiring some interpretation along the line and cannot be expected to accurately identify all animals in heat without some visual detection as well. Heat Watch is the only system that has been shown to be equal to visual detection in accuracy. I find heat detection aids most useful for non-synchronized animals. What is the most efficient or economical would vary with operation and facilities, value of AI calves, labor availability, and size of operation. For a small purebred herd that did not synchronize and owned no bulls, Heat Watch may be a very sound investment. For many of the large heifer development operations that I know, visual detection is used. In that case the same labor that detects heat also helps sort off animals that need to be bred. When the help isn’t sorting, they are checking heat, resulting in efficient use of labor for the entire day.

46. When using synchronization systems in a cow herd, is there any long term detrimental biological effects of hormone use in cows? Any long term effects on their reproductive tract? Production of oocytes? Increase incidence of reproductive diseases or structural abnormalities? (This is after repeated synchronizations.)
Answer (SJ): No there does not seem to be long term problems. Used according to label, most of the products used for synchronization are metabolized fairly quickly so there would be no residues to cause long-term effects. Many of the products are identical to what is produced naturally and we just give it at a time of our choice. Even if MGA is fed for a long period of time, as soon as it is cleared from the system, the animal resumes normal estrous cycles. Some ET donor cows seem to have problems if they are in the collection process for a long time. The problems usually relate to the fact the cow is not lactating and becomes rather fat and in some cases adhesions can develop on ovaries that had extensive response to superovulation.

47. What breeds respond most favorably to fat supplementation besides the Limousin x Piedmontese combination?

Answer (RF): The heifers were from cross-bred cows sired by either Hereford, Limousin or Piedmontese. Other breed differences related to fat supplementation have not been studied.

In this case, there was a diet X sire breed interaction on percentage of heifers pubertal at beginning of breeding.

<table>
<thead>
<tr>
<th>Sire</th>
<th>low fat</th>
<th>high fat</th>
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<tbody>
<tr>
<td>Hereford</td>
<td>74.4</td>
<td>76.3</td>
</tr>
<tr>
<td>Limousin</td>
<td>69.8</td>
<td>60.5</td>
</tr>
<tr>
<td>Pied</td>
<td>76.2</td>
<td>97.6</td>
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</tbody>
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Backfat was greatest for Hereford > Limousin > Piedmontese
So, there appears to be more going on than just a fatness issue. There may be inherent breed differences for fat requirements, similar to findings that there are breed differences for mineral requirements, but the relationship is not well understood, nor has it been tested appropriately.

48. Has PMSG been considered for use: 1.) At time of removal of CIDR, 2) at day of taking off MGA, 3) at day 7, 4) as a consideration to inc. levels in non-cycling cows GPS, 5) on repeat AI synchronization at time of CIDR removal. Has PMSG been considered as a source of LH, Estradiol?

Answer (CL): Yes, some consideration has been made for using PMSG and at least one laboratory is using PMSG as a possibility for synchronization – research results may be available within the next year or two. However, I am not familiar with the preliminary results yet.

49. CIDRs - do you see the same increase in P₄ levels with used vs. new CIDRs and timing/overall success? Are these detrimental effects of early pregnancy check 28-30 days to pregnancy rate, i.e. working stress, etc?

Answer (SJ): The CIDR approved in the US was designed with 1.38 g of P₄ to reduce residual P₄ in the insert because of environmental concerns. I have had producers tell me that the CIDR can be reused multiple times, which could be based on things they have heard from those using the 1.9 g CIDR in other countries. Whether or not a used CIDR may impact timing and or success of a synchronization program will partly depend on if the animal has a CL or not.
used CIDR placed in a cow in late diestrus or proestrus may prevent ovulation but not deliver enough P₄ so that follicle turnover occurs. More animals at this stage of the cycle might make the difference more noticeable. So results would depend on how the “used” CIDR was incorporated in a synchronization scheme (and how many prior uses) and if follicle control was incorporated. Situations are conceivable in which a used CIDR may not produce the same results as a new CIDR. This is particularly true if the number of uses was not accurately recorded or the cleaning process used resulted in significant P₄ loss from the insert. A new CIDR will provide a consistent response without exposing the cow to new pathogens or other problems from an unsanitary used CIDR.

The second part of the question must relate to reports of embryonic loss that were noted as a result of early pregnancy diagnosis at 30 days after AI. While I don’t think anyone has compared term pregnancy rates in animals that did or did not undergo early ultrasound, we have not noticed a corresponding drop in term pregnancy rates as this technology has been utilized. With skilled technicians and good facilities, there is very minimal stress involved in early diagnosis. That does not mean that stress can not cause pregnancy loss, but I don’t believe that stress is responsible for the embryonic loss reported in studies at this meeting.

50. Ralgro vs Synorex C effect on heifers.

Answer (SJ): If implants are used in potential replacement heifers, the choice of implants (Ralgro vs Synovex-C) is not as important as the fact that the animal only be implanted once and that they should not be implanted before one month of age for Ralgro or 45 days of age for Synovex-C. Ralgro implants given at birth will significantly decrease pregnancy rates of yearling heifers. When given once at 2-3 months of age, Synovex-C has been shown to increase weaning and yearling weights, not change age at puberty, increase yearling pelvic area but not precalving pelvic area, slightly delay average calving date (indication of first service conception rate) and have no effect on pregnancy rates compared to unimplanted controls.

51. Do you use same MGA dose (0.5 mg/hd/day) for cows and heifers?

Answer (SJ): Yes, the same dose of MGA (0.5 mg/hd/day) is used for both cows and heifers.

52. Are there any practical options for synch range beef heifers?

Answer (RF): I suspect practical relates to the number of times animals have to be handled and the ability to feed MGA. There are several options as outlined in the next question.

53. What synch can be used successfully on heifers?

Answer (RF): The best system for synchronizing heifers is the MGA/PGF system, with MGA fed for 14 d and the PGF injection given 19 d after the last MGA feeding. If heifers are cycling well, the two shot PGF system (given 14 d apart) can provide satisfactory results. The CIDR program has been shown to be successful in heifers. The Select Synch program has not been recommended for heifers, but more data is emerging that indicates it can be an option provided you are prepared to heat detect and breed animals that come in heat before the PGF injection.
These early heats can be avoided if a CIDR is inserted the day of GnRH and removed at PGF injection, or if MGA is fed from the day after GnRH until the day before PGF. Also, addition of a progestin may increase pregnancy rates in the Select Synch protocol.

54. Has anyone followed the impact on the calves that are/have been removed for synch purposes? (i.e. weaning, post-weaning, or feedlot/carcass performance?)

Answer (RF): There is limited information on this, but what there is indicates no adverse effects on calf health or weaning weights provided calves are given some care during this time. (See question 55.)

55. Is it worth a 10% increase in pregnancy rates, utilizing calf removal, if the detrimental effects on weight gain and stress induced illnesses in the calves leads to greater economic losses to the producer?

Answer (CL): At this stage of age calves tend to be in a high immune status (more than at weaning!) and will recover from any gain lost during the synchronization period. A more important question is: “Is it worth the time, effort, and strain on facilities to separate cows and calves for 48 hours?” Perhaps, perhaps not!! I feel this is an individual producer’s question to answer depending on their goals from synchronization.

Answer (RF) There does not appear to be a negative effect on calf health or weaning weights, some additional care may be required for the calves such as fresh, clean water and good quality grass hay, also some have recommended feeding a “sweet feed” to calves during the separation period.
Financial support for this program has been provided by:

USDA

Fort Dodge

Pharmacia Animal Health

Special acknowledgement is extended to Dr. Ralph L. Brinster, Richard King Mellon Professor of Reproductive Physiology, Laboratory of Reproductive Physiology, The School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA for permission to use the extremely unique image illustrating a state-of-the-art reproductive technology as a component of the cover page of these proceedings. This image was featured previously as the cover page for *Science*, Vol. 296, No. 5576, 21 June 2002.

“Male germline stem cells carrying modified genes (blue), transplanted to recipient seminiferous tubules (bottom), produce rat spermatozoa (middle). These sperm can fertilize newly ovulated oocytes (top) to generate transgenic animals. How species achieve their most fundamental goal – procreation – is the focus of the special section in this issue. [Image: J. Hayden, RBP (Bio-Graphics) and R. L. Brinster]”
Multi-State Extension Committees: Where Do They Come From?

As one of 19 agencies within the United States Department of Agriculture (USDA), the Cooperative State Research, Education, and Extension Service (CSREES) partners with the land-grant universities and other academic institutions and organizations “to advance knowledge for agriculture, the environment, human health and well-being, and communities”. Working together, Agricultural Experiment Station Directors, Cooperative Extension Agriculture and Natural Resource Leaders, and CSREES personnel create and administer Multi-State Research/Extension Committees.

A Multi-State Research Committee (NC-113) on “Methods to Increase Reproductive Efficiency in Cattle” was formed through the North Central Region of Agricultural Experiment Station Directors (http://www.wisc.edu/ncra/). From this collection of scientists, a North Central Region Extension Bovine Reproductive Task Force, a Multi-State Extension Committee, was envisioned. As a result of interested scientists participating in this Multi-State Extension Committee, this “Applied Reproductive Strategies in Beef Cattle Workshop” was created in the spirit of integrating research, education, and extension activities to disseminate timely, pertinent, and state-of-the-art scientific information for producers and their technology providers.